

7th Edition

Food

The Chemistry of its Components

Tom Coultate

Foreword by Professor Richard Pagni, University of Tennessee, USA



ROYAL SOCIETY
OF **CHEMISTRY**

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By

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Dedication

for
my grandsons
Isaac and Luke

Foreword to the Seventh Edition

Life originated on Earth, by as-yet poorly understood chemical and physical processes, at least 3.7 billion years ago. Over eons of time, these original life forms evolved into countless species, past and present. There are so many species alive today that it has proved difficult to enumerate the characteristics that they all have in common. In other words, what constitutes life? For example, are viruses – such as the SARS virus that causes COVID-19 – alive? Everyone would agree, however, that all organisms require food to survive, thrive and replicate.

Humans are no different in this regard, also requiring food to function, but food is so much more to us. Just imagine the time when someone discovered cooking over an open fire, thus making some things palatable and others tastier. It is hard to imagine this extraordinary event occurring in other animal species. We are constantly barraged by references to food on TV, radio and the internet, in books and magazines and, of course, in grocery stores. Just think of words such as diet, cuisine, vegan, recipe, fine dining, wine, Michelin stars, gourmet, ethnic, and thousands more, that we hear all the time. Whether one eats to live or the other way around, one cannot escape the milieu of food. If you are like me, you may wonder what is in food that is essential to our well-being, what makes eating and drinking so enjoyable to some, what happens when we cook, and why are there regulations about what can and cannot be in food. This is the information one hopes to find in a book on food science.

Almost 20 years ago I favorably reviewed an earlier edition of Tom Coultate's book on food in the *Journal of Chemical Education* and find the current edition to be likewise comprehensive, well-written and containing many useful structures, tables, graphs and references. There are the things of interest for the technically minded, but also things of a more general interest for those with little or no knowledge of chemistry or biology. Even a casual perusal of the book will yield delights that will make our eating experience more rewarding. The book will make a nice text for courses in food science and as a supplement to select courses in chemistry and biology.

Congratulations, Tom, on a job well done!

Richard Pagni

Preface to the Seventh Edition

In 1980 when the Royal Society of Chemistry first invited me to write this book the proposed readership was clear; namely chemistry teachers whose pupils were in their final two years of secondary school before going on to study chemistry at university level. Apparently these teachers were badly in need of background information to help them prepare their students for the new topic of Food Chemistry that had appeared on some of their syllabuses. By the time the first edition actually appeared (in 1984) the need for an up to date textbook of introductory food chemistry to support my own BSc Food Science students (at what was then the Polytechnic of the South Bank, London) had also become apparent. To my surprise the favourable response to the first and subsequent editions came from a much wider readership than was originally envisaged, not only in the UK but around the world. As a result demand for new editions has continued, including translations into Spanish, Portuguese and Chinese. Interesting new topics have demanded attention and chapters devoted water, minerals, undesirables and enzymes have been added.

My determination to ensure that the chemical formula is included for virtually every food substance mentioned in the text may make the contents look a little intimidating. However I have always tried to make the actual words as approachable as possible, lending them to being ‘read’, even ‘browsed’ as well as ‘looked up’. In spite of appearances readers with only modest recollections of school chemistry should find much that is within their grasp, especially in the later chapters.

Selections of texts for ‘Further Reading’ (books and review articles) are appended to each chapter, wherever possible restricted to 21st century editions. Extensive references to research literature are not included but each chapter concludes with a selection of relatively recent review articles that will provide access to the original research. By the use of citation indexing the same article may continue to provide an entry into the most up to date literature for many years. The temptation to include addresses of relevant and authoritative websites has generally been resisted since, unlike the printed scientific literature, websites tend to be rather ephemeral and are unlikely to remain available throughout the lifetime of a textbook. Undoubtedly the internet can provide a

wealth of information but it must be used with very great caution. It has always been the case that one should not necessarily believe something just because it was printed in a book, but such cynicism is absolutely essential when looking at websites. One exception is legislative and other official information published by government authorities and other similar organisations. This material is frequently very difficult to obtain in print and appropriate websites, accessible in 2023, are listed in Appendix II. The appropriate authorities, such as, in the UK, the Food Standards Agency, must always be consulted when accurate details of the legal position regarding, for example, the use of a particular food additive, are called for.

E Numbers

It will not have escaped the notice of readers of this book that the UK has now left the European Union. However, it appears that the possible implications of this departure on the regulation of food standards, food additives *etc.* in the UK are still some years away from serious consideration. It seems quite likely that within a few years the E prefix (“E” meaning “Europe”) in the numbering of permitted food additives on food product labels will change. Throughout this book all references to legislation and regulation refer to the situation that prevailed at the time of writing, *i.e.* 2022/23.

A Brief Note on Concentrations

The concentrations of chemical components are expressed in a number of different styles in this book, depending on the context and the concentrations concerned. Some readers may find the following helpful.

- (a) However they are expressed, concentrations always imply the amount contained, rather than added. Thus “5 g of X per 100 g of foodstuff” implies that 100 g of the foodstuff contains 5 g of substance(s) that are not X.
- (b) The abbreviation “p.p.m.” means “parts per million” *i.e.* grams per million grams, or more realistically milligrams per kilogram. One “p.p.b.”, or part per billion, corresponds to one microgram per kilogram.
- (c) Amounts contained in 100 g (or 100 cm³) are often referred to as simple percentages. Where necessary the terms “w/w”, “v/v” or “w/v” are added to indicate whether volumes or weights or both are involved. Thus “5% w/v” means that 100 cm³ of a liquid contains 5 g of a solid, either dissolved or in suspension. Although the millilitre (mL), cubic centimetre (cc) and litre (L) are no longer officially regarded as SI units they are widely used, by both the scientific community and the general public. Although the replacement for the mL, ‘the centimetre cubed’ (“cm³”) is widely recognised there is little sign that the cubic decimetre, or “dm³” has taken over from the litre, except in school and college teaching laboratories that must always be seen to ‘toe the party line’. As might be expected different abbreviations for the litre are in use on the different sides of the Atlantic, in the USA “L” is preferred, in Europe (and in this book) “l”. (d) Very often a strictly

mathematical style is adopted, with “per” expressed as the power of minus one. Since mathematically:

$$x^{-1} = 1 / x$$

5 mg kg⁻¹ becomes a convenient way of writing 5 micrograms per kilogram. This brief but mathematically rigorous style comes into its own when the rates of intake of substances such as toxins have to be related to the size of the animal consuming them, as in “5 milligrams per day per kilogram body weight”, which abbreviates to: 5 mg per day per kg body weight.

The term “body weight” may be abbreviated to “bw” or in some contexts omitted altogether. A concentration of, *e.g.* 10 mg per cubic centimetre, cm³, would be written: 10 mg cm⁻³.

Tom Coultate

Acknowledgements

This book's original objective, in the 1980s, was to provide background material for school teachers called upon to teach "food chemistry", a newly developed addition to the sixth form chemistry syllabus. However, it quickly became apparent that students of food science and related disciplines at polytechnics and universities also found it useful. To my surprise, this initial success has continued over the years, culminating in this seventh edition, with a three-fold increase in size, translations into several languages, and adoption as a course text in universities and colleges around the world. This success is owed to a number of factors, particularly the ongoing enthusiastic support from the Royal Society of Chemistry. Janet Freshwater, now Associate Publisher, has been my mentor and friend ever since, newly graduated, she was called upon to look after the third edition.

All this could not have been achieved without four decades of encouragement and support from my wife, Ann, and other members of my family. Over this period of time, I have enjoyed the advice and encouragement of innumerable friends and colleagues, starting with Dr David Rosie, who thoroughly reviewed the first edition. Dr (ultimately Emeritus Professor) Martin Chaplin, who joined me at South Bank Polytechnic (now London South Bank University) in 1988, has been a source of advice ever since. In particular, his contributions to the water chapter have proved invaluable. I first met Dr Lucy Foster when she was an undergraduate student on the BSc Food Science course at South Bank in the late 1980s. However, more recently, she has been able to enlighten me on many of the most recent developments in food chemistry, particularly its regulatory aspects.

I must also express my thanks to Professor Richard Pagni, of the Department of Chemistry at the University of Tennessee, for contributing such a generous Foreword to this edition.

Tom Coultate

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1 Introduction

During the eighteenth and nineteenth centuries, food became an important target for scientific investigators, particularly chemists. Alongside the medical profession, they realised that this knowledge was essential if dietary standards, and with them health and prosperity, were to improve. Inevitably, it was the food components present in large amounts – the carbohydrates, fats and proteins – that were the first organic, as opposed to mineral, nutrients to be described in chemical terms. However, it was also widely recognised that much of the food, and drink, available to the general public was likely to have been either contaminated accidentally or deliberately adulterated, often with the objective of enhanced profits for merchants and shop-keepers. Some of the chemists of the day took the blame for this:

There is in this city [London] a certain fraternity of chemical operators who work underground in holes, caverns and dark retirements ... They can squeeze Bordeaux from the sloeⁱ and draw champagne from an apple.

The Tatler (1710)

Fortunately, by the beginning of the nineteenth century, chemists had begun to expose some of the malpractices of food suppliers. For example, in 1824, a local newspaper in the north of England reported on the chemical analysis of some confectionery sold to local children:

One pennyworth of red balls, called we believe, Nelson or Waterloo Balls, – there were no such fine names in our lick-finger days, – yielded on analysis 30 grains of an indissoluble matter resembling red-lead,ⁱⁱ which on being fused with the blow-pipe produced 24 grainsⁱⁱⁱ of lead in a metallic state ...

ⁱBordeaux is a red wine from southwest France. Sloes are the dark purple berries of *Prunus spinosa*, a species of woody shrub also known as blackthorn. They are too astringent to be suitable for direct human consumption, but are traditionally used as a flavouring and colouring for a variety of gin.

ⁱⁱLead oxide, Pb_3O_4 .

ⁱⁱⁱ24 grains = 1.55 g.

A quarter of a century later, a famous survey was published^{iv} that revealed the extent to which the confectioners routinely borrowed colourings from the palettes of painters (of both pictures and houses). Lead chromate (yellow), lead oxide (red), Prussian Blue (a mixture nominally described as ferric ferrocyanide), copper oxychloride (green), copper arsenite (green) and mercuric sulfide (scarlet) were all in common use as food colours. Unfortunately, the major incentive for the development of the chemical analysis of food and drink was actually financial rather than public health. The UK government's activities were largely funded by excise duties on alcohol and tea and large numbers of chemists were employed to protect this revenue from the effects of the dilution and adulteration of wines and spirits.

As physiologists and physicians began to relate their findings to the chemical knowledge of foodstuffs, the need for reliable analytical techniques increased. Twentieth century laboratory techniques were essential for the isolation and study of vitamins and the other components that occur in similarly small amounts, including the natural and artificial colouring and flavouring compounds. Until the use of gas chromatography became widespread in the 1960s, the classical techniques of wet chemistry were the rule, but since that time increasingly sophisticated instrumental techniques have taken over. The latest methods are now so sensitive that many food components can be detected and quantified at such low levels (parts per billion, *i.e.* 1 microgram per kilogram, are commonplace) that one can have serious doubts as to whether the presence of a particular pesticide residue, or environmental toxin, at the detection limits of the analysis can really have any biological or health significance. It is perhaps some consolation for the older generations of food chemists that some of the methods of proximate analysis^v that they struggled with in the 1960s are still in use today, albeit in automated apparatus rather than using extravagant examples of the laboratory glassblower's art.

By the middle years of the twentieth century, it *appeared* that most of the questions being asked of food chemists by nutritionists, agriculturalists and the general public had been answered. This was certainly true as far as questions of the "What is this substance and how much is there in ...?" variety were concerned. Such questions not only concerned the obviously desirable nutrients, but also the substances that give our foods their characteristic – and desirable – flavours, colours and textures. At the same time, chemists were called upon to address the growing lists of undesirable substances, either naturally occurring or the products of chemical synthesis, that find their way into our food.

Throughout the second half of the twentieth century, biochemistry moved on from the two-dimensional structural formulae that abound in textbooks (such as this one) to the three-dimensional views that describe the shapes of molecules, even of the very largest proteins and polysaccharides. This has allowed us much deeper insights into the interactions between molecules – not only in living tissues, but also in the once-living tissues that constitute our food after we have harvested, preserved, processed and cooked it. We now recognise that these interactions between individual food components may

^{iv}A. H. Hassall, *Food and Its Adulterations: Comprising the Reports of the Analytical Sanitary Commission of 'The Lancet' for the Years 1851 to 1854 Inclusive*, 1855.

^vThe determination of total fat, protein, carbohydrate, ash (as a measure of metals) and water.

reduce, or enhance, their nutritional value as well making them more or less appealing or digestible. Much of the stimulus for this type of enquiry has come from the food manufacturing industry. For example, for many of us the observation that the starch in a dessert product provides a certain amount of energy (possibly more than we actually require) has been overtaken in importance by the need to know which type of starch will give just the right degree of thickening, and what is the molecular basis for the differences in behaviour between one starch and another. Furthermore, that dessert product must have a long shelf life, look as attractive and taste (nearly) as good as the version served in an expensive restaurant or its home-made equivalent.

Many of the changes in that take place in food materials during processing, storage *etc.* are definitely chemical in nature, such as the irreversible reactions involved in caramelisation or rancidity. Other changes may involve less permanent types of chemical interaction, such as when hydrogen bonds are involved in the formation of polysaccharide or protein gels. Some of the changes that occur in food materials, during storage or processing, are more biological in character, involving reactions catalysed by enzymes. Many of these reactions depend on the enzymes naturally present in food raw materials and have been unwittingly exploited for centuries, but only understood in recent years. However, in recent decades food processing operations that depend the use of enzymes from other sources, particularly microorganisms, have become increasingly significant. It is therefore important that this book includes a chapter devoted to the mechanics, and applications, of enzyme-catalysed reactions in the context of food product manufacture.

In recent years, we have had to come to terms with much wider aspects of food production and supply. Ordinary consumers may aspire to the gourmet dishes that popular media tell us are the normal diet of the rich and famous. However, the populations of less affluent nations similarly, and rightly, aspire to the varied, and often meat-containing, diets enjoyed elsewhere. We can no longer ignore the environmental and economic implications of these pressures – for example, the supply of soya protein for feeding cattle or the clearance of natural forests for oil palms. Of course, food chemistry alone cannot answer these questions, but as readers of this book will discover, it can make valuable contributions to the considerations of these issues.

Issues of flavour and texture may seem superficial, even cosmetic, compared with the dual roles of food as a supplier of essential nutrients and as a vehicle for natural and synthetic toxins. However, these apparently superficial aspects of food are actually of great importance. Nutritionists, physiologists and other scientists now recognise what consumers have always known – there is much more to the business of feeding people than assembling a list of nutrients in the correct proportions. This might be obvious in the supply of food in relatively affluent communities, but it is just as true if one is engaged in famine relief. To satisfy a nutritional need, a foodstuff must be acceptable and recognised for what it is, and that means looking and tasting “right”. We are now much more aware of two other aspects of our food. First, as some of us have become more affluent, our food intake is no longer limited by our income and we have begun to suffer from the Western disease of over-nutrition. Food scientists are now called upon to devise butter substitutes with minimal fat content, low-sugar, but tasty, breakfast cereals and low-salt/low-fat snack foods.

Closely associated with this issue is the intense public interest in the “chemicals” in our food. In the 1950s, the author’s first chemistry teacher, Mr Crosland, refused to allow the word “chemical” to be used as a noun. It is the author’s view a great pity that this attitude has never found wider acceptance. The general public’s limited appreciation of the language of chemistry continues to leave much to be desired – a situation the popular media and its self-appointed pundits never hesitate to exploit. One result is that it is unlikely that many people would readily buy coleslaw from the delicatessen if it carried a label like this.

Active ingredients:

Ethanoic acid, α -D-glucopyranosyl-(1,2)- α -D-fructofuranose, *p*-hydroxybenzyl and indolymethyl glucosinolates, *S*-propenyl and other *S*-alkyl cysteine sulfoxides, β -carotene (and other carotenoids), phosphatidylcholine.

All the substances in this list have the unifying characteristic that they are naturally occurring components of one or other of the raw materials that consumers would expect to find in coleslaw, whether or not it was a commercial product. Of course, many modern food products also contain ingredients that can be described as “additives”. According to the UK Food Standards Agency, additives are “Ingredients that are added to foods to carry out particular functions” without being nutrients in their own right. These functions are normally considered to include a food’s safety, freshness, taste, texture or appearance. The functions of particular types of food additives are dealt with in this book from a chemical point of view, but details of their regulation and legislation, which, of course, vary from country to country, are largely outside its scope and the expertise of its author.^{vi}

The issue of chemicals in food is also closely linked to the pursuit of “naturalness” as a supposed guarantee of “healthiness”. The enormous diversity of the foodstuffs consumed by *Homo sapiens* as a colonist of this planet makes it difficult to define the ideal diet. Diet-related disease, including starvation, is a major cause of death, but it appears that although our choice of diet can certainly influence the manner of our passing, diet has no influence on its inevitability. As chemists work together with nutritionists, doctors, epidemiologists and other scientists to understand what it is we are eating and what it does to us, we will steadily increase our understanding of the essential compromises the human diet entails. After all, our success on this planet is to some extent at least owed to our extraordinary ability to adapt our eating habits to what is available in the immediate environment. Whether that environment is an arctic waste, a tropical rain forest or a hamburger-infested inner city, humans actually cope rather well.

^{vi}But see M. Saltmarsh, *Saltmarsh’s Essential Guide to Food Additives*, Royal Society of Chemistry, London, 5th edn, 2020 and <https://www.food.gov.uk/safety-hygiene/food-additives>

This book sets out to introduce the chemistry of our diet. Chapters 2–5 and 13 cover food's macro-components – the carbohydrates, lipids, proteins and water – and these chapters are more obviously chemical in character. These are the substances whose chemical properties exert the major influence on the obvious physical characteristics of foodstuffs. If we are to understand the properties of food gels, we are going to need a firm grasp of the chemical properties of polysaccharides. Similarly, we will not properly understand the unique properties that cocoa butter gives to chocolate without becoming involved in the crystallography of triglycerides.

Chapters 6–11 are devoted to substances drawn together by the nature of their contribution to food, positive or negative, rather than by their chemical classification. For example, on the one hand we have colours, vitamins, *etc.*, and on the other mycotoxins, packaging residues, *etc.* Although this means that less attention can be devoted to the chemical behaviour of these substances individually, there is still much that the chemists can contribute to our understanding of flavour, appearance and nutritional value. Science is rarely as tidy as one might wish and it is inevitable that some food components have found their way into chapters where it might be thought they do not really belong. For example, some of the flavonoids mentioned in Chapter 6 make no contribution to the colour of food. However, in terms of chemical structure and behaviour, they are closely related to the flavonoid anthocyanin pigments and Chapter 6 may be regarded as a good a location for them as any.

Chapter 12 is devoted to enzymes. It is the reactions that enzymes catalyse that reveal their presence to us, rather than their chemical nature. All life depends on the activity of enzymes and every foodstuff, being biological in nature, comes to us in its raw state containing a multitude of different enzymes. Most we can ignore, some we need to inactivate to ensure the stability of nutrients, *etc.*, but others we have learned to exploit in the production of many food products.

The final chapter, Chapter 13, is devoted to water. Apparently the simplest of all our food's components, and certainly the most abundant, the actual complexities of water's behaviour and interactions with other food components have, until recently, tended to be overlooked.

This book does not set out to be a textbook of nutrition; this is outside the field of the author's expertise. Nevertheless, food chemists cannot ignore nutritional issues and wherever possible the links between the subtleties of the chemical nature of food components and nutritional and health issues have been pointed out. Similarly, students of nutrition should find a greater insight into the chemical background of their subject valuable. It is also to be hoped that people who campaign for the reduction or increase of this or that component of our diet to improve our health will gain a better appreciation of exactly what it is they are demanding, and its wider potential implications.

Food chemists should never overlook the fact that the object of their study is not just another, albeit fascinating, aspect of applied science. It is all about what we eat, not just to provide nutrients for the physical benefit of our bodies, but also to give pleasure and satisfaction to the senses. Not even the driest old scientist compliments the cook on the vitamin content of the food on his or her plate – it's the flavours and the textures, and the company around the table, which win every time.

Further Reading

H. McGee, *On Food and Cooking*, Unwin Hyman, London, 1984.

J. Burnett, *Plenty and Want: A Social History of Diet in England from 1815 to the Present Day*, Routledge, London, 3rd edn, 1989.

M. Toussaint-Samat, *History of Food*, Blackwell, Cambridge, MA, USA, 1992.

2 Sugars

Sugars such as sucrose and glucose, together with polysaccharides such as starch and cellulose, are the principal components of the class of substances we call carbohydrates. In this chapter we will be concerned with the sugars and some of their derivatives; other sugar derivatives such as the polysaccharides will be considered in Chapter 3. Although chemists never seem to have the slightest difficulty in deciding whether or not a particular substance should be classified as a carbohydrate, a concise formal definition has proved elusive. The empirical formulae (*i.e.* the ratio of each type of atom in the molecule) of most of the carbohydrates we encounter in foodstuffs approximate to $(\text{CH}_2\text{O})_n$, hence the name. It is simpler to regard them as aliphatic, as opposed to aromatic, polyhydroxy compounds that carry a carbonyl group (and, of course, the derivatives of such compounds). The special place of sugars in our everyday diet will be apparent from the data presented in Table 2.1.

Sugars are automatically associated in most people's minds with sweetness, and it is this property that normally reveals to us their presence in a food. In fact there are a great many foods, in particular elaborately processed products, where the sugar content may be less obvious. Fortunately, it is now common to find information about the total sugar content (as in: "Total carbohydrates ..., of which sugars ...") on the labels of packaged food products, but this tells us little about which sugars are present. In fact, only a few different sugars are actually common in foods, as shown in Figure 2.1.

It is often convenient to have an approximate measure of the total sugar content of food materials such as fruit juices. For example, farmers need to be able to monitor the accumulation of sugars in fruit as it ripens to help decide when it is best to start picking or harvesting. The chemical reactions of sugars that can be adapted for analytical purposes hardly lend themselves to generating instant results in the field. Instead, the reasonable assumption is made that sugars will account for almost all the dissolved solids in a fruit juice. The refractive index of sugar solutions is directly related to their sugar content and can readily be measured, in the field or factory, with a simple hand-held optical refractometer. These instruments report the dissolved solids content as ° Brix, where 1° Brix is equivalent to a sucrose concentration of 1% w/v, *i.e.* 1 g contained

Table 2.1 The total sugar contents of a variety of foods and beverages consumed in the UK. These figures should be regarded as typical rather than absolute values for the particular food concerned. The levels of sugars in eggs, cured or fresh meat, poultry, game and fish, and fats and oils are nutritionally insignificant, even though traces can sometimes be revealed by browning at high temperatures, caused by the Maillard reaction (see Section 2.6). (Data from: <https://www.gov.uk/government/publications/composition-of-foods-integrated-dataset-cofid>).

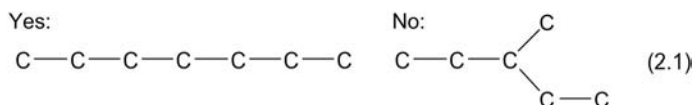
Food	Total sugars (%)	Food	Total sugars (%)
White bread	3.0	New potatoes	1.1
Cornflakes	7.3	Old potatoes	0.9
Sugar-coated cornflakes	38.3	Canned baked beans	4.8
Digestive biscuits	17.5	Frozen peas (uncooked)	5.7
Gingernuts (biscuits)	31.3	Broccoli (raw)	1.9
Rich fruit cake	49.6	Beetroot (raw)	7.0
Cow's milk (whole)	4.6	Onions (raw)	6.2
Human milk	7.2	Peanuts	6.2
Cheese (Cheddar)	0.1	Bananas	18.1
Cheese (processed)	5.0	Grapes	16.1
Yoghurt (plain)	7.8	Oranges	8.2
Ice cream (dairy)	22.0	Cooking apples (raw)	8.9
Cheesecake (frozen)	25.0	Eating apples (raw)	11.6
Burger, beef, raw without bun	2.7	Raisins	62.6
Honey	76.4	Beer (bitter)	2.2
Jam	69.0	Lager	1.5
Plain chocolate	62.6	Red wine	0.2
Cola	10.9	White wine (medium sweet)	3.0

in 100 cm³ of sugar solution; a reading of 20° Brix would indicate that the solution contained 20 g of sugar per 100 cm³.

2.1 Monosaccharides

The monosaccharides constitute the simplest group of carbohydrates and, as we shall come to see, most of them can be referred to as sugars. Monosaccharides have a backbone of three to eight carbon atoms, but only those with five or six carbon atoms are commonly encountered.

Monosaccharides that contain a carbonyl group have the suffix “-ose”, and in the absence of any other identification the number of carbon atoms is indicated by terms such as triose, tetrose, *etc.* The chain of carbon atoms is always straight, never branched (2.1):



All but one of the carbon atoms carries a hydroxyl group, the exception being the carbon forming the carbonyl group. It is the presence of a carbonyl group that confers reducing (*i.e.* readily oxidised) properties on monosaccharides and many other sugars, and the carbonyl is often referred to as the “reducing group” when considering sugar structure. The prefixes “aldo-” and “keto-” show whether the carbonyl carbon is the first or a subsequent carbon atom, *i.e.* whether the sugar is an aldehyde or a ketone.

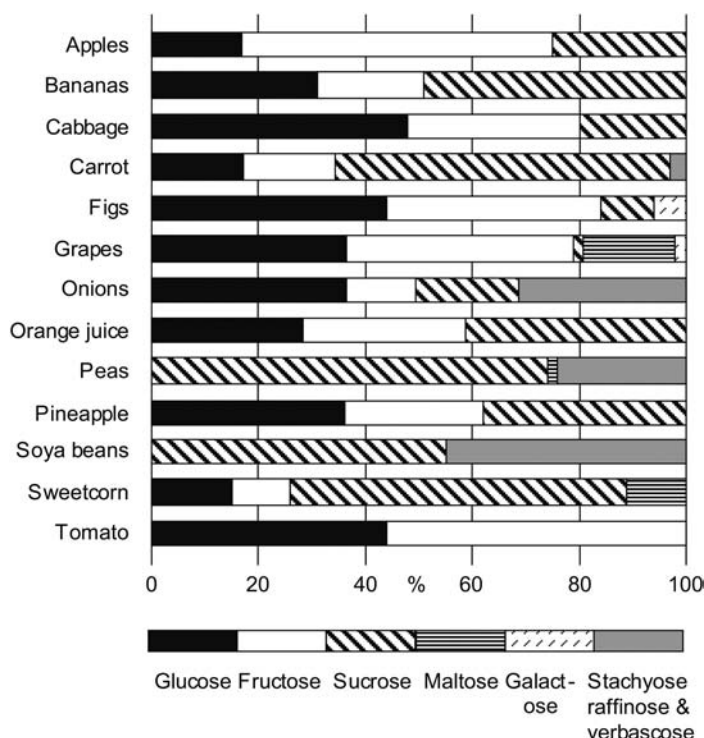
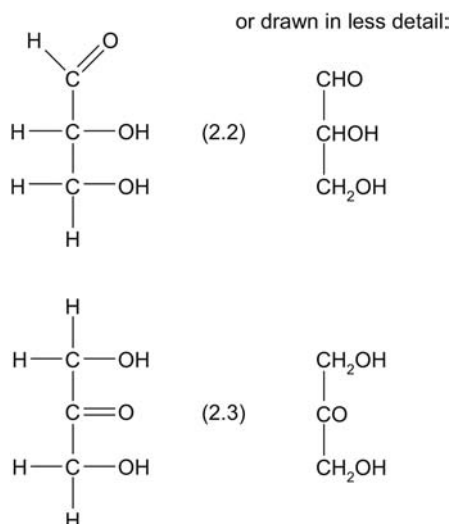
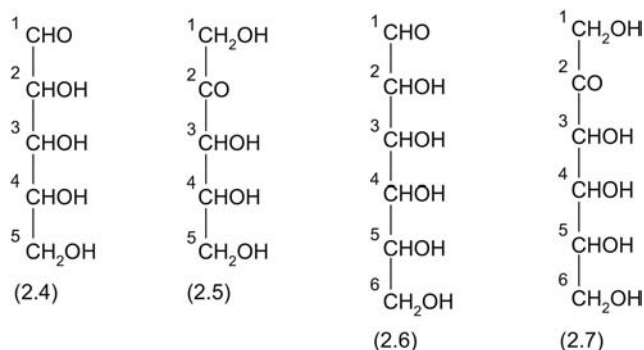


Figure 2.1 Representative percentage distribution of sugars in the total sugar content of various foods derived from plants. There is considerable variation in these proportions, depending on the variety, season, etc. The contribution of sugars to the total weight of plant material also varies widely between different species.

Thus we refer to sugars, for example, as aldohexoses or ketopentoses. To complicate matters further, the two triose monosaccharides are hardly ever named in this way, but are referred to as glyceraldehyde (2,3-dihydroxypropanal, (2.2)) and dihydroxyacetone (dihydroxypropanone, (2.3)):



Of greatest concern to us are the aldoses [(2.4) and (2.6)], ketoses [(2.5) and (2.7)], pentoses [(2.4) and (2.5)] and hexoses [(2.6) and (2.7)], shown here with conventional numbering of the carbon atoms:



The carbon atom in each CHOH unit carries four different groups and is therefore asymmetrically substituted, *i.e.* the molecule has no plane of symmetry. The effect of this on the simplest monosaccharide, glyceraldehyde, is shown in Figure 2.2.

These two molecules appear at first sight to be identical, in that all the elements of the structure are present in each and all of these are in the same spatial relationship. In fact, the two molecules are mirror images of each other. However, there is no way in which one of these could be rotated so that the two structures could be superimposed upon each other. Having four different groups attached to the central carbon atom means that the molecule as a whole has no plane of symmetry, in other words it is asymmetric, and its mirror images cannot be superimposed on each other. There is an obvious analogy in the relationship between one's left and right hands, and this gives rise to such isomersⁱ being referred to as chiral, from the Greek word for hand. Mirror image pairs of asymmetric substances like this are known as enantiomers, from the Greek word for opposite.

All substances that contain asymmetric centres are classified as members of either a D-series or an L-series, depending on whether their chemical structure is derived from D-glyceraldehyde or L-glyceraldehyde, respectively. The prefixes D- and L- are derived from, but are not abbreviations of, the words dextrorotatory and laevorotatory. These terms refer to the phenomenon of optical activity possessed by asymmetric molecules. Both crystals and solutions of optically active substances rotate the plane of polarised lightⁱⁱ when it passes through their crystals or solutions. Enantiomeric pairs of substances, such as D- and L-glyceraldehyde, rotate polarised light in opposite directions, in this particular case to the right (clockwise) and left (anticlockwise), respectively. Measurements of optical rotation, using an instrument known as a polarimeter, made valuable contributions to the analysis of sugars in foods in the years before sophisticated

ⁱ Isomers: each of two or more substances having the same formula, but a different arrangement of atoms in the molecule and, as a result, different chemical, physical or biological properties.

ⁱⁱ Polarised light is light that has been passed through a polarising filter. Unpolarised light consists of waves vibrating in all directions perpendicular to the direction of the light, whereas the wave motion of polarised light is restricted to a single direction. Polaroid™ sunglasses are a familiar example of polarising filters. Readers requiring a more detailed treatment of this topic, which is outside the scope of this book, may wish to consult an advanced textbook of organic chemistry.

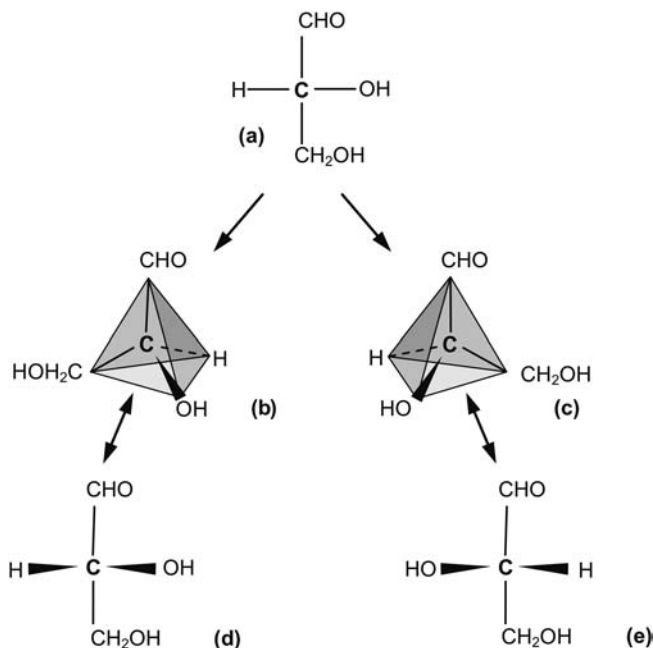


Figure 2.2 Glyceraldehyde. The simple two-dimensional structural formula (a) is compared with the two possible three-dimensional structures, (b) and (c). By convention, bonds shown as simple straight lines (e.g. C-CHO) lie in the plane of the page; bonds shown as a solid wedge (e.g. C-OH) project towards the reader in front of the plane of the page; and bonds shown as a dashed line (e.g. C---H) project behind the plane of the page. The four groups attached to the central carbon atom lie at the vertices of a tetrahedron. The tetrahedral portrayal is simplified in (d) and (e). Formulae (b) and (d) show D-glyceraldehyde, (c) and (e) show L-glyceraldehyde.

methods such as high-performance liquid chromatography (HPLC) took over in food analytical laboratories.

Unfortunately, one cannot automatically equate membership of the D-series with rotation to the right, so the symbols (+) and (−) are used to denote rotation to the right and left. The old-fashioned names, dextrose and laevulose, are derived from the dextro-rotatory and laevorotatory properties of D(+)-glucose and D(−)-fructose, respectively. Figure 2.3 shows how the formulae in Figure 2.2 are converted into the more convenient Fischer projection used in Figure 2.4 and elsewhere.

When monosaccharides contain more than one asymmetric carbon atom, their inclusion in the L- or D-series is based on the configuration of their highest numbered asymmetric carbon atom (e.g. carbon 4 in a pentose or carbon 5 in a hexose), the one furthest from the carbonyl group. Almost all naturally occurring monosaccharides belong to the D-series.

Although only a few of them are relevant to food or even common in nature, it is helpful to understand the structural relationships within the full set of aldose monosaccharides, as shown in Figure 2.4. A corresponding table of ketose sugars may be drawn up, but with the obvious exception of D-fructose (2.8), none of the ketoses is of much significance in food. The names of ketose sugars are derived by changing the “-ose” of the corresponding aldose sugar to “-ulose”, as in D-xylulose (2.9). Dihydroxyacetone and fructose are exceptions to this convention.

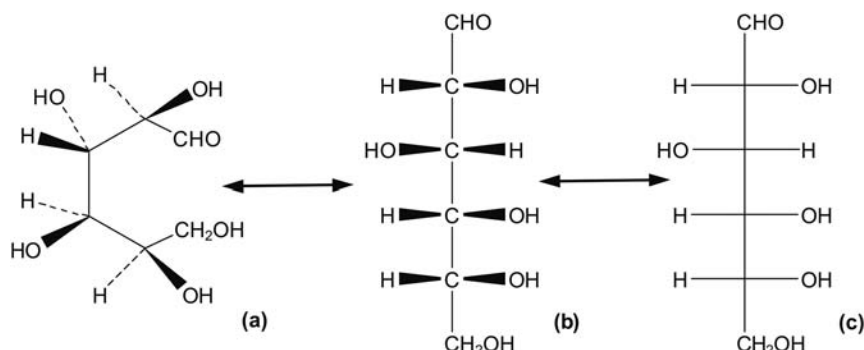


Figure 2.3 Projections of the structural formula of glucose. Versions (a) and (b) are comparable to glyceraldehyde, as shown in Figure 2.2. However, it is the Fischer projection (c) that is most often used by carbohydrate and food chemists. Bonds drawn horizontally project forwards from the plane of the paper, and bonds drawn vertically are either in, or project behind, the plane of the paper. Asymmetric carbon atoms are not shown explicitly, but are implied by the intersection of the vertical and horizontal lines.

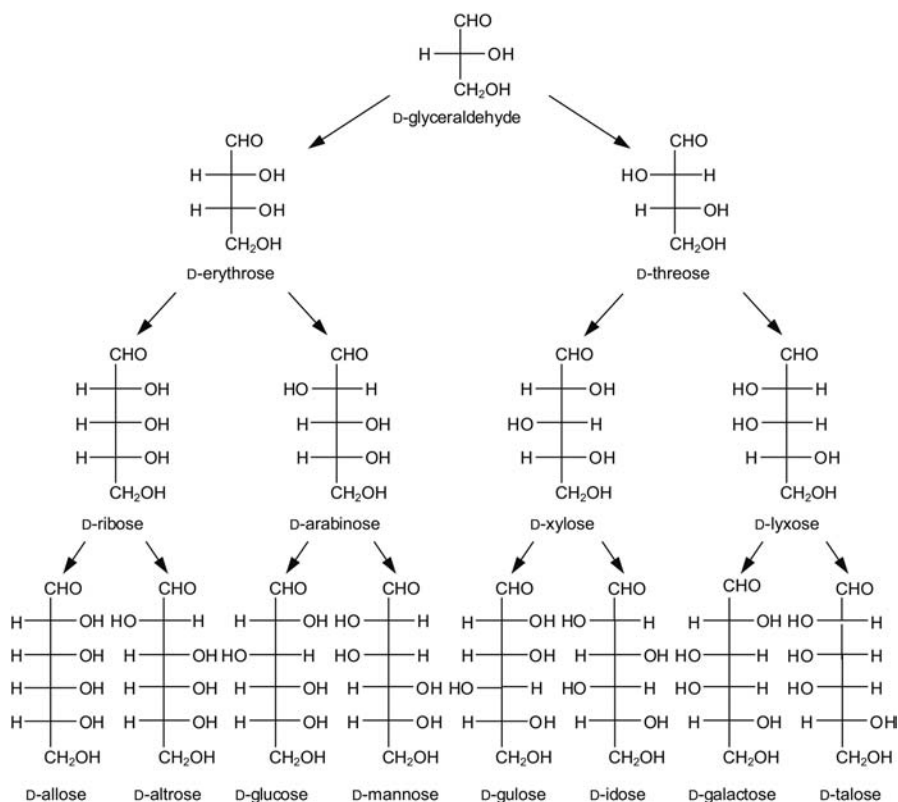
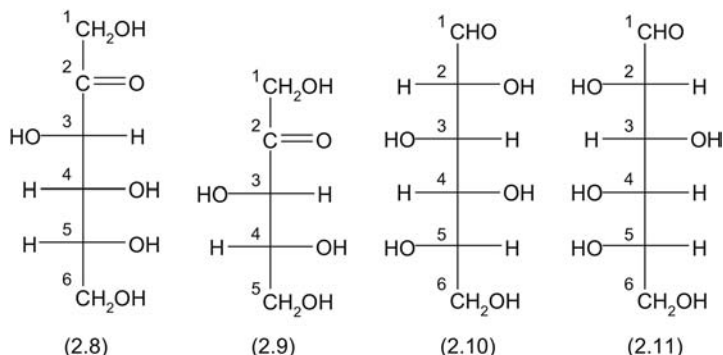


Figure 2.4 Configurations of the D-aldoses. For anyone who wishes to remember the structures or names of the aldoses, the sequence, reading downwards and from left to right, may be remembered by use of the traditional, and dreadful, mnemonics “Get Raxl!” and “All altruists gladly make gum in gallon tanks”. (Despite prompting in previous editions, the author, on behalf of innumerable students, is yet to hear of improved versions of these mnemonics.)

It is important to remember that the monosaccharides of the L-series are related to L-glyceraldehyde (see Figure 2.2) and have configurations that are mirror images of the corresponding D-series sugars. Thus, L-glucose is (2.11) rather than (2.10), which is, in fact, L-idose.



The straight-chain structural formulae used so far in this chapter do not account satisfactorily for some monosaccharide properties. In particular, their reactions, while indicating the presence of a carbonyl group, are not entirely typical of carbonyl compounds generally. The differences are explained by the formation of a ring structure in which the carbonyl group has reacted reversibly with one or other of the hydroxyl groups at the far end of the chain. In aldose sugars, the resulting structure is known as a hemiacetal (Figure 2.5(b)).

Figure 2.6 shows how a number of different structural isomers of glucose and fructose can be formed. Six- and five-membered rings are referred to, respectively, as pyranose and furanose, from the structures of pyran (2.12) and furan (2.13).

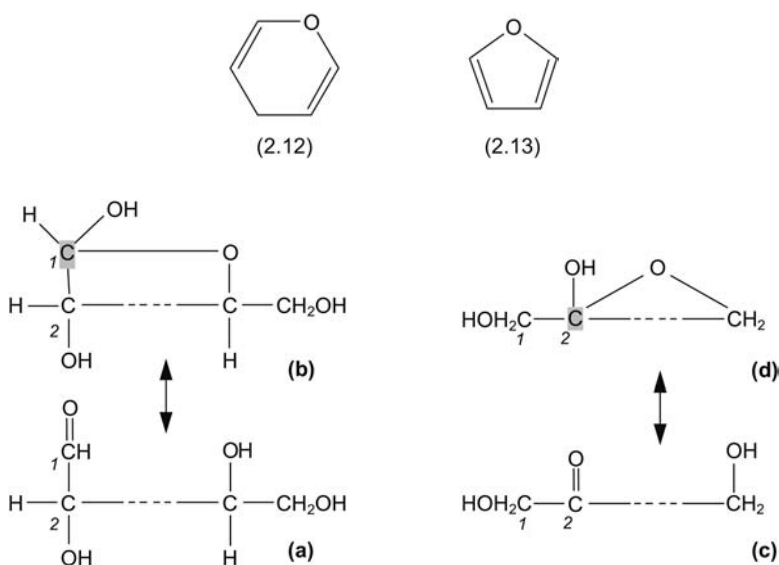


Figure 2.5 Formation of ring structures in aldose, (a) and (b), and ketose, (c) and (d), monosaccharides. These freely reversible reactions only occur rapidly and spontaneously when a sugar is in solution, but only a very tiny proportion of the sugar is in the open-chain form, (a) or (c), at any one moment. The newly formed asymmetric centres in the ring structures are highlighted.

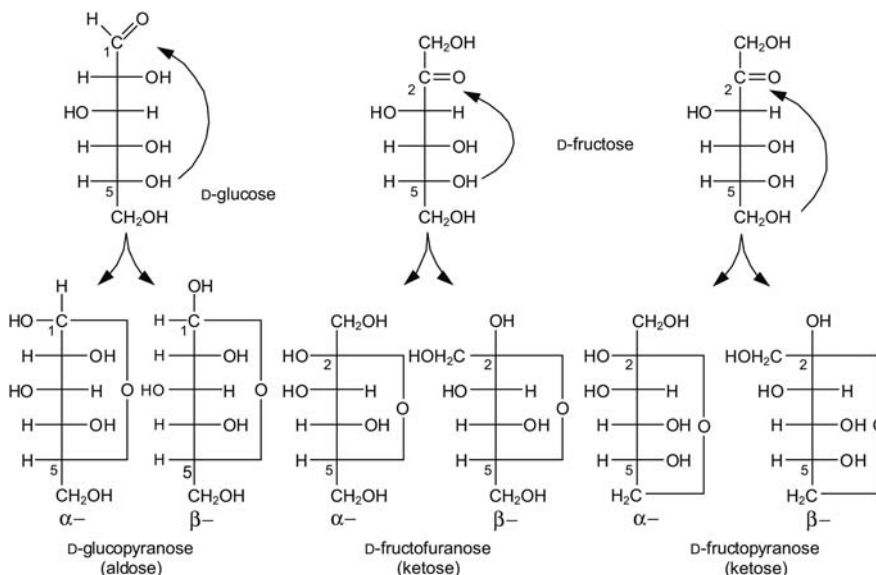


Figure 2.6 Formation of pyranose and furanose ring forms of D-glucose and D-fructose. Only the pyranose forms of D-glucose occur in significant amounts. This representation of the ring form has its shortcomings: C–O bonds are actually about the same length as C–C bonds and certainly do not turn through 90° halfway along. Translating the formulae of other monosaccharides from the open chains of Figure 2.4 into the more realistic Haworth rings shown in Figure 2.7 is best achieved by comparison with glucose, rather than by resorting to the three-dimensional mental gymnastics recommended by some classical organic chemistry texts.

Another result of hemiacetal formation, also shown in Figures 2.5 and 2.6, is that what was previously the carbonyl carbon now has four different substituents, *i.e.* it has become a new asymmetric centre. This means that the ring form of a monosaccharide will occur as a pair of optical isomers, its α - and β -anomers.

The α - and β -anomers of a particular monosaccharide differ in optical rotation. For example, α -D-glucopyranose, which is the form in which D-glucose crystallises out of aqueous solution, has a specific rotationⁱⁱⁱ of +112°, whereas that of β -D-glucopyranose, which can be obtained by crystallisation from pyridine solutions of D-glucose, is +19°. Elaborate rules are used to decide which of a pair of anomers is to be designated α and which β . However, in practice, we observe that α -anomers have their reducing group (*i.e.* the C-1 hydroxyl of aldose sugars and the C-2 hydroxyl of ketose sugars) on the opposite face of the ring to C-6, whereas β -anomers have their reducing group on the same face as C-6. Careful inspection of the formulae in Figures 2.6 and 2.7 will show how this works out in the potentially tricky L-sugars and in furanose rings.

ⁱⁱⁱThe specific rotation of a substance at 20 °C using light of the D-line of the sodium spectrum is given by the expression.

$$[\alpha]_{\text{D}}^{20} = \frac{100\alpha}{l \times c}$$

where α is the rotation observed in a polarimeter tube of length l (dm) and at a concentration of c (g per 100 mL).

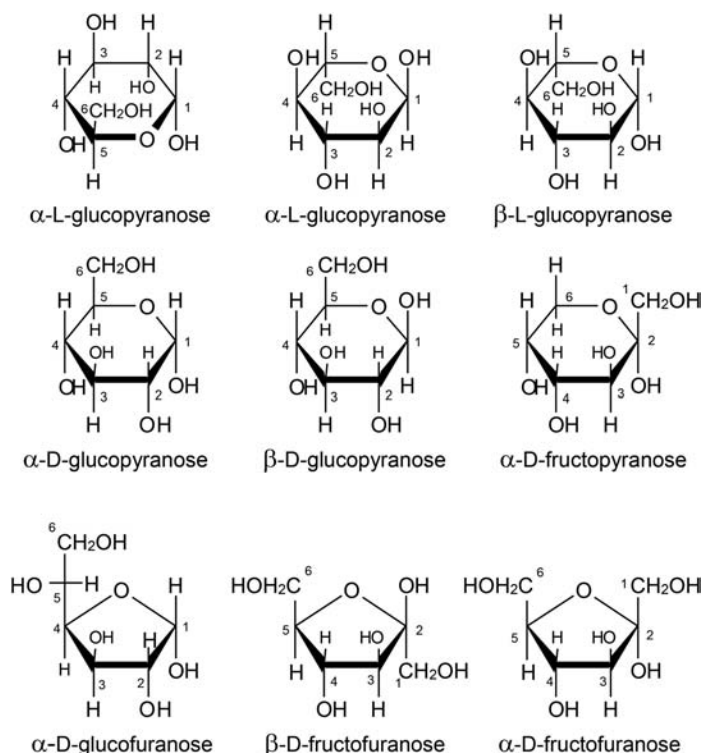


Figure 2.7 Isomers of glucose and fructose. The obvious shortcomings of the formulae used so far in this chapter to represent molecular structures can be partially resolved by the adoption of the Haworth convention for ring structures. The ring is treated as planar and drawn to appear perpendicular to the plane of the page. These structures may be compared with those in Figure 2.6. The numbering of carbon atoms can be correlated with that in the open-chain structures (2.6) and (2.7). It should be remembered that only a few of the isomers shown occur naturally in significant amounts, and also that not all the possible isomers have been included.

When crystals of either α - or β -D-glucose are dissolved in water, the specific rotation is observed to change until, regardless of the form it started with, the solution finally gives a value of $+52^\circ$. This phenomenon is known as *mutarotation*. The transition of one anomer to another proceeds through the open-chain aldehydo form, and it is clear that it is this isomer that is involved in the sugar reactions that are typical of carbonyl compounds, even though in aqueous solutions only 0.02% of D-glucose molecules are in this form.

The structural relationships between L- and D-isomers, α - and β -anomers, and pyranose and furanose rings in both aldose and ketose sugars, are by no means easily mastered. If available, the use of a set of molecular models will help to clarify the issues, but the structural formulae set out in Figure 2.7 illustrate the essential features of the terminology in this area.

Over the years chemists have synthesised innumerable monosaccharide derivatives, but only a few of these occur naturally or have particular significance in food. Oxidation

of the carbonyl group of aldose sugars leads to the formation of the “-onic” series of sugar acids and, depending on the exact conditions, various other products. Additional aspects of the chemical reactions of the reducing group are dealt with in the first of the Special Topics later in this chapter.

The enzyme glucose oxidase catalyses the oxidation of the β -anomer of D-glucose to D-gluconolactone (2.14). This hydrolyses spontaneously to D-gluconic acid (2.15), with hydrogen peroxide as a by-product. In the popular analytical technique (Figure 2.8) a second enzyme, peroxidase, is included to measure the quantity of hydrogen peroxide and thus the concentration of glucose. Besides its obvious applications in food analysis, a similar reaction has been used in the test strips used to measure glucose concentrations in the blood of people with diabetes.^{iv} Glucose oxidase is also used in a process for removing traces of glucose from the bulk liquid egg used in

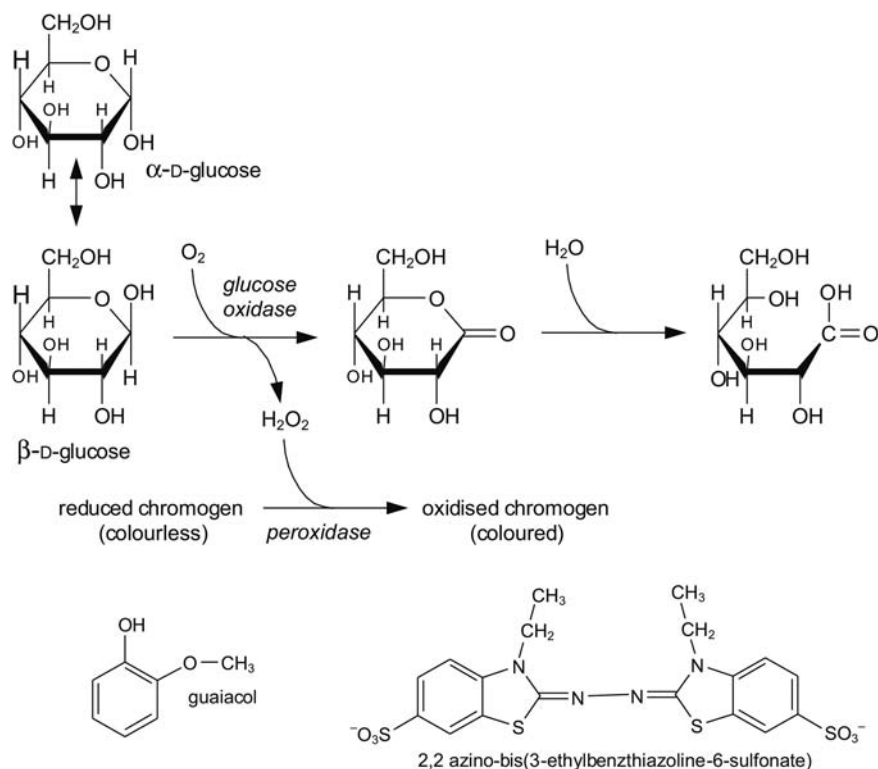
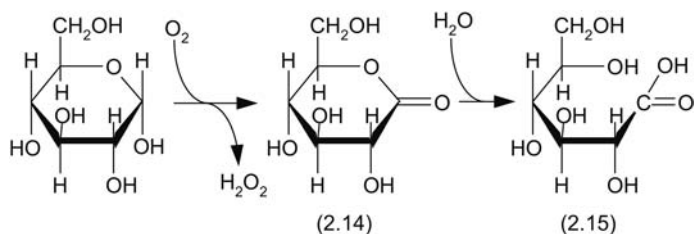


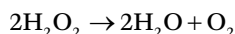
Figure 2.8 Enzymic determination of glucose. The original colour reagent (chromogen) used with this method was guaiacol, but nowadays 2,2-azino-bis(3ethylbenzthiazoline-6-sulfonate), more commonly known as PeridTM, which gives a beautiful emerald green colour, is the most popular.

^{iv}These test strips have now been superseded by a faster, more consistent type. The enzyme used is a glucose dehydrogenase that couples the oxidation of glucose to the reduction of the coenzyme FAD to $FADH_2$ (see Chapters 8 and 12). In turn, the $FADH_2$ reduces ferricyanide to ferrocyanide that, in turn, delivers an electron to an electrode. The enzyme, ferricyanide, the electrolyte and the electrodes are mounted into thin, disposable, plastic strips that receive the blood sample at one end and deliver a signal to the meter from the other end.

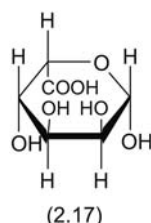
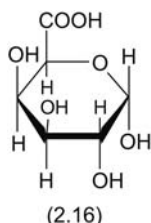
commercial bakeries and elsewhere to prevent the Maillard reaction taking place (see Section 2.6).



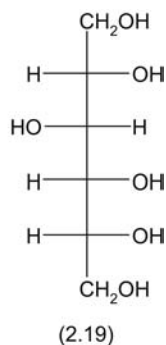
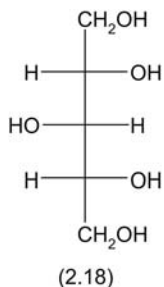
The hydrogen peroxide and the enzyme catalase are also included (added separately) to ensure sufficient molecular oxygen is available for the reaction. Catalase catalyses the reaction:



The “-uronic” series of sugar acids are aldohexoses with a carboxyl group at C-6, such as D-galacturonic acid (2.16) and L-guluronic acid (2.17). These are important as constituents of polysaccharides such as pectins and alginates, respectively, but are of little interest in their own right.

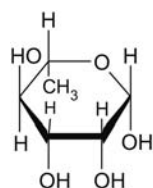


Reduction of the carbonyl group to a hydroxyl gives sugar alcohols such as xylitol (2.18) and sorbitol (2.19). Small amounts of sorbitol occur widely in fruit (around 4% and 12% of the total sugars in apple and pear juices, respectively) and its presence, or absence, can be used to assist in the authentication of fruit juices suspected of adulteration. Sugar alcohols are extensively synthesised on an industrial scale from the corresponding aldose sugars by reduction with hydrogen. Sorbitol and other hydrogenated sugars are also used as low-calorie, *i.e.* non-metabolised, sweeteners and are considered in more detail in Chapter 7.

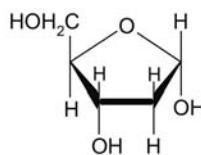


Sorbitol is particularly important as the starting point for the industrial synthesis of ascorbic acid (vitamin C, see Chapter 8, Section 8.8). Sugar alcohols have

been used to replace sugars in diabetic and other calorie-reduced food products. Although sweet, these are not absorbed in the small intestine and do not reach the bloodstream. Unfortunately, their eventual arrival in the large intestine can, if large amounts have been consumed, provoke “osmotic diarrhoea”, when the normal transfer of water from the colon’s contents into the bloodstream is impaired. These products are not recommended and nowadays people with diabetes are instead encouraged to drastically reduce their intake of cakes, confectionery, *etc.* Reduction at other positions in the sugar molecule gives deoxy sugars such as L-rhamnose (6-deoxy-L-mannose) (2.20), an important minor constituent of pectins, and 2-deoxy-D-ribose (2.21), the sugar component of DNA.



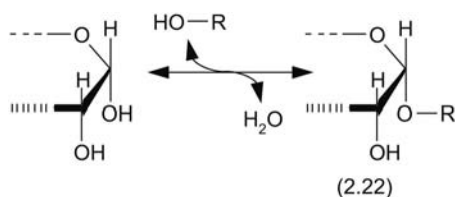
(2.20)



(2.21)

The most important derivatives of monosaccharides are those in which the hemiacetal, or reducing group, forms a glycosidic link (more strictly known as an acetal) with a hydroxyl group of another organic compound (2.22).

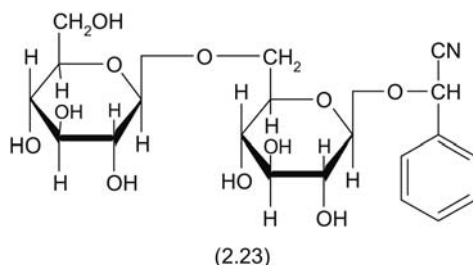
Glycosidic links are stable under ordinary conditions, but are readily hydrolysed under acidic conditions or in the presence of appropriate hydrolytic enzymes. The formation of the glycosidic link has the effect of fixing the hemiacetal structure in either the α or β configuration and, of course, abolishing mutarotation. As we will see later in the description of oligosaccharides and other compounds having glycosidic links, it will be necessary when describing a particular structure to specify whether the link in a compound is in the α or β configuration. The glycoside shown (2.22) is in the α -configuration.



(2.22)

Although any compound containing a glycosidic link is strictly speaking a glycoside, the term is usually reserved for a class of compounds that occur naturally in plants. These particular glycosides have a sugar component linked to a non-sugar component termed the aglycone. Flavonoids, found widely in plants, normally occur as glycosides with a sugar linked to one of their phenolic hydroxyl groups. The anthocyanin pigments of plants, considered in Chapter 6, are good examples. Many other well-known naturally occurring glycosides are plant toxins (see Chapter 10) such as solanine, the cyanogenic glycosides such as amygdalin (2.23) (found in bitter almonds) and the glucoside of α -hydroxyisobutyronitrile (in cassava, see Chapter 10). The phenolic hydroxyl groups of flavonoids can react spontaneously with proteins through the hydroxyl groups of tyrosine residues. Blocking the phenolic hydroxyls with sugar residues maintains their contribution to the biological value of the flavonoid

structure while avoiding their potentially destructive effect on the activity of enzymes, *etc.* Similarly, the presence of the sugar prevents the cyanogenic glycosides from releasing their cyanide until the circumstances are appropriate.



2.2 Oligosaccharides

When a glycosidic link connects the reducing group of one monosaccharide to a hydroxyl group of another, the result is a disaccharide. Further such linkages will give rise to trisaccharides, tetrasaccharides, *etc.*, the oligosaccharides,^v and, ultimately, polysaccharides. The polysaccharides, in which hundreds or even thousands of monosaccharide units may be combined in a single molecule, are considered in Chapter 3. Here we will be considering the di-, tri- and tetrasaccharides that commonly occur in our food.

Even when a disaccharide is composed of two identical monosaccharide units, there are numerous possible structures. This is illustrated in Figure 2.9, which shows five of the more important glucose–glucose disaccharides. Although the configuration of the hemiacetal involved in the link is no longer free to become reversed, it should not be overlooked that the uninvolved hemiacetal (*i.e.* the one in the right-hand ring of the reducing sugars as they are portrayed in Figure 2.9) is still subject to mutarotation in aqueous solution. Hence the first four of these sugars occur as pairs of α - and β -anomers. Furthermore, these four sugars show reducing properties similar to those of monosaccharides. In these cases it is the carbonyl group of the right-hand ring, as drawn in Figure 2.9, that provides the reducing properties. The best known of these reducing properties is the ability to reduce Cu^{2+} ions in alkaline solutions, such as Fehling's solution, to give a reddish-brown precipitate of cuprous oxide. This provides a useful laboratory test to distinguish these reducing sugars from non-reducing sugars – notably trehalose and, as we shall see, sucrose.

Not all of the five disaccharides shown in Figure 2.9 occur in nature. Maltose, together with a small proportion of isomaltose, occurs in syrups obtained by the partial hydrolysis of starch, in which similar patterns of glycosidic links are found. Maltose also occurs in some fruits, notably grapes (see Figure 2.1). Cellobiose is formed during the acid hydrolysis of cellulose. Gentiobiose commonly occurs as the sugar component of glycosides, and trehalose is found in yeast.

^vChemists do not recognise a fixed numerical boundary between “oligo” and “poly”. The classical Greek origins of these prefixes are usually translated as “few” and “many”, respectively, and chemists apply these prefixes on this basis.

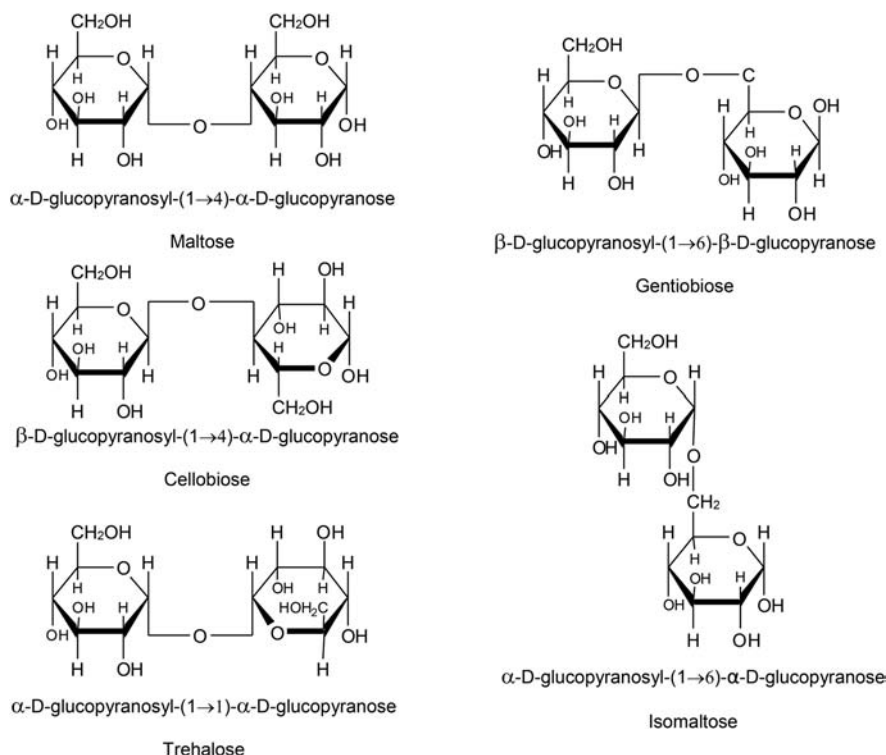
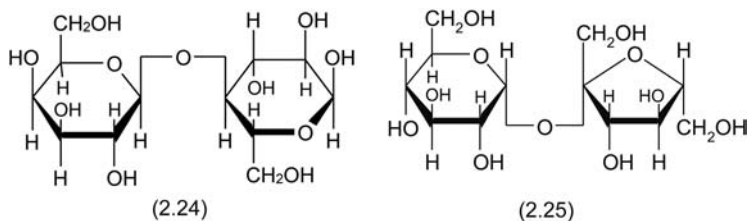


Figure 2.9 Disaccharides of glucose. Both trivial and systematic names are given. In the cases of the reducing oligosaccharides, maltose, cellobiose, isomaltose and gentiobiose, only one of the two possible anomers is shown.

The scientific names given to oligosaccharides are inevitably rather clumsy. The formally correct procedure is to name a disaccharide as a substituted monosaccharide. Thus lactose (2.24), which consists of a glucose unit carrying a D-galactose unit (in the β configuration) on its C-4 hydroxyl group, is 4-*O*- β -D-galactopyranosyl-D-glucopyranose. This system becomes very cumbersome when applied to higher oligosaccharides (those with three or more monosaccharide units) and most food scientists prefer to place the details of the link between the monosaccharides where it belongs, *i.e.* between them. Lactose is in this case, and in practice more frequently, described as β -D-galactopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose. In sucrose (2.25), the glycosidic link occurs between the reducing groups of *both* monosaccharide components, glucose and fructose, so that the usual formal name, α -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-fructofuranose could be reversed to β -D-fructofuranosyl-(2 $\rightarrow$ 1)- α -D-glucopyranose, but no one ever bothers.



Lactose and sucrose are two of the most important food sugars. Lactose is the sugar present in milk^{vi} (approximately 5% w/v in cow's milk) and is, of course, a reducing sugar. Human milk, with 6.2–7.5% w/v lactose, has one of the highest lactose concentrations of any mammal's milk. The lactose contents of the milk of a number mammalian species, and of a range of products made from cow's milk, are shown in Table 2.2.

Sucrose is the familiar “sugar” of the kitchen and commerce, and occurs widely in plant materials. The sucrose we buy as “sugar” has been extracted from sugar cane (*Saccharum officinarum*) or sugar beet (a variety of *Beta vulgaris*), but sucrose is also abundant in most plant materials, particularly fruit. In terms of their nutritional value, biology and most of their chemistry, the two are completely indistinguishable. However, one of the Special Topics later in this chapter explains how they can be distinguished and the potential value of doing so. Although glucose in the form of its 6-phosphate derivative is the initial product of photosynthesis, it is normally converted into sucrose before transport from the leaves to the remainder of the plant. Most plants convert the sucrose to starch (see Chapter 3) in the form of insoluble granules for long-term storage as an energy reserve, but some plants, such as sugar beet and sugar cane, retain sucrose in solution. The sucrose and other sugars (see Figure 2.1) in fruit are resynthesised from starch as the fruit ripens.

Since the glucose and fructose units are joined through their hemiacetal groups, sucrose is not a reducing sugar. Under mildly acid conditions, or by the action of the enzyme invertase, sucrose is readily hydrolysed to its component monosaccharides. This phenomenon is termed *inversion*, and the resulting mixture *invert* sugar, due to the effect of the hydrolysis on its optical rotation properties in solution. The specific rotation values for sucrose, glucose and fructose are +66.5°, +52.7° and –92.4°, respectively, so we can see that a dextrorotatory solution of sucrose will give a laevorotatory solution of invert sugar with specific rotation –39.7°.

Table 2.2 The lactose contents of mammalian milk and dairy products made from cow's (*i.e.* bovine) milk. The lactose contents of cheeses can vary widely depending on the time allowed for the cheese to mature. With the exception of the value for Stilton cheese, the figures shown here are representative of the values quoted by N. S. Scrimshaw and E. B. Murray, *Am. J. Clin. Nutr.*, 1988, **48**, 1099. The value for Stilton was quoted by M. C. E. Lomer *et al.*, see Reviews, under Further Reading. Another aspect of the carbohydrate content of human milk is considered in Special Topics.

Mammalian milk	Lactose (% w/w)	Dairy product	Lactose (% w/w)
Human	7.0	Butter	0.9
Cow	4.8	Yoghurt (whole milk)	4.4
Goat	4.4	Camembert cheese	0–0.5
Sheep	4.8	Edam cheese	0–1.4
Reindeer	2.5	Cheddar cheese	0–2.1
Marine mammals (various)	0 ^a –2.6	Stilton cheese	0.1

^aSealion (*Zalophus* sp.) milk is notable for the total absence of lactose.

^{vi}As secreted by female mammals for feeding their young; on the other hand, so-called “milks” derived from plant sources such as soya beans or coconuts never contain lactose.

Traditionally, invert syrup is manufactured by acid hydrolysis of sucrose using citric acid. The speed of the hydrolysis is highly dependent on both the pH and the temperature. A reduction of the pH from 4 to 3, or an increase in temperature of 20 °C, will in either case increase the rate by a factor of six. The resulting syrup will not crystallise, even if manufactured with a solids content as high as 80%. Inversion can also be carried out using the enzyme invertase, which occurs in the cell walls of yeast. Boiled sweets and other confectionery products depend upon the non-crystallising property of invert syrups. Sucrose is dissolved in invert syrup and the mixture is then boiled to reduce the total water content. The result is a glass structure, a supercooled liquid that is malleable when hot, but sets to form a glass-like solid as it cools. In this state, the solute molecules do not form the stable ordered associations characteristic of a crystal, but remain totally disordered. However, the water content is too low for the system to remain fluid, although it remains in strict chemical terms a solution. Flavours and colours are blended in as the mass cools and is rolled out, cut and moulded into the finished shape.

In modern confectionery, some or all of the invert sugar is often replaced by glucose syrups, otherwise known as corn syrups, because corn (*i.e.* maize) starch is the raw material normally used in their manufacture. In times past glucose syrups were made by the acid hydrolysis of starch. Maize starch, or other starchy raw materials such as potatoes, were heated with dilute sulfuric acid to hydrolyse the α -1 $\rightarrow$ 4 and α -1 $\rightarrow$ 6 glycosidic links between the glucose units (see Chapter 3). One might expect complete hydrolysis to glucose to result, but, in fact, the reaction does not go to completion. As the reaction proceeds, the concentration of sugars becomes very high and there is a tendency for the reverse reaction to occur. However, the reverse reaction is in no way constrained to re-form the α -1 $\rightarrow$ 4 and α -1 $\rightarrow$ 6 glycosidic links present in the original starch. In consequence, an accumulation of oligosaccharides takes place, based on the full range of possible linkages, α or β , 1 $\rightarrow$ 1, 1 $\rightarrow$ 2, 1 $\rightarrow$ 3, *etc.*, because many of these are much more resistant to acid hydrolysis. These oligosaccharides are unlikely to be utilised in the human digestive process, but their presence does ensure that the syrups, known commercially as liquid glucose,^{vii} do not crystallise. Modern commercial cake recipes also take advantage of the refusal of glucose syrups to crystallise. They help the crumb of the cake to retain moisture and thereby remain soft and palatable for longer.

In the nineteenth century, it was common for crude syrups (sold as “brewing sugar”), manufactured by the hydrolysis of potato starch with sulfuric acid, to be used as adjuncts in beer-making. Adjuncts were, and remain, cheap sources of fermentable carbohydrates that reduce the brewer’s dependence on expensive malted barley. This practice could have unfortunate consequences. In 1890, in the midlands and north of England, there was an incidence of over 6000 cases of arsenic poisoning among beer-drinkers, including 70 deaths. This was traced to a Salford brewery that had used contaminated brewing sugar. The crude sulfuric acid used to hydrolyse the starch had been manufactured from iron pyrites (ferrous sulfide) contaminated with arsenic.

^{vii}In the food industry, the terms “glucose” and “liquid glucose” are used to refer to the mixed products of starch hydrolysis. The otherwise obsolete term “dextrose” is used in the food industry to denote pure D-glucopyranose in the crystalline state.

Nowadays, enzymic hydrolysis using thermostable α -amylases and other enzymes has superseded acid hydrolysis, making syrups available with highly controllable compositions, ranging from almost pure glucose to mixtures dominated by particular oligosaccharides. These enzymic methods, described in greater detail in Chapter 3, can also be used to produce syrups resembling invert syrup, known as “isosyrups”. These are manufactured by using the enzyme glucose isomerase (see Chapter 12) to convert glucose to an equilibrium mixture of approximately equal parts glucose and fructose.

The inversion of sucrose is also an essential part of the process of making jam and other preserves. In making jam, the fruit, together with a large amount of sugar, is boiled for a prolonged period. During this period the fruit is softened and its pectin (see Chapter 3), plus any extra added pectin, is brought into solution. At the same time, a considerable proportion of the fruit's original water content is removed. The mild acidity leads to the hydrolysis of up to half of the added sucrose to glucose and fructose. This has a number of beneficial effects. First, the total number of sugar molecules is increased by up to 50%. This gives a modest increase in sweetness (see Chapter 7), but, most importantly, it increases the proportion of water that is bound to the sugar molecules and therefore unavailable to support the growth of microorganisms (see Chapter 13). The invert sugar is much more soluble in water than the original sucrose and the presence of invert sugar actually represses the crystallisation of sucrose. It is therefore easy to reach the very high solids levels (and correspondingly low water activity levels) that are needed for long-term stability.

The prolonged high temperatures, reaching 105 °C during boiling, can have a drastic effect on the colour and flavour of the fruit. Commercial jam-makers often reduce this effect by carrying out the boil at reduced pressure, driving off the water at a lower temperature. It is also common to replace some of the sucrose with glucose syrups prepared from starch (see Chapter 3). These contain significant proportions of glucose oligosaccharides, such as maltose, maltotriose (*i.e.* three glucose units linked α -1 $\rightarrow$ 4), isomaltose and maltotetraose. The presence of these oligosaccharides represses glucose crystallisation and enables high total sugar contents to be reached without excessive heating.

One group of higher oligosaccharides, the galactose derivatives of sucrose, deserves particular attention.^{viii} Their structures are shown in Figure 2.10. They are well known for their occurrence in the seeds of legumes, such as peas and beans, and some other vegetables (see Figure 2.1), and present particular problems in the utilisation of soya beans. Soya milk may contain up to 4 mg and 6 mg of raffinose and stachyose, respectively, per 100 ml. These oligosaccharides are neither susceptible to the digestive enzymes of the human small intestine nor absorbed into the bloodstream. Instead, they go on to become a feast for anaerobic bacteria in the large intestine, the colon.

These bacteria produce large quantities of hydrogen and some carbon dioxide as by-products of the metabolism of these sugars. Much of the carbon dioxide and hydrogen diffuses through the walls of the colon into the bloodstream and ultimately leaves *via* the lungs, but plenty remains to cause the flatulence traditionally associated with

^{viii} Another group of oligosaccharides, derivatives of lactose having two or more additional galactose residues, largely replace lactose in the milk of marsupials such as kangaroos and wallabies.

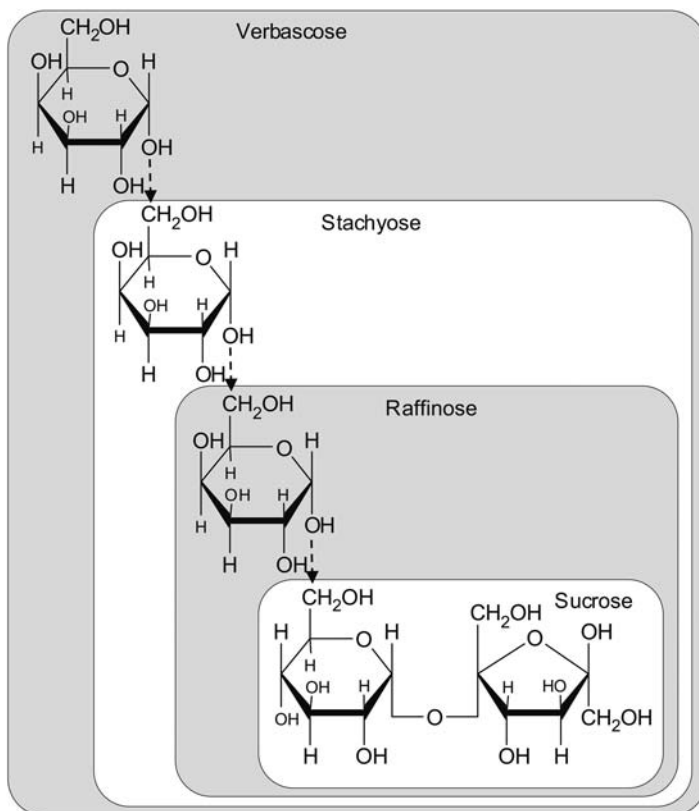


Figure 2.10 The raffinose family of oligosaccharides. Their structures are based on the repeated attachment (shown by the dashed arrow) of α -D-galactopyranosyl units to the vacant C-6 hydroxyl group of sucrose.

eating large quantities of beans. In recent years, some medical gastroenterologists have begun to suspect that this bacterial activity may, in a proportion of sufferers, be a cause of irritable bowel syndrome. A similar fate befalls the lactose in milk consumed by people who are not able to secrete the enzyme lactase (β -galactosidase) in their small intestine. Some details of the occurrence of lactose in the milk of various mammalian species, and also in dairy products made from cow's milk, are given in Table 2.2. All humans, like other mammals, possess this enzyme as infants, but there are wide variations between different ethnic groups in the extent to which individual adults secrete the enzyme. Less than 5% of adults from north-western Europe, but 95% or more of adults from the Far East, are lactase deficient, with other ethnic groups distributed between these extremes. Although rarely explicitly stated, the underlying assumption has been that, during the course of human evolution, particular ethnic groups have lost the ability to utilise lactose as adults. However, it is now widely accepted that a number of different mutations conferring lactase persistence (*i.e.* persisting from infancy into adulthood) arose spontaneously in Europe, Africa and parts of Asia, mostly around 6000–8000 years ago, but possibly as recently as 2000 years ago. Such a mutation

would inevitably confer massive evolutionary advantages on humans who were already engaged in farming cattle (or sheep, goats, *etc.*)

Lactose intolerance (the term used by the medical profession for the absence of lactase and its dietary consequences) is actually more widespread than these data might indicate. It can develop in previously lactose-tolerant people, particularly elderly patients, as an after-effect of some intestinal disorders or infections. Fungal lactases (see Chapter 12) are now widely used to treat milk to meet the needs of lactose-intolerant consumers for both fresh (*i.e.* pasteurised) milk and other dairy products such as butter and cheese. Domestic cats have traditionally been regarded as partial to a saucer of cow's milk, but in fact adult cats are often unable to digest its lactose. Milk treated similarly with lactase, so-called 'cat milk', is now marketed, enabling our pets to avoid the otherwise inevitable gastrointestinal upsets.

2.3 Sugars as Solids

Sugars in their crystalline state make important contributions to the appearance and texture of many food products, particularly confectionery, biscuits and cakes. The supermarket shelf usually offers customers a choice of crystal sizes in granulated (400–600 μm) and caster (200–450 μm) sugars and a powdered form for icing (average 10–15 μm).^{ix}

The relative proportions of undissolved sugar crystals and sugar solution (syrup) control the texture of the soft cream centres of chocolates, but a special problem is that of sealing the soft centre inside the chocolate coating. The answer is the use of invertase. This is an enzyme obtained commercially from yeast (*Saccharomyces cerevisiae*) that catalyses the hydrolysis of sucrose to glucose and fructose. In the manufacture of soft-centred chocolates, the mixture used for the centre contains a high proportion of finely milled sucrose suspended in just sufficient glucose syrup (see Chapter 3), together with appropriate colourings and flavourings, to produce a very stiff mouldable paste. Also included is a small amount of invertase. After the paste has been stamped out into suitable shapes, it is coated with melted chocolate. During the ensuing weeks, and before the chocolates are sold to the public, the invertase hydrolyses a proportion of the sucrose. The glucose and fructose are considerably more soluble in water than their parent sucrose and, as they dissolve, they alter the solid-liquid balance of the centre, giving it its familiar soft creamy texture. Invertase is one of only two enzymes that are treated in European legislation as food additives (E1103). Unlike other enzymes used in food processing, it is unaffected by the other processing operations normally carried out after its addition (*e.g.* coating with chocolate) and is detectable in the finished product.

The normal crystalline forms of most sugars are anhydrous and consist of only one anomer. However, at temperatures below 50 °C glucose crystallises from aqueous

^{ix}In the USA, caster sugar is known as superfine granulated sugar. Similarly, icing sugar is usually known as confectioners' sugar in the USA. The distinction, and overlap, between the term "icing" as used in the UK, and its approximate trans-Atlantic equivalent "frosting" need only concern cooks and confectioners.

solutions as the monohydrate of the α -pyranose anomer. Likewise, D-lactose crystallises as the monohydrate of the α -anomer. Although fairly soluble (about 20 g per 100 mL at room temperature), α -D-lactose monohydrate crystals are slow to dissolve and occasionally form a gritty deposit in evaporated milk. The texture of sweetened condensed milk is also highly dependent on the size of the lactose monohydrate crystals.

Boiled sweets, also known as “hard candies” are examples of a physical state described as a “glass”, with both liquid and solid characteristics. In the manufacture of boiled sweets, sucrose is dissolved in either glucose syrup (see Chapter 3, Section 3.6.2) or, less commonly, invert syrup (see Section 2.2). The mixture is then boiled down to raise the total sugar content to around 95% w/v. This operation has to be conducted in a sealed vacuum pan at reduced pressure so that the temperature remains low and caramelisation (see Section 2.5) is kept to a minimum. In spite of the very low water content, the presence of trace amounts of a great variety of different oligosaccharides from the invert or glucose syrups prevents the sucrose from crystallising. The result is a super-cooled liquid or “glass”. While it remains warm, the mass is sufficiently malleable to have flavourings and colours blended into it before being extruded into moulds and allowed to cool and set hard. These considerations apart, the food chemist is primarily interested in sugars in aqueous solution.

2.4 Sugars in Solution

Until a few decades ago, our knowledge of sugar chemistry was dominated by the chemical reactions of sugars and by the ability of chemists to create a bewildering array of chemical derivatives, few of which are relevant to food. There is now much more interest in the behaviour of the sugars themselves in the apparently “simple” environment of an aqueous solution. Studies using nuclear magnetic resonance (NMR) spectroscopy, and predictions based on thermodynamic calculations, have now revealed a great deal. The Haworth representations of the configuration of sugar rings used so far in this chapter have the advantage that they are fairly easy to draw freehand and can demonstrate optical relationships very clearly. However, for many purposes formulae based on the cyclohexane chair structure are needed (Figure 2.11).

In Figure 2.11, many of the hydrogen atoms or hydroxyl groups in the chair structures are circled. These are those in the so-called *axial* positions, *i.e.* their C–H bonds lie approximately parallel to the axis of the ring. The un-circled hydrogen atoms and hydroxyl groups are *equatorial*, *i.e.* their C–H bonds project outwards towards the equator of the ring. An added complication, also shown in Figure 2.11, is that there are two possible chair structures, known as *conformations*, for any given anomer. In Figure 2.11(d) and (e), we have the two conformations for β -D-glucose. The crucial difference between them is that (d), designated $C1^x$, has all of its hydroxyl groups and the $-\text{CH}_2\text{OH}$ group in equatorial positions. By contrast, the so-called $1C$ conformation (e) has these

^xThe designations $C1$ or $1C$ were introduced by R. R. Reeves as part of a system for naming different conformations. For most purposes the designation of a particular structure as $C1$ or $1C$ is most easily achieved by comparison with the two structures shown in Figure 2.11. If the sugar ring is portrayed as usual with the ring oxygen towards the right and “at the back”, then the conformation is $C1$ when carbon-1 of the ring is below the plane of the “seat” and $1C$ when carbon-1 lies above this plane.

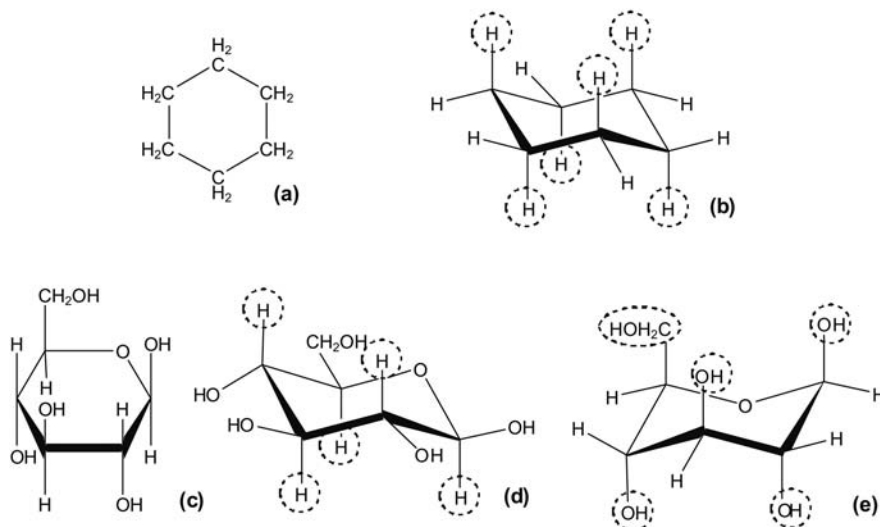
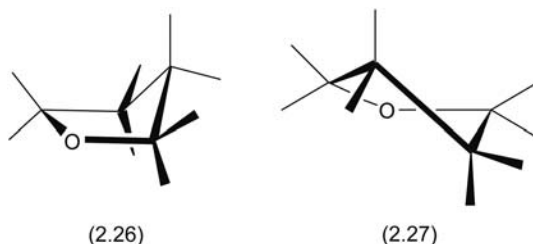


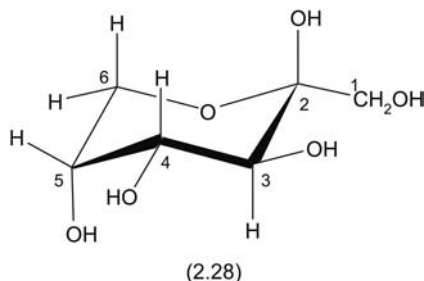
Figure 2.11 Chair structures for cyclohexane and glucose. Formula (a) is a simple two-dimensional representation of cyclohexane. With the carbon atoms shown with tetrahedrally distributed bonds (b), the result is the well-known chair structure. A similar relationship exists between the familiar Haworth structure of α -D-glucopyranose (c) and its corresponding chair structures (d) and (e). Here the square seat of the chair is formed by carbons 2, 3 and 5 and the ring oxygen with carbon 4 is the headrest.

groups in axial positions. In spite of the very different appearances of these two molecules, none of the asymmetric centres differs in configuration.

Theoretical studies have shown that the favoured conformation will be the one that has the greater number of bulky substituents, such as $-\text{OH}$ or $-\text{CH}_2\text{OH}$, in equatorial positions and where neighbouring hydroxyls are as far apart as possible. This has been borne out by experimental work and it immediately explains why less than 1% of D-glucose molecules in solution are in the $1C$ conformation and why the β -anomer is preferred for glucose. The correct description of a furanose ring is more difficult. There are two most probable shapes, described as the envelope (2.26) and twist (2.27) forms.



It seems best to assume that, in solution, furanoses are present as an equilibrium mixture of many rapidly interconverting forms, such as those just described, that do not differ greatly from each other and no one form is dominant. In fact, D-fructose occurs predominantly in the $1C$ conformation of the β -pyranose form (2.28), which has the bulky hydroxymethyl group ($-\text{CH}_2\text{OH}$) in the equatorial position.



Of the eight D-aldohexoses, only D-glucose has a possible conformation in which all of its hydroxyl groups, and the hydroxymethyl group, are equatorial. This is almost certainly the explanation for the dominant role of glucose in living systems, both as a metabolic intermediate and as a structural element. The apparent untidiness of its configuration when displayed in either straight-chain or Haworth formulae is obviously irrelevant to its actual state in living systems. This issue is given further consideration in the third of the Special Topics at the end of this chapter.

The conformation and anomer proportions of a particular sugar in solution are not only a function of the intramolecular non-bonding interactions between atoms that have been mentioned so far, but are also dependent on interactions between the sugar molecule and the solvent, which, in foodstuffs, is usually water. The fact that there is 37% of the α -anomer of D-glucose at 25 °C in aqueous solution, but 45% when the solvent is pyridine, is just one indication of the existence of strong interactions between water and sugar molecules. As we shall see, the binding of water to sugars and polysaccharides is an important contributor to the properties of many foodstuffs.

The most obvious indication of the binding of water by sugars is that concentrated solutions of sugars do not obey Raoult's law. This law states that the relative lowering of the vapour pressure of the solvent is equal to the molecular fraction of the solute in solution. The observed vapour pressure can be as much as 10% below the expected figure. Comparison of the observed with the expected values permits a fairly straightforward calculation of the proportion of water molecules that are no longer effectively part of the bulk of the water of the system, *i.e.* those that are apparently bound to the sugar molecules directly by hydrogen bonds. The resulting *hydration numbers* approach two and five molecules of water per molecule of sugar for glucose and sucrose, respectively.

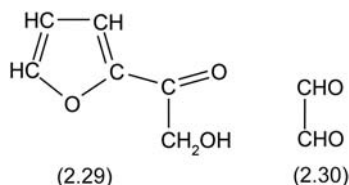
The techniques of NMR and dielectric relaxation are alternative tools for examining water-binding. They have revealed a primary hydration layer surrounding a sugar molecule that contains an average number of water molecules rather higher than that deduced by departures from Raoult's law. For example, ribose gives a value of 2.5, glucose 3.7, maltose 5.0 and sucrose 6.6. The water involved in hydration is not necessarily part of a permanent structure. NMR techniques will reveal water molecules that remain hydrogen-bonded for as little as 1 ms as "bound" water.

Further aspects of the binding of water by sugars, and other food components, are considered in Chapter 13. The sweetness that sugars impart to our food is discussed in Chapter 7.

2.5 Decomposition

It is not only intact sugar molecules themselves that make a positive contribution to the properties of our diet: the products of their thermal decomposition are also important food components in their own right. When sugars are heated to temperatures above 100 °C a complex series of reactions ensues, known as caramelisation, that gives rise to a wide range of flavour compounds in addition to the brown pigments we particularly associate with caramel. A related series of reactions, referred to as the Maillard reaction, occurs when amino compounds are also present and is considered in Section 2.6. Since both of these groups of reactions are particularly associated with the generation of brown pigments in food, they are often collectively known as *non-enzymic browning reactions*. This distinguishes them from the *enzymic browning reactions* (that do not involve sugars) that occur in fruit and vegetables, dealt with in Chapter 6.

The first stage of the high-temperature breakdown is a reversible isomerisation (memorably known as the Lobry de Bruyn–Alberda van Eckerstein transformation) of an aldose or ketose sugar, *via* their open-chain forms, to a 1,2-*cis*-enediol intermediate (Figure 2.12). If glucose is gently heated in the presence of dilute alkali, then this reaction leads to the formation of a mixture of glucose, mannose and fructose.



The enediol is readily dehydrated in a neutral or acidic environment *via* the sequence of reactions shown in Figure 2.13. Hydroxymethylfurfural (HMF, 2.29) is by no means the only product of this pathway. Highly reactive compounds, particularly α -dicarbonyl compounds such as 3-deoxyosone (see Figure 2.13) and ethanedial (also known as glyoxal or oxaldehyde) (2.30), in which pairs of carbonyl oxygen atoms are carried by neighbouring carbon atoms, occur as intermediates in these sequences and can give rise to a range of cyclic products. They also react with amino compounds such as amino acids in the important Strecker degradation reactions discussed in the following.

HMF can be readily detected in sugar-based food products that have been heated, such as boiled sweets, honey that has been adulterated with invert syrup, and Golden Syrup.^{xi} The brown pigments that characterise caramel and some other foods arise from a poorly defined group of polymerisation reactions that involve both HMF and its precursors. These pigment molecules have very high molecular masses and, in consequence, are not absorbed from the intestine. Burnt sugar has always been a popular brown colouring because it can be easily made in the home and therefore has a “natural” image. The commercially manufactured caramels that are used as food colours are manufactured in processes based on the Maillard reaction, considered in the next section.

^{xi}Syrup for domestic consumption is produced by the acid hydrolysis of sucrose at high temperature and is marketed in the UK as Golden Syrup.

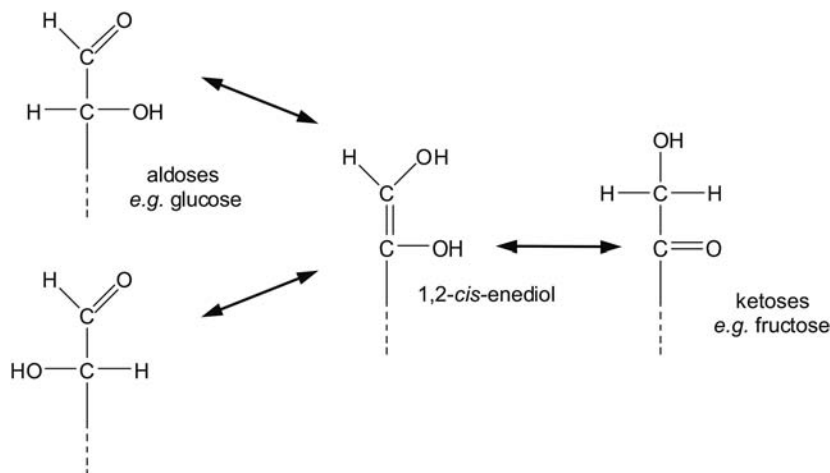


Figure 2.12 The Lobry de Bruyn-Alberda van Eckerstein transformation.

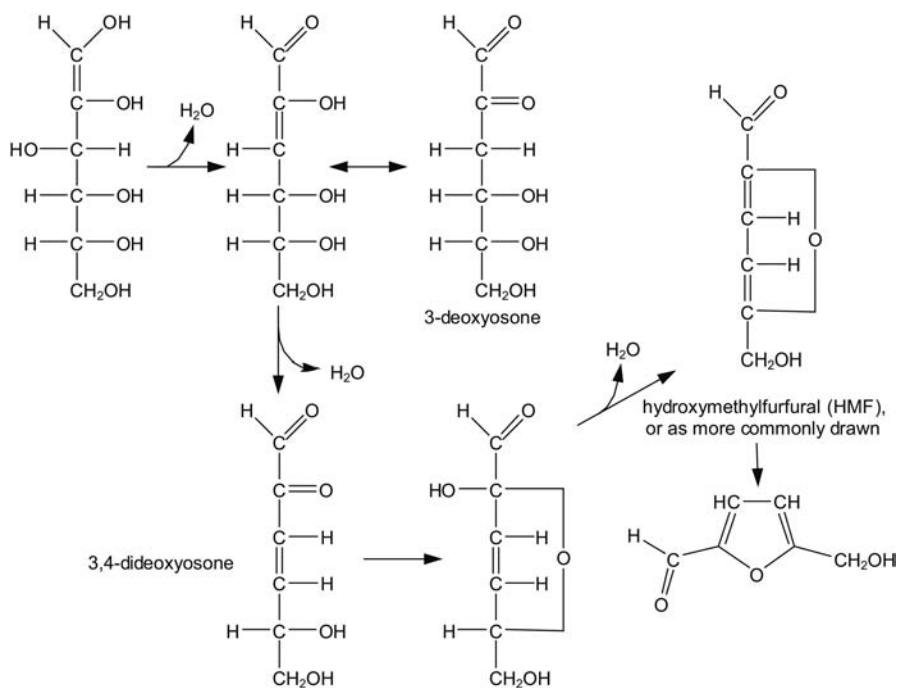
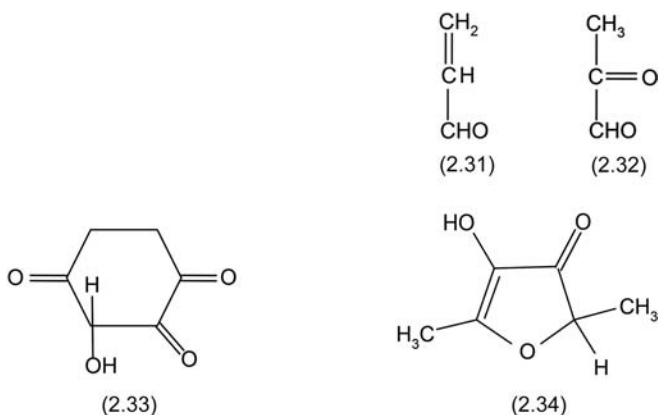


Figure 2.13 Formation of hydroxymethyl furfural by the sequential dehydration of a hexose enediol. Dehydration can occur at other sites, leading to other products such as hydroxyacetyl furan (2.29).

The characteristic acrid smell of burnt sugar can be ascribed to small amounts of a vast range of other breakdown products, of which glyoxal (mentioned earlier and also known as ethanedial), acrolein (propenal, (2.31)), pyruvaldehyde (2-oxopropanal, (2.32)) and (glyoxal, (2.30)) are typical. The caramel odour itself is reportedly due to

two particular cyclic compounds, acetylformoin (2.33) and 4-hydroxy-2,5-dimethyl-3-furanone (HDMF) (2.34).



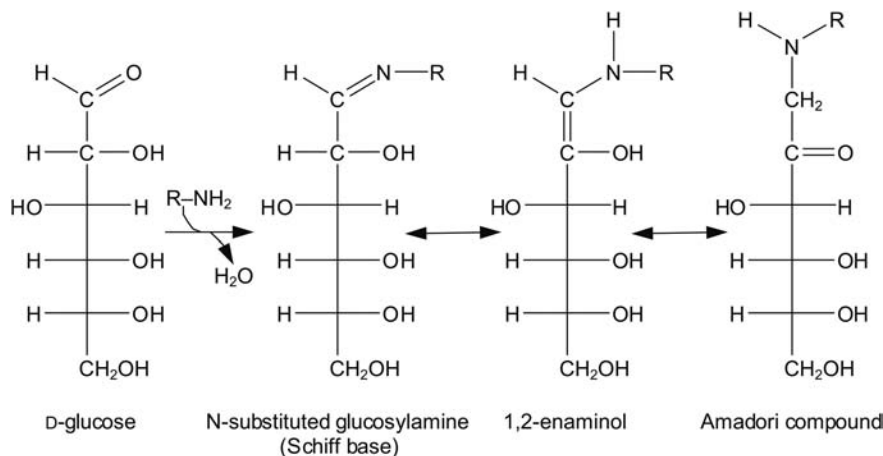
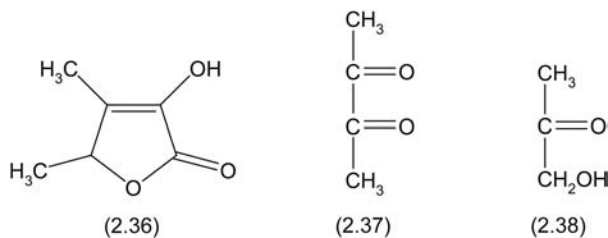


Figure 2.14 Reactions of glucose in the first stage of the Maillard reaction. R-NH₂ can be any compound with a free amino group, but in food systems this will normally be either a free amino acid or the side-chain amino group of an amino acid such as lysine incorporated in a protein.

encountered earlier in the case of caramelisation. If the pH is >7, then the Amadori compound breaks down by a slightly different route that can result in a range of end-products, notably maltol and isomaltol, and various other α -dicarbonyl compounds.

Of course, in a real food situation, a clear distinction between these two pathways would never occur and a considerable variety of ring compounds are likely to appear, some of which, such as acetylformoin (2.33), have already been mentioned in the context of caramelisation. One particular compound, sotolon, 4,5-dimethyl-3-hydroxyfuranone (2.36), has been identified as the key compound in the aroma of raw cane sugar.



Although sotolon's burnt sugar aroma, detectable at levels of a few parts per billion, is regarded as highly desirable in many foods, particularly confectionery, it can be responsible for off-flavours in others, especially at higher levels. Maltol and isomaltol are contributors to the flavour of many cooked foodstuffs.

Disintegration of the α -dicarbonyl molecules also contributes to the flavour of foodstuffs through the formation of substances such as diacetyl (butanedione, (2.37)) and acetol (hydroxypropanone, (2.38)), as well as pyruvaldehyde and glyoxal, which have already been mentioned.

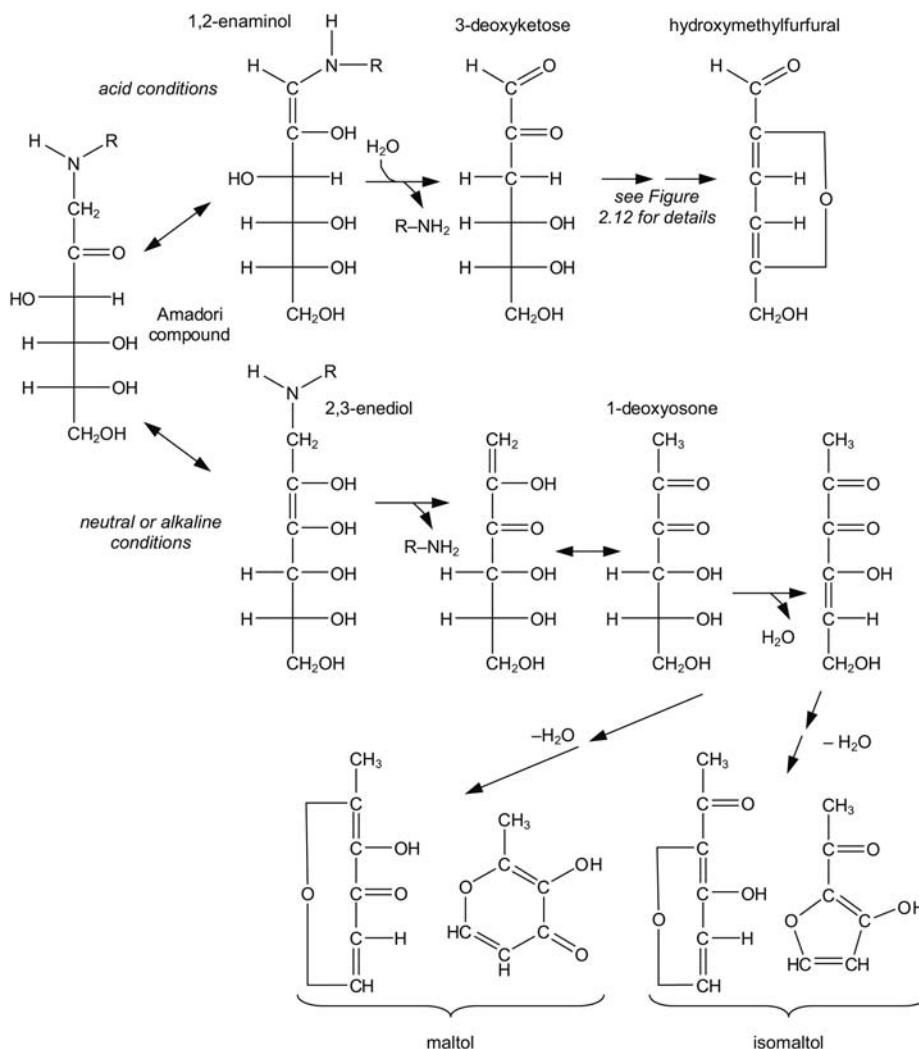


Figure 2.15 Breakdown of the Amadori compound in the Maillard reaction. In the formula of HMF shown here, the carbon chain is arranged in a straight line. This has the virtue of showing the structural relationship of HMF to the original glucose, but should be compared with the version shown in Figure 2.13. Similarly, both portrayals of the end-products, maltol and isomaltol, are shown here.

A most important class of volatile flavour compounds arises by an interaction at elevated temperatures of α -dicarbonyl compounds with α -amino acids, known as the Strecker degradation, shown in Figure 2.16. The volatile aldehydes derived from amino acids in this reaction make a major contribution to the attractive odour of bread-, cake- and biscuit-making. The pyrazines and other heterocyclic compounds that are often the other end-product of the Strecker degradation also make important contributions to food flavours, *e.g.* those of chocolate and roasted meat (see Figure 7.9).

It is now being recognised that the process of “sealing” pieces of meat before they are casseroled is another exploitation of the Maillard reaction. The authors of cookery

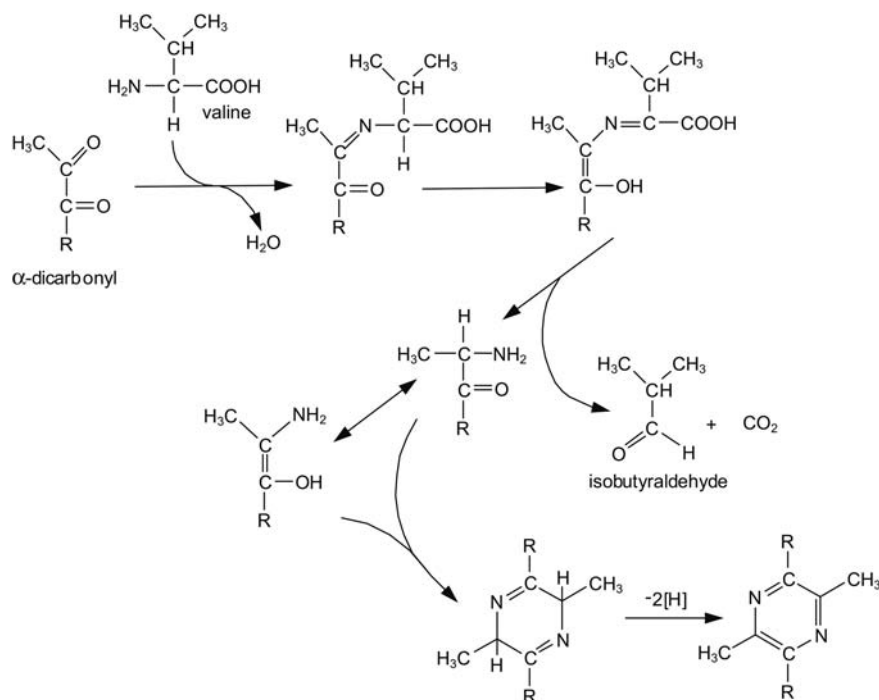


Figure 2.16 The Strecker degradation. A free amino acid (in this case illustrated by valine) reacts with an α -dicarbonyl compound generated elsewhere in the Maillard reaction. The reaction bears a superficial resemblance to transamination (see Figure 8.10). After rearrangement, the carbon skeleton of the amino acid is liberated as a volatile aldehyde. After two ex-sugar fragments (not necessarily identical, as shown here) condense, the product is readily oxidised to give the pyrazine derivative.

books and television chefs have always recommended that meat should be briefly fried before being added to a casserole or similar dish where subsequent cooking will be in an aqueous matrix. The cooked fatty surface was assumed to seal in the flavours of the meat. In reality, what is happening is that frying heats the surfaces of the meat to high temperatures, resulting in large-scale flavour generation *via* the Maillard reaction and Strecker degradation. Whether the various flavour compounds stay inside the meat or end up in a fatty/meat juice mixture in the pan is irrelevant; a good cook will ensure the entire contents of the pan reach the casserole.

Complex reaction sequences starting with 2,3-enaminols and involving a second amino acid in a Strecker degradation sequence have been suggested as the source of the range of pyrroles and pyridines that can also arise in the Maillard reaction. These are important as they are believed to form the monomers of the polymerised brown pigments (melanoidins) that characterise the Maillard reaction. Actual food situations are far more complex than the model systems using purified starting materials studied in laboratories, but the structures shown in Figure 2.17 may well turn out to be a close approximation to the brown pigments we find in our food.

Low water concentrations, when the reactants are more concentrated, favour the Maillard reaction, implicated in the browning of bread crust and the less welcome

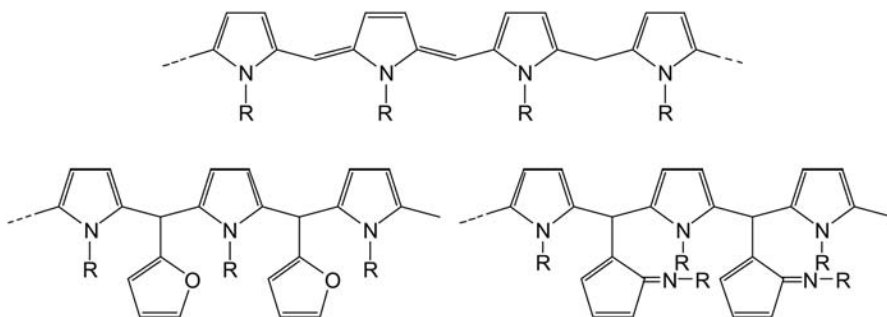


Figure 2.17 Postulated structures for the brown melanoidin pigments formed in the Maillard reaction. In these structures, R represents any of the possible sugar fragments, such as $-\text{CH}_3$ or $-(\text{CHOH})_2\text{CH}_2\text{OH}$, that arise during the course of the reaction. All of the structures shown here are likely to be found in a single melanoidin molecule. It has been estimated that around 95% of molecules initially participating in the Maillard reaction eventually end up in melanoidin polymers. (Based on the proposals of R. Tressl, *et al.*, in *The Maillard Reaction in Foods and Medicine*, ed. J. O'Brien, H. E. Nursten, M. J. C. Crabbe and J. M. Ames, Royal Society of Chemistry, 1998, pp. 69–75.)

discoloration that occurs from time to time in the production of powdered, dehydrated egg or milk. These foodstuffs are ones in which sugars are heated in the presence of protein, the source of the amino groups. The amino groups most often involved are those in the side-chains of lysine and histidine, although the α -amino groups of any free amino acids will participate.

While most of the effects of the Maillard reaction can be regarded as favourable, there can be adverse nutritional consequences. When food materials containing protein and reducing sugars are exposed to only slightly elevated temperatures, possibly during prolonged storage, the reaction may not go all the way to coloured end-products and may be undetected by the consumer. However, the resulting glycosylated amino acids are unavailable to human metabolism. If a sufficient proportion of the protein's surface is blocked by glycosylation, then the entire molecule may become unavailable because the proteolytic digestive enzymes cannot gain access to the peptide bonds (see Chapter 5). Lysine is the most reactive of the vulnerable amino acids, followed by arginine, and then tryptophan and histidine.

Heating to reduce or eliminate its normal population of microorganisms is a near universal method of extending the useful life of milk. The Maillard reaction, involving lactose and the amino groups on the surface of milk proteins, is an unwanted side-effect of the heat treatment of milk. Although it does not normally go beyond the formation of a stable Amadori compound, such as α -deoxylactulosyl-lysine (Figure 2.18), this can still make the lysine nutritionally available. Modest losses (up to 2%) of lysine result from both pasteurisation and the ultra-high-temperature (UHT) treatment of milk,^{xii} but the more aggressive heating involved in some milk processes, such as sterilisation, evaporation or roller-drying, can result in as much as 25% of the lysine being lost.

^{xii}Modern pasteurisation uses the high temperature short time (HTST) process in which the milk is heated to 72 °C for 15 s. The UHT process involves heating at >135 °C for 1–2 s.

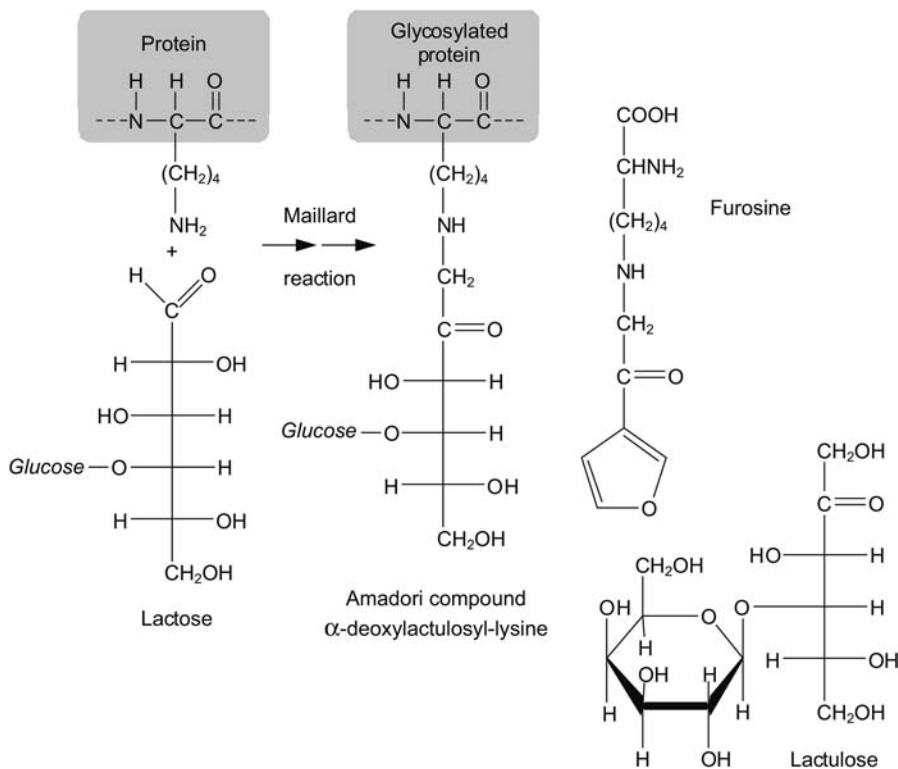


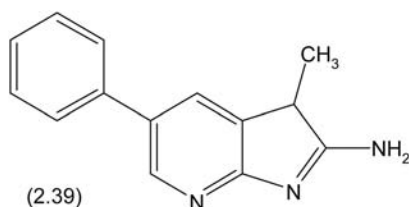
Figure 2.18 Lactose reactions during milk processing. The extent of glycosylation in a food protein can be estimated from the furosine content of the hydrolysate after it has been subjected to acid hydrolysis. Also, at high temperatures small amounts of lactose undergo the Lobry de Bruyn-Alberda van Eckerstein transformation (see Figure 2.12) to give lactulose, *i.e.* the glucose component of the disaccharide is converted from glucose to fructose, shown here for clarity in the open-chain structure. Measurements of furosine and lactulose levels can be important in establishing the processing history of commercial milk products.

A mixture of glucose and casein (the principal protein of milk) has been shown to lose as much as 70% of its lysine over a period of five days at temperatures as low as 37 °C. Conditions such as these may well occur during the transport of powdered milk for famine relief in the tropics. Lysine, histidine and tryptophan must all be present in the diet, *i.e.* they are all indispensable (or essential) amino acids (see Chapter 5). Poor vegetarian diets are often deficient in lysine, which makes this an important issue.

The disease diabetes mellitus provides a very important example of protein glycosylation taking place at an unexpectedly low temperature, *i.e.* blood heat. Semi-automatic devices based on enzymes (Figure 2.8), provide an “instant” measurement of a patient’s blood glucose concentration at the time of the test. An insight into blood glucose concentrations over the preceding few months can be obtained by measuring the proportion of the patient’s haemoglobin that has become glycosylated *via* the Maillard

reaction. Normally, about 3–7% of haemoglobin is in the glycosylated form (known as HbA_{1c}), but higher percentages of HbA_{1c} are found when diabetes has resulted in persistently elevated levels of blood glucose. Many of the complications affecting people who have had diabetes for a long time, including degeneration of the peripheral vascular system, sight loss (retinopathy) and kidney failure, are now known to be the result of crucial proteins in these tissues becoming glycosylated.

The Maillard reaction can pose other health problems not strictly related to nutrition. More than 20 complex heterocyclic amines, undoubtedly the products of Maillard-type reactions, have been identified in cooked meat, fish and poultry, particularly when grilled or fried, as well as in model systems. Many of these amines have been shown to be mutagenic in tests with bacteria (in the well-known Ames test) and must be regarded as potential, if not actual, carcinogens. Although the concentrations of these amines in grilled food is often very low (<1 ppb), one of them, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (2.39), has been detected at 500 ppb in flame-cooked chicken. High temperatures (200–300 °C) and fairly long cooking times are required for the formation of heterocyclic amines; they are not produced during ordinary stewing, roasting or baking. It is very difficult to estimate the risks of human cancer from these compounds in real foods, as opposed to model systems, but one current risk estimate is 1 in 10 000 over a normal lifetime and the typical consumption of foods cooked at high temperatures. Although the humble burger is grilled at high temperatures, the short cooking times make it less of a hazard than might be expected. In recent years, food chemists have become aware of another potentially hazardous food contaminant, acrylamide, which has now been shown to arise in the Maillard reaction. This is the subject of the last of the Special Topics in this chapter.



Caramel is produced commercially for use as a food colour in a vast range of food products, not just the “gravy browning” still found in some British domestic kitchens. Around 10 000 tons are used annually in Britain. Until the issue of the “Southampton Six” synthetic dyes arose in 2007 (see Chapter 6, Section 6.7), caramels accounted for all but 5% or so of the quantity of artificial colours used in food and drinks. Caramels are manufactured commercially by heating sucrose with catalytic amounts (a few per cent) of acid or alkali (Class I caramel), sulfite (Class II), ammonia (Class III) or ammonia plus sulfite (Class IV). All four are permitted food additives in Europe, numbered E150a, b, c and d, respectively, as well all the other jurisdictions listed in Table 6.4. The different classes of caramel differ slightly in properties and consequently find different applications, especially in drinks. For example, Class I and II caramels are used in spirits such as whisky, Class III in beer and Class IV in soft drinks, notably cola.

2.7 Special Topics

2.7.1 Reducing Group Reactions

Textbooks of general organic chemistry give details of numerous chemical reactions of sugars, particularly those involving the carbonyl group. Many of these have little or no relevance to modern food chemistry, even though our present understanding of the structure of sugars was initially dependent upon their study. Until the advent of sophisticated chromatographic methods, notably HPLC, the most important applications of these reactions were in food analysis to identify, and sometimes quantify, the sugars present in a particular food material. The monosaccharide carbonyl group is readily oxidised, hence the reference to *reducing* sugars, *i.e.* sugars that can reduce oxidising agents (oxidants). The reaction between reducing sugars and Cu^{2+} ions complexed in alkalis (*e.g.* Fehling's solution or Benedict's reagent) is frequently used in school laboratory experiments, but its quantitative versions, the titrimetric (Lane and Eynon) or gravimetric (Munson and Walker) methods are now rarely used. HPLC is usually used in analytical laboratories, but methods relying on the specificity of enzymic catalysis (see Chapter 12) are widely used when the amounts of particular sugars are to be determined.

2.7.2 Cane Sugar, Beet Sugar and Tequila

The fundamental reaction of photosynthesis in all green plants is the reaction, catalysed by the enzyme ribulose-1,5-biphosphate carboxylase, of carbon dioxide with ribulose-1,5-biphosphate to form two molecules of 3-phosphoglycerate. After a complex series of reactions, these are converted to fructose-6-phosphate and, eventually, into glucose and sucrose. Although this part of the process, known as the Calvin cycle, is common to all plants, two groups of plants have evolved specialised sequences of reactions that carry out the initial trapping of carbon dioxide from the atmosphere before it is passed on to the Calvin cycle enzymes. One such group consists of the so-called "tropical grasses", which include maize, *Zea mays* ("corn" in North America), and sugar cane, *Saccharum officinarum*, the traditional source of sucrose. These use the Hatch and Slack pathway for the first stage of carbon dioxide fixation. Another pathway, known as crassulacean acid metabolism (CAM), is used by the succulents, of which only two species, the pineapple, *Ananas comosus*, and the blue agave, *Agave tequilana*, are of interest to us. The leaves of the agave plant are the source of the sugary syrup that is fermented and then distilled to give tequila, the famous Mexican spirit. Both these variants of normal photosynthesis give improved efficiency under tropical conditions. By contrast, sugar beet, a variety of the common beet, *Beta vulgaris*, uses the normal Calvin cycle. This grows in a wide range of temperate climates and has been an alternative source of sugar, especially in continental Europe, since the middle of the eighteenth century.

In terms of chemistry, biochemistry, human nutrition and health, there is no difference between cane and beet sugars once they have been purified to the colourless crystalline materials we recognise as granulated or caster sugar. Although in isolation the distinction between cane and beet sugar is purely academic, it is important from

commercial and regulatory points of view to be able to discriminate between them. The method depends upon the 1% of all naturally occurring carbon atoms (in organic compounds, as atmospheric carbon dioxide and minerals such as calcium carbonate) that occurs as the non-radioactive isotope ^{13}C . Almost all the rest is the ^{12}C isotope. Most chemical and biological systems, including human metabolism, do not distinguish between the two isotopes, but the enzymes of the Calvin cycle, to a small extent, do. As a result, plant materials have very slightly different proportions of ^{12}C and ^{13}C depending on the photosynthetic pathway involved in their synthesis. These proportions, shown in Table 2.3, can be measured with sufficient precision by a mass spectrometric technique, stable isotope ratio analysis. This makes it possible to discriminate between cane and beet sugar and to detect whether cane sugar has been dishonestly used to supplement the natural sugar content of a fruit product.

This method has been successful in demonstrating the illegal addition of cane sugar to grape juice prior to fermentation and in detecting the addition of corn syrup and cane sugar to maple syrup and citrus juices. Unfortunately, cane sugar that finds its way into pineapple juice or the tequila fermentation is much harder to detect. A number of other food authenticity or adulteration issues have now been tackled by this technique. A good example is the distinction between vanillin, the essential component of the highly valued natural flavouring vanilla extracted from the seed pods of vanilla orchids (*Vanilla* sp.) and synthetic vanillin. Vanilla orchids utilise crassulacean acid metabolism in photosynthesis, whereas synthetic vanillin is synthesised from precursors derived from non-CAM plant materials, such as the lignin of conifer wood, and is, unsurprisingly, very much less expensive.^{xiii}

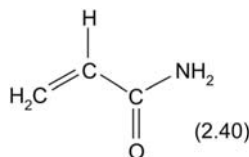
2.7.3 Acrylamide

In 1992, construction started on the Hallandsås railway tunnel on the west coast of Sweden. Progress had already been severely delayed by geological problems when, in 1997, it was discovered that local farm animals were dying prematurely and construction workers were being taken ill. The cause was traced to a sealing compound, polyacrylamide, used in the construction. Unfortunately, this had not polymerised properly and the monomer, acrylamide (2.40) had leached out into local water courses.

Table 2.3 Proportion of the stable carbon isotope ^{13}C in the total carbon of plant materials compared with an arbitrary international standard of a fossil calcium carbonate (belemnite).

	International standard	Tropical grasses, including sugar cane and maize	Crassulaceae, including agave and pineapple	All other plants, including sugar beet and grapes
^{13}C (%)	1.1112	1.0991	1.0975	1.0837

^{xiii} *Food Forensics: Stable Isotopes as a Guide to Authenticity and Origin*, ed. J. F. Carter and, L. A. Chesson, CRC Press, London, 2017.



As part of their investigations into the poisoning, Swedish scientists discovered that ordinary non-smoking^{xiv} members of the public, examined to provide baseline data, also showed evidence of unexpectedly high levels of acrylamide exposure, although nothing like the levels found among the tunnel workers. By 2002, this was traced to foodstuffs that had been exposed to high temperatures as part of their normal cooking procedures, in particular starchy foods that had been fried.

At that time, the toxic effects of acrylamide were regarded as well established. Exposures at high levels in industrial accidents were known to have caused neurological damage. However, no neurotoxic effect was expected from the acrylamide levels found in food. Prolonged exposure at lower levels has been shown to induce tumours in laboratory animals, but there is no convincing evidence of acrylamide causing cancer in humans. Nevertheless, the widespread occurrence of a known carcinogen in a wide variety of heated foods caused considerable concern. In 2007, a large study in the USA showed that the incidence of at least one common cancer, that of the breast, was unaffected by the relative amounts of acrylamide-rich foods consumed over a long period. The UK Food Standards Agency (FSA) has estimated that the minimum levels reported to cause cancer in laboratory rats are at least 1000 times greater than current dietary exposure levels in the UK.

In more recent epidemiological studies, no evidence has been found for a positive association between the incidence of a wide range of cancers (including those of the breast, prostate, larynx and ovary) and the dietary intake of acrylamide. However, despite the continuing confidence of responsible authorities around the world that the levels of acrylamide in human diets do not constitute a significant hazard, the levels of acrylamide in a wide range of foods are being monitored on a regular basis.

Initial studies, fully endorsed by subsequent investigations, incriminated starchy foods subjected to high temperatures (either during domestic cooking or commercial processing) as sources of acrylamide. The European Food Safety Authority (EFSA) and the UK FSA regularly publish data on the acrylamide contents of a wide range of potential food products. These include a variety of potato products, especially crisps and French fries, cereal products, such as popcorn and rye crispbread, as well as infant foods and coffee. Interpretation of these data is complicated by the need to ensure that researchers and regulators agree on the names of food products.^{xv} A more significant issue is the wide variation in the acrylamide levels found at different times

^{xiv}Acrylamide is known to occur in tobacco smoke.

^{xv}The European Commission has recommended that the term “potato crisps” should refer to crunchy thin slices of deep-fried/baked potato usually eaten as snacks, whereas “French fries” refers to batons of deep-fried potato usually served as an accompaniment during a meal.

in supposedly identical products. The data quoted in Table 2.4 refer to food products sold in the UK, but data from elsewhere in the world tell a similar story. Even within a single class of food product in a single year, acrylamide levels in individual samples may vary widely.

It is not easy to establish the total intake of acrylamide from dietary sources by different sections of the population. However, the EFSA was able bring together data on total intakes from the ten most relevant food groups for the period 2007–2009 in 20 European Union countries (plus Norway). The study involved the analysis of 10 366 different food samples and patterns of food consumption by different age groups over the three-year period. The results, summarised in Table 2.5, did not change significantly over time.

It is now generally accepted that acrylamide in foods is a product of the Maillard reaction between sugars, such as glucose, and the amino acid asparagine. To react with the sugar, the asparagine must be free, *i.e.* not part of the structure of a protein (Figure 2.19). Other potential pathways for the generation of acrylamide have been

Table 2.4 Levels of acrylamide found in samples of raw and cooked foodstuffs in the UK, 2014–2018, reported by the UK FSA. The right-hand column shows the total number (*n*) of samples analysed in each in these categories over the five-year period.

	2014	2015	2016	2017	2018	<i>n</i>
Food category	Acrylamide ($\mu\text{g kg}^{-1}$)					
French fries sold as ready to eat	157	167	208	156	200	190
Potato crisps and potato-based crackers	779	691	617	631	621	135
Fresh (<i>i.e.</i> not crisp) bread	14	22	18	23	17	135
Breakfast cereals (excluding porridge)	174	146	154	221	184	120
Biscuits, crackers, crispbread and similar (excluding pastries and cakes)	347	299	306	207	223	146
Coffee and coffee substitutes	455	411	539	375	577	92
Baby foods, other than processed cereal-based foods	12	13	12	9	20	118
Processed cereal-based foods for infants and young children	60	31	21	17	48	116

Table 2.5 Estimated dietary intakes of acrylamide by sections of the European population in the period 2007–2009 (*EFSA J.*, 2011, **9**, 2133). The ranges of values shown are the national mean values for the countries contributing data to this aspect of the survey.

Age group (years)	Mean exposure ($\mu\text{g per kg body weight per day}$)	Major contributors
Adults (aged 18+)	0.31–1.1	Fried potatoes (incl. French fries), coffee and bread
Adolescents (aged 11–17)	0.43–1.4	Fried potatoes, bread and potato crisps or biscuits
Children (aged 3–10)	0.70–2.05	
Infants (aged 1–3)	1.2–2.4	

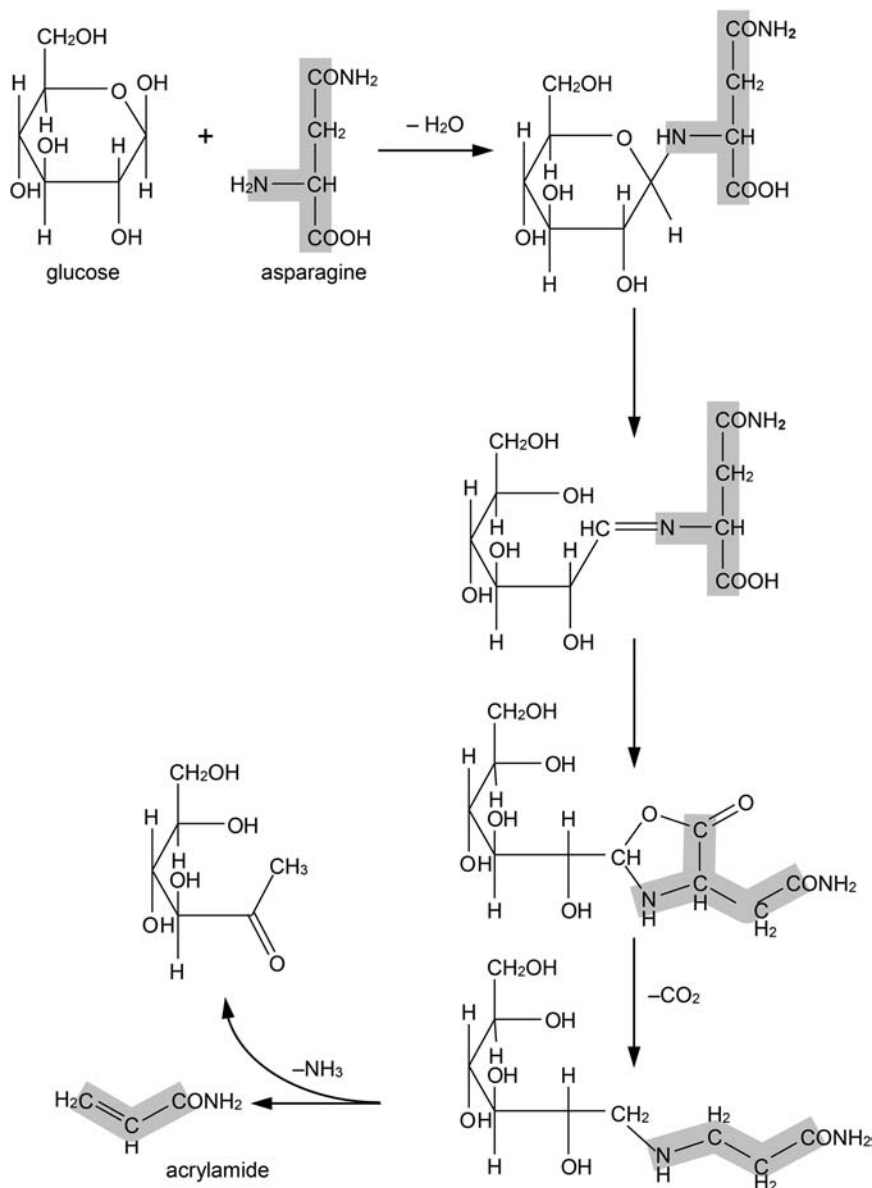


Figure 2.19 The generally accepted route for the formation of acrylamide by reaction with reducing sugars. The backbone carbon and nitrogen atoms of asparagine have been shaded to make their progress through the reactions easier to follow.

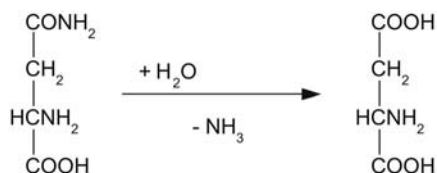
described from time to time, notably one involving acrolein (2.33), a breakdown product of fats subjected to high temperatures, as the starting material. Although there is some evidence for the operation of these alternative pathways, they are believed to make only a very minor contribution to the acrylamide content of food products.

The degree to which the acrylamide content of cooked or industrially processed food constitutes a hazard to human health is largely unresolved. However, food manufacturers and government authorities around the world have been actively seeking methods to reduce its levels. Unfortunately, both reactants, *i.e.* free asparagine and reducing sugars, are natural components of the plant materials from which we derive a great many of our most popular foods. For example, free asparagine is particularly abundant in potatoes (200–500 mg per 100 g fresh weight), varying widely with cultivation method, cultivar and storage time, all factors which affect the quality of potatoes cooked in domestic kitchens as well as those processed in industrial plants.

One drawback to the efforts of plant breeders and agricultural scientists to reduce natural asparagine levels in potatoes is that asparagine is a major contributor to the flavour of potatoes and potato products. The high temperatures involved in frying ($>120\text{ }^{\circ}\text{C}$) that are critical to acrylamide generation are also essential to the desirable flavours and textures of our crisps and chips. The sugar content of sliced potatoes can be reduced by washing and blanching and that this can give major reductions in the levels of acrylamide in fried potato crisps (see Scheme 2.1).

Another promising technique for asparagine reduction depends on the enzyme asparaginase. This enzyme, obtained commercially from fungi, catalyses the removal of the side-chain amino group, converting asparagine into aspartic acid, which does not participate in the reactions leading to acrylamide. Potentially useful reductions in free acrylamide using asparaginase have been reported in the manufacture of bread (by including asparaginase in the dough), coffee (by washing the beans in an asparaginase solution before roasting) and similarly washing sliced potatoes before frying. The high cost of this enzyme treatment for relatively low-cost bulk raw materials appears to have prevented its adoption to date. Modest reductions in acrylamide levels are also being achieved by other modifications of processing procedures – for example, by the careful control of temperatures in frying operations.

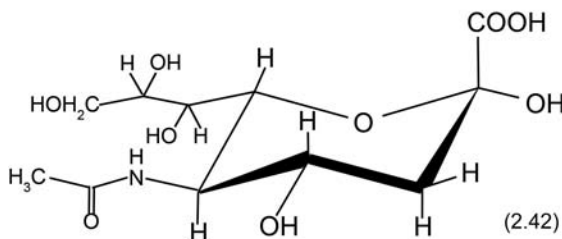
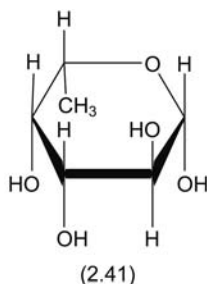
In the manufacture of bread and other baked goods, asparagine can be incorporated into the dough, achieving reductions in the acrylamide content of the final product claimed to be up to 90%. In 2019, a novel baking yeast variety was developed (without the use of genetic modification techniques) that secretes greatly enhanced levels of asparaginase. Its use is claimed to reduce acrylamide levels in baked goods by approximately 5%, without requiring any alterations to the manufacturing processes. It is unfortunate that, as shown by the data in Table 2.5, bread is not a major source of dietary acrylamide.



Scheme 2.1.

2.7.4 Human Milk Oligosaccharides

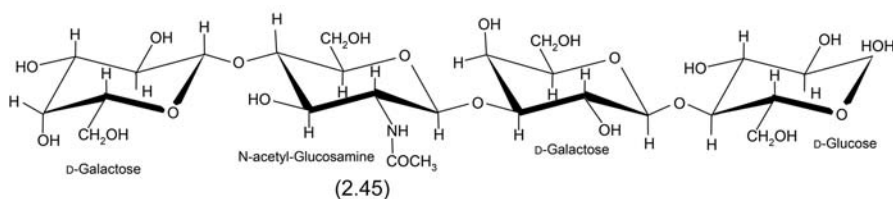
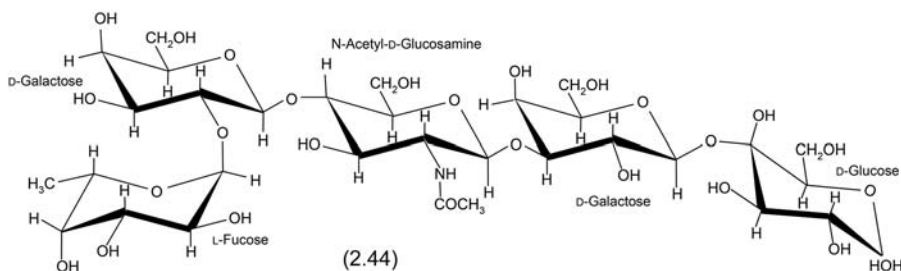
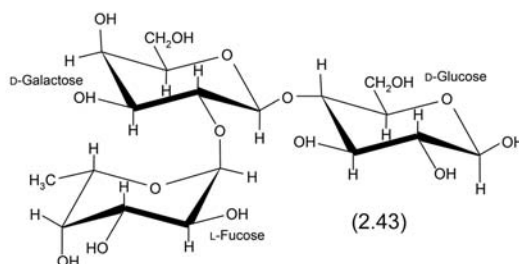
The contribution of the disaccharide lactose (2.24) to the role of milk was mentioned earlier in this chapter. However, while this sugar is by far milk's most significant carbohydrate component, milk is nowadays recognised as also containing a diverse range of other oligosaccharides. The amounts of these other oligosaccharides in the milks of most mammalian species, including the cattle, goats and sheep that have been consumed by humans for thousands of years, are fairly small (about 0.05 g L^{-1} in cow's milk). However, the milk of *Homo sapiens* is uniquely rich in these carbohydrates, nowadays always referred to as "human milk oligosaccharides" (HMOs), with between 8 and 12 g L^{-1} , depending on the stage of lactation, although levels in human colostrum can reach 20 g L^{-1} . Five monosaccharides are the principal building blocks of HMOs: D-glucose, D-galactose, N-acetylglucosamine (3.1), L-fucose (2.41), and N-acetylneuraminic acid (2.42).



The most striking feature of the HMOs is their structural variety. More than 200 different oligosaccharides have been identified in human milk, although only 15 of these occur in significant amounts. In terms of chemical structure, all are oligosaccharide derivatives of lactose carrying up to 30 other monosaccharide units in a bewildering variety of arrangements. The most abundant of these are 2-fucosyllactose, 2'-FL, (2.43) and lacto-N-fucopentaose, LNF I, (2.44) at $2\text{--}3 \text{ g L}^{-1}$ and $0.5\text{--}1.5 \text{ g L}^{-1}$, respectively. It is now well established that, unlike lactose, the HMOs are not absorbed from the small intestine as might be expected, but go on into the colon where they are consumed by highly specific strains of bifidobacterial, such as *Bifidobacterium infantis*, *B. bifidum* and *B. breve*, leading to the formation of short-chain fatty acids, notably butyric acid. This activity lowers the pH of the contents of the colon and inhibits the growth of other, potentially harmful, bacteria. This is in striking contrast to the fate of the lactose that reaches the large intestine of people with lactose intolerance, which is caused by a failure to absorb lactose from the small intestine. There is also evidence to suggest that some of these oligosaccharides may also interfere with the binding of pathogenic

bacteria to the lining of the intestine, providing some protection for the infant against disease.

Two HMOs, 2-fucosyllactose and lacto-*N*-neotetraose (LNnT) (2.45) are now available in commercial quantities for inclusion in milk products for bottle-fed babies (“formula” in the USA). They are manufactured by the fermentation of genetically engineered microorganisms. In the EU, they are both currently permitted as “Novel Foods” and in the USA have Generally Recognised As Safe (GRAS) status. A third HMO, 3'-sialyllactose (3'SL) also has GRAS status in the USA.



2.7.5 D-Allulose

Until recently, the monosaccharide D-allulose (D-ribo 2-ketohexulose), often known as D-psicose, was regarded as an oddity that was known to occur naturally in trace amounts in a variety of fruits and related products, notably figs (*Ficus carica*), jackfruit (*Artocarpus heterophyllus*), raisins and maple syrup. However, in recent years, it has become possible to synthesise it in commercially viable quantities as a novel sweetening agent.

In chemical terms, it can be regarded as either the ketose isomer of D-allose or the C-3 epimer^{xvi} of D-fructose (Figure 2.20). Although it crystallises as the β-D-pyranose, there

^{xvi}Epimers are pairs of optical isomers (see footnote i) that differ in the configuration of one asymmetric carbon atom, but in which the other asymmetric carbon atoms are otherwise identical. For example, D-glucose and D-galactose are C-4 epimers.

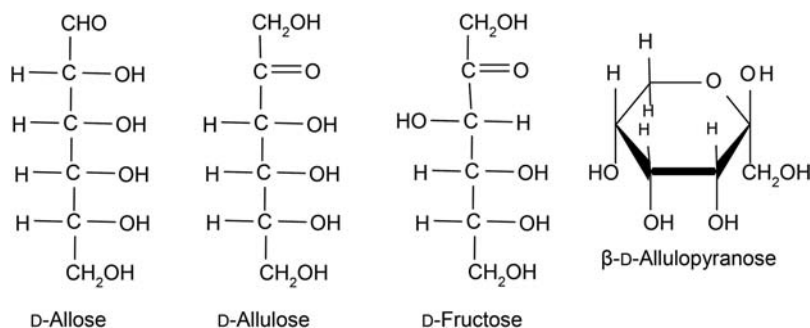


Figure 2.20 D-Allulose.

appears to be no certainty as to which structure predominates in its aqueous solutions, *i.e.* pyranose or furanose, α or β (see Section 2.1).

Allulose has approximately 70% of the sweetening power of sucrose, but has a very low calorific value. In humans, 1 g contributes 0.2–0.4 kcal, compared with about 4 kcal per gram for typical carbohydrates. It is readily absorbed from the small intestine into the bloodstream, being so similar to fructose, but is not then metabolised in the liver, muscles *etc.* Instead, it is excreted in urine. In nutritional calculations, the glycaemic index of D-allulose can be regarded as negligible.

Allulose is manufactured enzymically from fructose. Ketose-3-epimerases from any of several different bacteria are available to catalyse conversion. Allulose has been awarded GRAS status in the USA, although its status as a food additive in the UK and Europe had not, in 2022, been finalised. A notable feature of the legislation in the USA is that manufacturers of confectionery, cakes, *etc.* are permitted to omit allulose from the details of total and added sugars on Nutrition Facts labels. However, its relatively high cost probably precludes its use as a bulk ingredient in food products, such as cakes, in which sugar is an essential bulk ingredient. In soft drinks, colas, *etc.* it may prove to be an acceptable alternative to non-sugar sweeteners such as aspartame or cyclamate (see Chapter 9).

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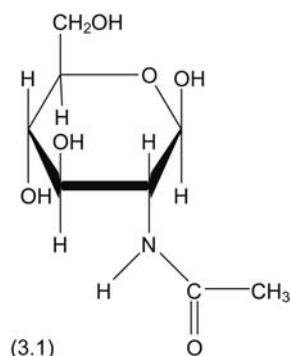
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3 Polysaccharides

In Chapter 2 we considered the properties of the mono- and oligosaccharides. We can now turn to the high molecular weight polymers of the monosaccharides: the polysaccharides. Polysaccharide structures show considerable diversity and are often classified according to features of their chemical structure – for example, whether the polymer chains are branched or linear and whether more than one type of monosaccharide is present. There is now a much better understanding of the relationship between the chemical structure of a polysaccharide and its physical properties, particularly with regard to its solutions, and the impact of this relationship on food materials is a major theme of this chapter. Despite their physiological importance, it is through this influence on the texture of food that polysaccharides often make the most immediate impact on the consumer.

The one common feature of the polysaccharides is that all those that are important to food scientists occur in plants. Polysaccharides have a relatively minor role in the physiology of animals. Glycogen, which is structurally very similar to amylopectin, acts as an energy and carbohydrate reserve in the liver and muscles.ⁱ The other animal polysaccharides are chitin, a polymer of *N*-acetylglucosamine (3.1), the fibrous component of arthropod exoskeletons, and the proteoglycans and mucopolysaccharides, which have specialised roles in connective tissues. The molecules of these animal polysaccharides include considerable proportions of protein.

ⁱMuscle tissue loses its glycogen in the ongoing *post-mortem* metabolism before it reaches the kitchen as meat, but fresh liver still contains 1–2% glycogen at the time it is sold.



Polysaccharides have two major roles in plants. The first of these, as an energy and carbohydrate reserve in the tissues of seeds and tubers, is almost always provided by starch. On the other hand, the data in Table 3.1 show that leafy plant foods, as exemplified by broccoli, contain only traces of starch. The second role of polysaccharides is to provide the structural skeleton of both individual plant cells and the plant as a whole. This role is filled by a wide range of different structural types of polysaccharides, which offer a correspondingly wide range of physical characteristics.

The contributions of polysaccharides to foods derived from plants are no less diverse. From a strictly nutritional point of view, starch is the only major plant polysaccharide that is *readily* digested in the human intestine and directly utilised as a source of energy. In fact, a high proportion of the human requirement for energy is met by the starch of seeds, such as wheat, rice and maize, and tubers, such as potatoes and cassava. However, it is now widely recognised that polysaccharides make a much greater contribution to human health than the mere provision of energy. Cellulose, pectins and hemicelluloses, among others, commonly referred to as “fibre”, are more correctly termed “non-starch polysaccharides” (NSPs). They have an essential role in the healthy function of the large intestine and in moderating the absorption of nutrients from the small intestine.

Table 3.1 Starch contents of a variety of foods consumed in the UK. These data should be regarded as typical values rather than absolute figures for the particular food, <https://www.gov.uk/government/publications/composition-of-foods-integrated-dataset-cofid>.

Food	Starch (%)	Food	Starch (%)
Wheat flour (wholemeal)	68.5	Peas (raw)	7.0
White bread	45.8	Broad beans (cooked)	11.2
White rice (uncooked)	83.6	Beetroot (raw)	0.6
Spinach (raw)	0.2	Broccoli	0.6
Maize (corn) flour	92.0	Carrots (young, raw)	0.2
Cornflakes	83.6	Chestnuts	24.6
Soya beans (raw)	4.8	Peanuts (raw)	6.3
Cassava (raw)	35.3	Walnuts	0.7
Potatoes (boiled, new)	14.8	Apples	Trace
Pasta (white, cooked)	30.5	Bananas	2.2

3.1 Starch

Reflecting its role in plants as a carbohydrate reserve, starch is found in greatest abundance in tissues such as tubers and the endosperm of seeds. It occurs in the form of granules, which are usually an irregular rounded shape, ranging in size from 2 to 100 μm . Both the shapes and sizes of the granules are characteristic of the species of plant and can help to identify the origin of a starch or flour.

Starch consists of two types of glucose polymer: amylose, which is essentially linear, and amylopectin, which is highly branched. These occur together in the granules, but amylose can readily be separated from starch solutions because it is much less soluble in organic solvents such as butanol. Most starches are 20–25% amylose, but there are exceptions: pea starch is around 60% amylose and the so-called waxy varieties of maize and other cereals contain little or no amylose.

Amylose consists of long chains of α -D-glucopyranosyl units linked, as in maltose, between their 1- and 4-positions (see Figure 3.1a). There is no certainty about the length of the chains, but they are generally believed to contain many thousands of glucose units, with typical average molecular weights between 2×10^5 and 2×10^6 . Amylose chains are not entirely linear, having a very small amount of the branching characteristic of amylopectin.

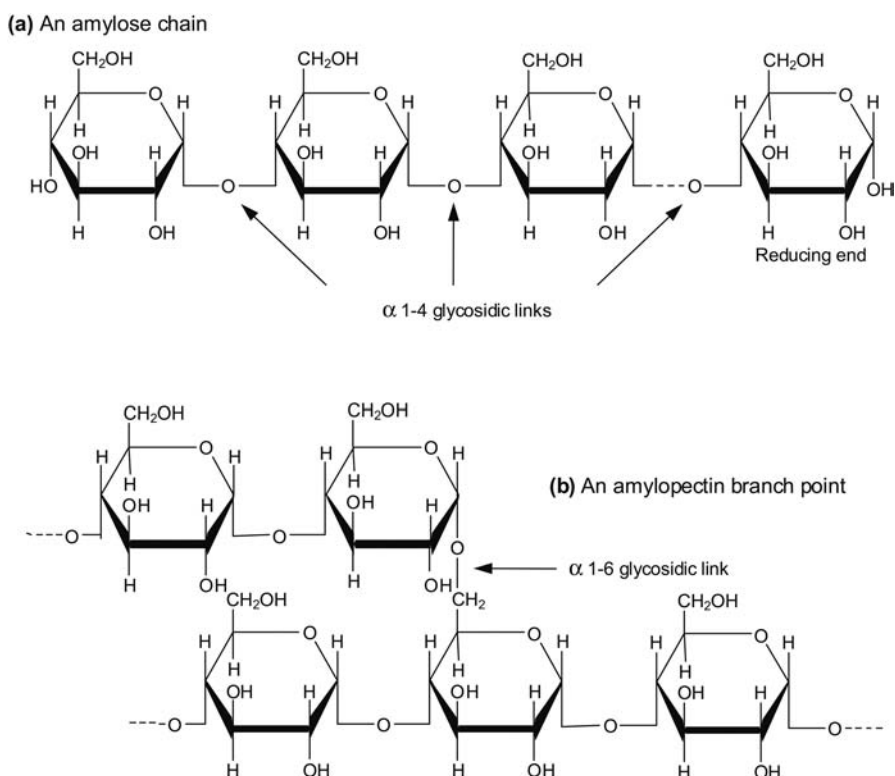


Figure 3.1 Chemical structure of amylose and amylopectin.

Amylopectin is a much larger molecule, comprising a million or so glucose units per molecule. As in amylose, the glucose units are joined by 1→4 glycosidic links, but about 4–5% of the glucose units are also involved in 1→6 links, creating the branch points shown in Figure 3.1b. This proportion of branch points results in an average chain length of 20–25 units. Over the years a great many hypotheses have been put forward to describe the arrangement of the branching of the chains in amylopectin, and for some time the tree-like arrangement proposed by Meyer and Bernfeld in 1940 was accepted. Since then, new insights have been provided by the dissection of the molecule into its individual component chains using the enzyme pullulanase, which is specific for 1→6 linkages. Although the chains do indeed have the expected overall average chain length, the molecule has been found to consist of two types of chain, the more abundant type having around 15 glucose units and the other about 40, referred to as the A and B chains, respectively. The so-called “cluster model” based on these data, and other supporting evidence, was proposed by Robin in 1974 and is now widely accepted (Figure 3.2a). Its essential feature is a skeleton of mainly singly branched A chains carrying clusters of B chains. In each molecule there is one chain, referred to as the C chain, which is unique in carrying a reducing end-group. As shown in Figure 3.2b and c, the amylopectin molecules are believed to be radially orientated in the starch granule, with the terminal reducing group towards the centre, possibly close to the central spot sometimes visible by light microscopy, the hilum. The clusters of short chains are believed to be distributed on the B chain skeleton, as shown in Figure 3.2b, leading to the formation of concentric domains of regularly orientated (crystalline) polysaccharide chains alternating with amorphous domains, shaded in Figure 3.2b, in which most of the 1→6 branch points are located. In the crystalline regions, pairs of neighbouring chains are believed to form short double helices with six glucose units per turn in each chain (Figure 3.2d).

When observed under a polarising microscope, starch granules show the Maltese cross pattern characteristic of birefringent materials. This confirms the presence of a high degree of molecular orientation. The crystallinity is confirmed by their X-ray diffraction patterns, which incidentally reveal that root starch granules are in general more crystalline than those from cereals. Another indication of the importance of the amylopectin fraction as the source of the crystallinity of starch granules is that waxy starches, which lack amylose, give X-ray diffraction patterns very similar to those of a normal starch. Surprisingly little is known of the arrangement of the amylose molecules within the starch granule. These are assumed to meander among the amylopectin molecules, orientated more or less radially. The granules of cereal starches contain about 1% lipid material, mostly in the form of lysophospholipids (see Chapter 4). The hydrocarbon chains of the lipid molecules are believed to lie along the axis of helical amylose chains, forming a complex not dissimilar in structure from the iodine–amylose complex.

Owing to the collective strength of the hydrogen bonds binding the chains together, undamaged starch granules are insoluble in cold water, but as the temperature is increased to what is known as the initial gelatinisation temperature, water begins to be imbibed. The initial gelatinisation temperature is characteristic of individual starches, but usually lies in the range 55–70 °C. As water is taken in, the granules swell and there is a steady loss of birefringence. Studies using X-ray diffraction show that complete

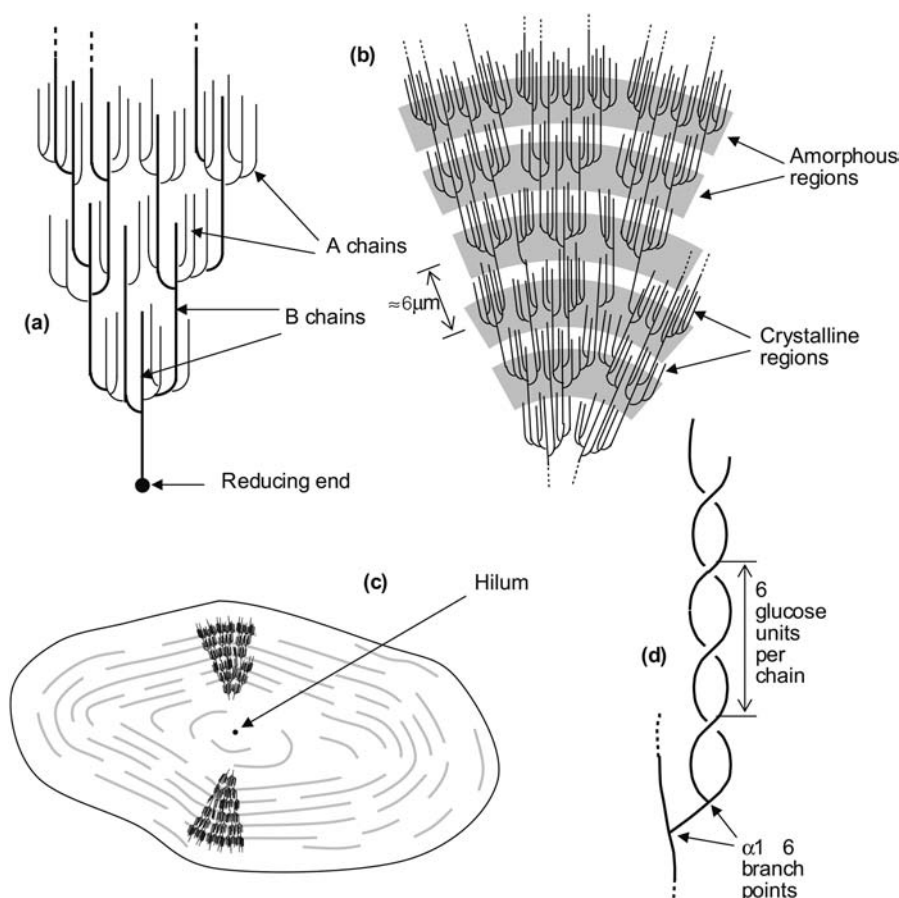


Figure 3.2 Amylopectin and the starch granule. (a) Essential features of the cluster model first proposed by Robin in 1974. (b) The organisation of the amorphous and crystalline regions (or domains) of the structure generating the concentric layers that contribute to the “growth rings” visible by light microscopy. (c) The orientation of the amylopectin molecules in a cross-section of an idealised entire granule. (d) The likely double-helix structure taken up by neighbouring chains and giving rise to the extensive degree of crystallinity in the granule.

conversion to the amorphous state does not occur until temperatures around 100 °C are reached. As swelling proceeds and the swollen granules begin to impinge on each other, the viscosity of the suspension/solution increases dramatically. The amylose molecules are leached out of the swollen granules and contribute to the viscosity of what is best described as a paste. If heating is maintained together with stirring, the viscosity soon begins to decrease again as the integrity of the granules is destroyed by the physical effects of the mixing. When the paste is allowed to cool, the viscosity increases again as new hydrogen bonding relationships between amylopectin and amylose are re-established to give a more gel-like consistency. These changes will be familiar to anyone who has used starch to thicken gravy or to prepare desserts such as blancmange. They can be followed in the laboratory using viscometers such as the Brabender amylograph,

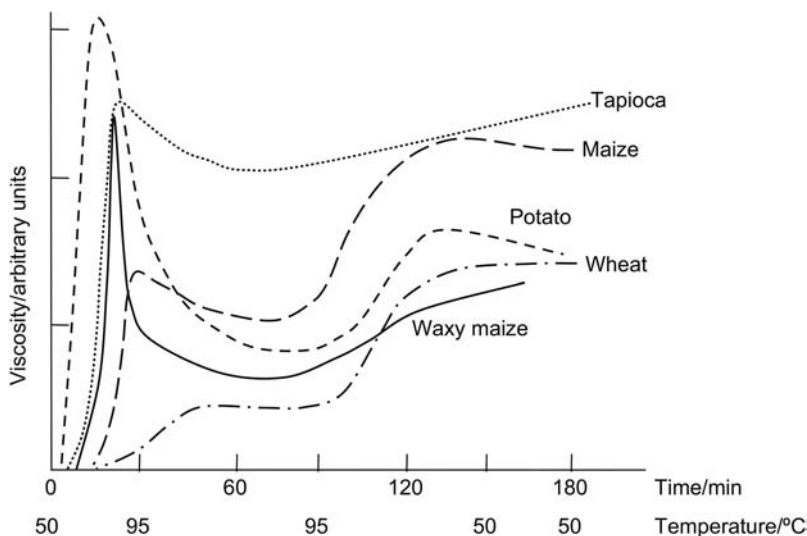


Figure 3.3 Brabender amylograph traces of the gelatinisation of several starches. The temperature is increased gradually from 50 to 95 °C over the first half-hour. It is then maintained at 95 °C for one hour before being allowed to decrease to 50 °C over the final half-hour period. (Reproduced by permission from Dr D. Howling and Applied Science Publishers.)

which apply continuous and controlled stirring to a starch suspension during a reproducible temperature regime. Typical Brabender amylograph results for several different starches are shown in Figure 3.3. The relationship between the variations in the molecular features of amylopectin from different sources and their gelatinisation properties have been studied intensively in recent decades. Higher gelatinisation temperatures and higher viscosity of the resulting starch pastes are generally associated with a greater proportion of the shorter internal B chains (see Figure 3.2a).

Solutions of starch have a number of characteristic properties, of which their reaction with iodine is the best known. Solutions of iodine in aqueous potassium iodide stain the surface of starch granules dark blue, but the reaction is most striking with the amylose component of dissolved starch. In the presence of iodine the amylose chains are stabilised in a helical configuration with six glucose units to each turn of the helix and the iodine molecules complexed along its axis. The blue colour of the complex depends on the length of the chain involved: with around 100 units $\lambda_{\text{max}}^{\text{ii}}$ is at 700 nm, but with shorter chains λ_{max} gradually decreases, until with only 25 units it is 550 nm. In view of this, it is not surprising that amylopectin (in the absence of amylose) and glycogen both give a reddish-brown colour with iodine.

When starch solutions or pastes are allowed to stand for a few hours, they begin to show changes in their rheological properties. Dilute solutions lose viscosity, but concentrated pastes and gels become rubbery and exude water. Both types of change are due to a phenomenon termed retrogradation, which involves only the amylose molecules.

ⁱⁱ λ_{max} is the wavelength of maximum light absorption.

Over a period of time, these molecules associate and effectively crystallise out. If one recalls that in starch solutions amylose tends to bind together the expanded amylopectin molecules within the granule structure, then the effects of retrogradation on the rheological properties are clearly inevitable.

Special efforts have to be made in a number of food products to avoid the problems that retrogradation could cause. For example, starch is often used commercially to thicken the juice or gravy of pies. In domestic kitchens wheat flour is similarly employed. If such a pie is frozen after manufacture, the amylose will undergo rapid retrogradation. When the pie is subsequently thawed, the starch paste will become completely liquid, revealing just how much, or how little, of the filling was actually meat or fruit, as well as making the pastry wet and soft. One answer to this has been to use starch from the “waxy” varieties of maize,ⁱⁱⁱ which consist only of amylopectin and therefore give pastes that will survive freezing and thawing.

Manufacturers of so-called “instant desserts”, popular with children in the 1970s and 1980s, also had to be wary of retrogradation. These starch-based dry mixes were designed to be blended with milk to give a blancmange-like dish without the need for cooking. A slurry of raw starch in water was passed over steam-heated rollers, resulting in rapid gelatinisation, immediately followed by drying to a film, which could be scraped off the roller and subsequently milled to a fine powder. The water was thus removed before the amylose molecules had the chance to become organised. The resulting pre-gelatinised starch dispersed fairly easily in milk, but no one would have claimed that this gave as good a texture as that of a proper blancmange.

In the manufacture of bread and other bakery products, the behaviour of starch is obviously very important. When flour is milled it is inevitable that a significant proportion of the starch granules will be physically damaged, scratched, cracked or broken. When the dough is first mixed, these damaged starch granules absorb some water. During bread-making, the kneading and proving stages prior to the actual baking allow time for the α - and β -amylases naturally present in flour to break down a small proportion of the starch to maltose and other sugars. These are fermented by the yeast to give the CO_2 that leavens the dough. Other baked goods such as cakes, made without yeast, rely on the CO_2 from baking powder^{iv} for the same purpose, together with air whipped into the mixture.

Once in the oven, the starch granules gelatinise and undergo varying degrees of disruption and dispersion. The α -amylase activity of flour persists until temperatures around 75 °C are reached, so that some enzymic fragmentation of the starch granules also occurs, particularly in bread. Among the factors that affect the degree of starch breakdown are the availability of water and the presence of fat. Obviously in a short-bread mixture containing only flour, butter and sugar, the coating of fat over the starch granules will limit the access of the small amount of water present and we find that the granular structure is still visible after baking. A roux is another example of fat being used to control the gelatinisation behaviour of starch.

ⁱⁱⁱThe term “waxy” is derived from the appearance of the starchy endosperm of the maize kernel when it is broken in two.

^{iv}Baking powder is a mixture of sodium hydrogen carbonate (NaHCO_3) and a weakly acidic salt, usually potassium hydrogen tartrate, which gives off carbon dioxide in the moist heat of the oven.

After one or two days, bread, and cakes with a low-fat content, start to go stale. Staling is often assumed to be a simple drying-out process, but it occurs just as readily in a closed container. What we are actually seeing is the retrogradation of the starch. The current view of bread staling is that it is a two-stage process. The first stage, marked by the transition from the soft “new bread” state, takes place only a few hours after baking and is believed to involve the retrogradation of the unbranched amylose fragments. The second stage, staling proper, involves retrogradation of a proportion of the amylopectin fragments. The crystallinity of the retrograded starch gives the crumb of stale bread its extra whiteness, and the increased rigidity of the gelatinised starch causes the lack of “spring” in the crumb texture. Retrograded starch can be returned to solution only by heating, *i.e.* by re-gelatinisation; stale bread can be revived by moistening and then returning it to a hot oven for a few minutes.

The phenomenon of retrogradation is a reminder that not all the starch in a foodstuff is necessarily available to us, *i.e.* readily digested and absorbed from the small intestine. The starch content of a foodstuff falls into a number of categories:

- RDS: Rapidly digestible starch, dominant in starchy foods cooked by moist heat, *e.g.* bread or potatoes.
- SDS: Slowly digestible starch, like RDS completely digested in the small intestine, but more slowly. Includes physically inaccessible amorphous starch, raw starch granules, or retrograded starch in cooked foods.
- RS: Resistant starch, potentially resistant to digestion in the small intestine. Resistant starch is further subdivided as follows:
 - RS1: Resistant due to physical inaccessibility, as in partly milled cereals and some very dense processed starchy foods. It is measured on the basis of enzyme digestibility before and after very thorough homogenisation or milling.
 - RS2: Uncooked granules, particularly resistant to enzyme action, *e.g.* raw potato and banana.
 - RS3: The most resistant starch, consisting of retrograded amylose formed during cooling of previously heated gelatinised starch.
 - RS4: Chemically modified starches incorporated into food products, described in detail in the first of the Special Topics at the end of this chapter. They are used either to provide specific physical/textural properties or to enhance the dietary fibre content (see —Section 3.4). Their susceptibility to enzyme breakdown depends on the type and degree of chemical modification.

All types of resistant starch are considered to be digestible by the microflora in the colon, at least to some degree. The resulting products of digestion, glucose and glucose-containing oligosaccharides, are subjected to bacterial fermentation, leading to the formation of hydrogen, carbon dioxide, methane, short-chain fatty acids (SCFAs) and other organic acids, notably lactic acid (5.8). The most important SCFAs are butyric acid [butanoic acid, $\text{CH}_3(\text{CH}_2)_2\text{COOH}$], propionic acid (propanoic acid, $\text{CH}_3\text{CH}_2\text{COOH}$) and acetic acid (CH_3COOH). Butyric acid is the favoured nutrient of the cells lining the colon and is now recognised as important in reducing the risk of colon cancer and

other disorders. Over 20% of the resistant starch in the diet may be fermented, whereas the corresponding figure for NSPs, dealt with later in this chapter, is less than 15%.

The fact that over 10% of the total starch in canned beans, and 2% of that in bread, comes into the resistant category suggests that the calculation of a food's nutritional value based on its chemical composition needs, in some cases, to be viewed with caution. Further nutritional implications of resistant starch are discussed under the heading Glycaemic Index in the Special Topics section at the end of this chapter.

Although the overall proportion of resistant starch in many foodstuffs has been determined, little information is available about the proportions of the different subdivisions. However, the data in Table 3.2 give a fairly accurate impression, which we shall see is in reasonable agreement with nutritional observations.

In partially milled cereal products that are eaten uncooked, such as muesli, some of the starch remains undigested simply because the amylases cannot gain access to it. The starch in raw potatoes and unripe bananas is in a particular crystalline form that is highly resistant to intestinal hydrolysis. Another type of resistant starch is formed when starchy materials are heated in the absence of abundant water – for example, during the

Table 3.2 Classification of food starches on the basis of digestibility.

A. Proportions of total resistant, *i.e.* RS1 + RS2 + RS3. (From: H. N. Englyst, S. M. Kingman and J. H. Cummings, *Eur. J. Clin. Nutr.*, 1992, **46**, S33.)

Food	Total starch (% dry matter)	Resistant starch (% total starch)	Food	Total starch (% dry matter)	Resistant starch (% total starch)
White bread	77	1.2	Potato, boiled, hot	74	6.8
Wholemeal bread	60	1.7	Potato, boiled, cold	75	13.3
Cornflakes	78	3.8	Spaghetti, freshly cooked	79	6.3
Porridge oats	65	3.1	Peas, cooked	20	25
Rye crispbread	61	4.9	Haricot beans, cooked	45	40

B. Detailed subdivision of starch types in a limited range of foods. The expressions RDS, SDS, *etc.*, are explained in the text. (From: British Nutrition Foundation, *Complex Carbohydrates in Food: Report of the British Nutrition Foundation Task Force*, Chapman and Hall, London, 1990.)

Food	Starch type (%)				
	RDS	SDS	RS1	RS2	RS3
White flour	38	59	—	3	Trace
Spaghetti	55	36	8	—	1
Shortbread	56	43	—	—	Trace
Canned chickpeas	56	24	5	—	14
Canned red kidney beans	60	25	—	—	15
Wholemeal bread	90	8	—	—	2
White bread	94	4	—	—	2

manufacture of breakfast cereals. This type also appears during the canning of beans or other legumes. There may be plenty of water in the can, but little penetrates into the beans. These prolonged high-temperature treatments damage the granule structure so that it becomes immune to the effect of digestive enzymes and is very reluctant to gelatinise. It must therefore be classed as an insoluble fibre component, even though it is not easy for analytical procedures to distinguish it from available starch.

3.2 Pectins

Pectins are the major constituents of the primary cell walls of plant tissues and also the middle lamella. The middle lamella is the material that lies outside the cell walls between cells and provides the packing material that helps to bind the cells together. In these sites, pectins comprise a substantial proportion of the structural material of soft tissues, such as the parenchyma of soft fruit and fleshy roots. As the structure of pectins can only be explored after they have been separated from the rest of the plant tissue, we cannot be certain as to the exact nature of pectins as they occur in plant tissues. The term protopectin is applied to the water-insoluble material in the plant that yields water-soluble pectin on treatment with a weak acid.

Most elementary accounts of pectins describe them as linear polymers of α -D-galacturonic acid (2.16), homogalacturonan,^v in which a proportion of the carboxyl groups are esterified with methanol. The proportion of carboxyl groups that are esterified varies between plant species from around 60%, *e.g.* those from apple pulp and citrus peel, to around 10%, *e.g.* that from strawberries. The molecular weight of pectin molecules can best be described as uncertain but high, probably around 100 000. During recent years, many studies of pectins have concentrated on commercially significant pectin sources such as citrus peels and apple pomace (*i.e.* the pulpy residue after the juice has been pressed out) and a much more complex picture has emerged, especially with regard to the occurrence of neutral sugars and complex branching chain structures. Nevertheless, the homogalacturonan chains illustrated in Figure 3.4 are still regarded as the dominant structural feature, constituting about 65–80% of the total pectin fraction. The simple structure shown in Figure 3.4 was always recognised by carbohydrate chemists to be an over-simplification. However, nowadays food scientists appreciate that the behaviour of pectins in food systems such as jam and confectionery can be properly understood only if minor components and structural features are also taken into consideration.

The importance of pectin in food products such as jam and other fruit preserves is its ability to form gels. Gels consist largely of water, but are stable and able to retain the shape given to them by a mould. We have already seen that sugar molecules, and by inference also polysaccharides, are effective at binding water. However, a stable gel

^vTerms such as galacturonan, galactans and arabinan describe polysaccharide chains consisting of galacturonic acid, galactose and arabinose, respectively. The “an” suffix is derived from “anhydro”, indicating that the links between the monosaccharide units are formed by loss of the elements of water.

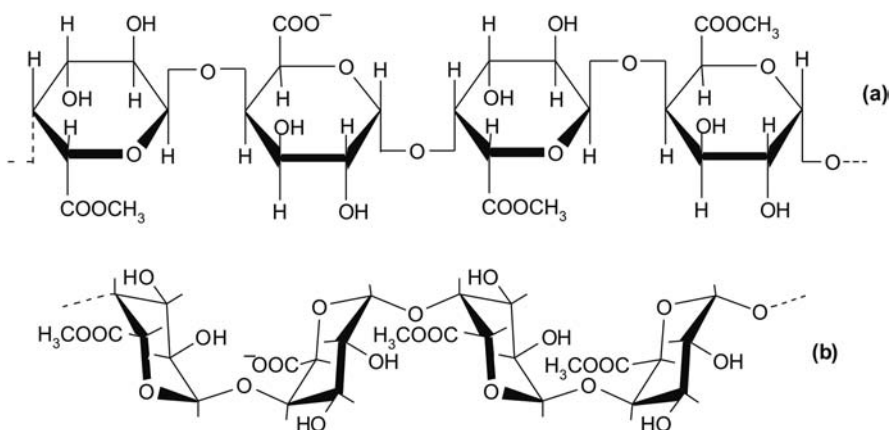


Figure 3.4 The polygalacturonan backbone of pectin: (a) shows four galacturonic acid units, three of which are methylated, drawn according to the Haworth convention and (b) shows the same structure with the galacturonate units drawn in their chair configuration to illustrate the zigzag shape of the chain. In (b), the hydrogen atoms directly attached to the rings have been omitted for clarity.

also requires a three-dimensional network of polymer chains with water, plus solutes and suspended solid particles, held in its interstices. It has often been assumed that simple interactions, such as single hydrogen bonds between neighbouring chains, were all that was required to maintain the stability of the network. The fact that the lifetime of an isolated hydrogen bond in an aqueous environment is only a tiny fraction of a second was overlooked. Obviously, attractive forces of this character provide an adequate explanation for the viscosity of the solutions of many polymers, such as the polysaccharide gums, but do not explain gel structure.

The concept of the junction zone was first introduced by Rees to explain the structure of these gels. His concept initially concerned gels formed by carrageenans, a group of polysaccharides considered later in this chapter, but it can be applied to most gel-forming polymers, including the pectins. Although single hydrogen bonds are not sufficiently durable to provide a stable network, a multiplicity of hydrogen bonds or other weak forces acting cooperatively can form the basis of the stability of crystal structures. Rees showed that polymer chains in a gel interact in regions of ordered structure, involving considerable lengths of the chains. These regions, the junction zones, are essentially crystalline in nature, and details of their structure have been revealed by X-ray crystallography.

The detailed structure of the junction zone is dependent on the nature of the polymer involved. In agar and carrageenan gels, the interacting chains form double helices that further interact to form “super-junctions”. The details of pectin junction zones are less clear, but it is accepted that interacting homogalacturonan chains pack closely together in a crystal-like array. However, our understanding of gel structures requires more than merely the ability of the polymer chains to pack together. Other structures are required to disrupt the associations between chains because without them all the chains would

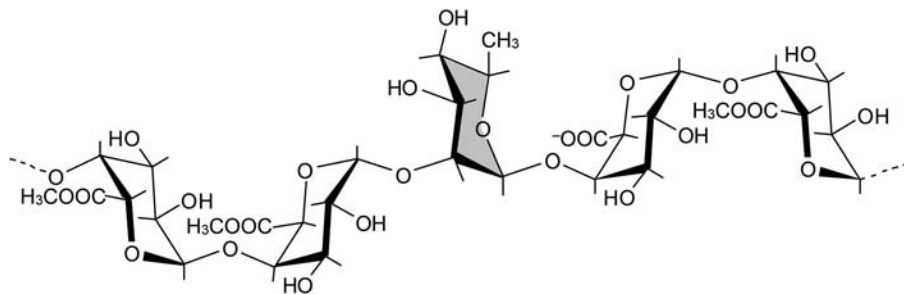


Figure 3.5 Insertion of an L-rhamnose residue (shaded) into a sequence of galacturonate residues: [...-4)-GalA- α -(1-4)-GalA- α -(1-2)-Rha- α -(1-4)-GalA- α -(1-4)-GalA- α -(1-...)] can be seen to disrupt the regular zigzag pattern. As drawn here, three of the galacturonate residues are esterified with methanol. The terms Rha and GalA are the conventional abbreviations for rhamnose and galacturonate, respectively.

simply pack into a single junction and precipitate out of solution, and a gel would not be formed. Two types of disruptive structures are recognised. One of these is referred to as an *anomalous residue*.^{vi} This consists of individual monosaccharide units with different linkages and/or other structural features which occur occasionally in the chain. The disruptive effect of a rhamnose residue inserted into a sequence of galacturonate residues is shown in Figure 3.5.

The second type of disruptive structure is branching in the polysaccharide chain. Even a single monosaccharide residue attached to an otherwise regular backbone would make the formation of a junction zone unlikely – and a long branch chain, like the ones already encountered in amylopectin, would make the formation of a junction zone impossible. The picturesque terms, *smooth* and *hairy*, have been adopted to describe, respectively, uninterrupted regular regions of polysaccharide chains and regions rich in branches. The potential effects of anomalous residues and branches on junction zone formation are illustrated in Figure 3.6.

The contribution of the hairy regions of the chain to the formation of gels is now clear. Without them, there would be nothing to prevent interaction taking place along the entire length of the chains, resulting in precipitation. The polymer chains linked at the junction zones provide a stable three-dimensional network in which water, solutes and suspended particles are bound or entrapped by the branched or irregular polymer chains. The retrogradation of the amylose component of starch pastes (see Section 4.1) is a perfect example of what happens when unbranched, uninterrupted chains are able to interact.

As many as 20% (but usually less) of the components of a pectin may be neutral sugars, including L-rhamnose (2.20), D-glucose, L-arabinose (3.2), D-xylose (3.3) and D-galactose (3.4). L-Rhamnose residues are found in all pectins.

^{vi}In this context, the terms residue and unit both refer to a monosaccharide molecule that is a component of a polysaccharide or oligosaccharide chain.

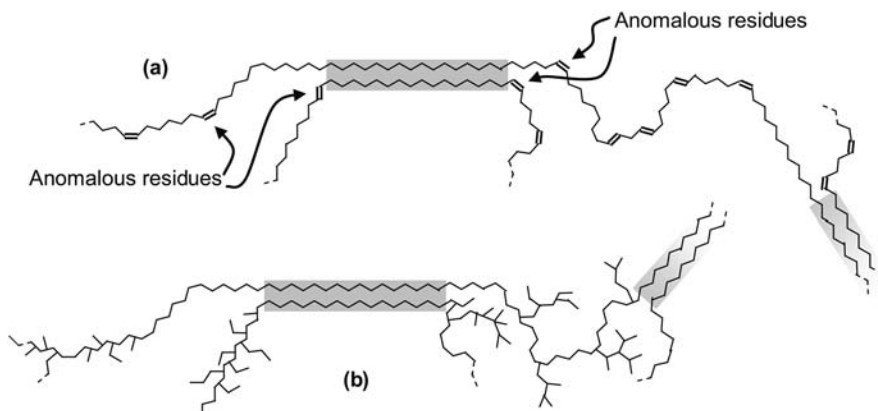
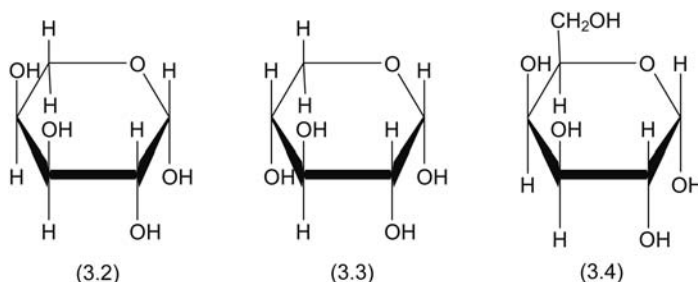
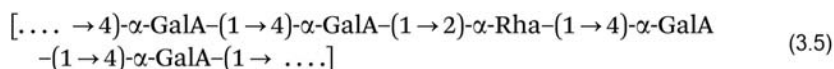


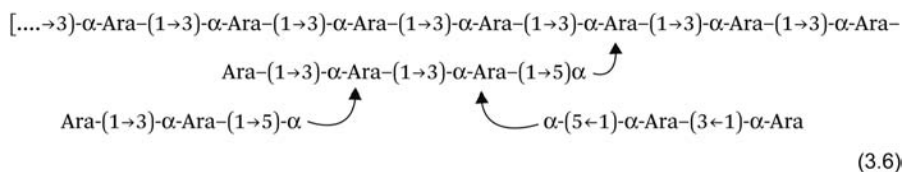
Figure 3.6 Effect of (a) the insertion of anomalous residues and (b) the attachment of side-chains in otherwise homogenous polymer chains on the formation of junction zones (shaded).



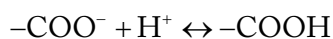
Particular neutral sugars tend to occur in particular situations, such as in specialised chain structures – for example, rhamnose residues are most often found in rhamnogalacturonan chains, *i.e.* chains of alternating rhamnose and galacturonic acid residues (3.5):



Arabinose units are found in highly branched chains that have an arabinan backbone carrying further short, and often themselves branched, arabinan chains linked to the 5-position of the backbone arabinose units (3.6). There are more details of these and other pectin components in the Special Topics section at the end of this chapter.



It may have been inferred from the discussion so far that pectins form gels fairly readily, but anyone who has been involved in jam-making, either at home or commercially, will know how untrue this is. The presence of pectin, solubilised when the fruit is boiled, is not enough in itself. For successful jam-making, we must meet other conditions as well. Ideally, the pectin should be highly esterified, with at least 70% of the carboxyl groups methylated, and present at a concentration of at least 1% w/v. The acid arising from the fruit, or added separately, must maintain the pH below 3.5 and ideally close to 3.0. Citric acid or malic acid, each of which occur naturally in many fruits (see Chapter 7), are the acidifying agents normally used. The total sugar content must be at least 50% w/v. Although the relationships between these requirements and the procedures of jam-making can be readily discerned, the reasons behind them are less obvious. In fact, pectin is a reluctant participant in gel formation. The successful formation of junction zones requires that the affinity of the pectin chains for each other must be maximised. Keeping the pH low has two valuable effects. The concentration of hydrogen ions is high at low pH, which tends to repress the ionisation of the galacturonate carboxyl groups:



This means that the tendency of the negatively charged COO^- groups to repel each other is eliminated. Furthermore, any charged groups such as COO^- tend to attract clusters of water molecules, which would also prevent the pectin chains getting close to each other. The methyl esterification of the carboxyl groups has the same beneficial effect in preventing ionisation and similarly encourages junction zone formation. By contrast, the high concentration of sugar molecules competes for water and has a beneficial effect on gel formation.

During the boiling phase of jam-making, a considerable proportion of the sucrose (both added and from the fruit) is hydrolysed to its parent monosaccharides, glucose and fructose (see Chapter 2). This increases the total proportion of bound water molecules, *i.e.* those not available for binding to the pectin chains, thereby enhancing the process of gel formation.

It is usual for commercial jam manufacturers to add extra pectin to improve the gel strength. This pectin will normally have been extracted from apple pomace or citrus peel and treated to modify its properties to suit particular applications. So-called high-methoxy pectins are 50–75% esterified, whereas low-methoxy pectins are 25–50% esterified. The amount of pectin added varies with the proportion and type of fruit being used. For example, strawberries and raspberries, which are low in pectin, require higher levels of added pectin (about 0.1–0.4%, depending on the fruit content of the jam) than medium pectin fruits such as oranges (*i.e.* in marmalade) and apricots, or high-pectin fruits such as apples or gooseberries, which require only 0.2% added pectin or none at all. Pectins with a range of degrees of esterification (*i.e.* the proportion of galacturonate residues esterified with methanol) are available commercially. Rapid-set pectins, around 70% esterified, are used in jam-making. Slow-set pectins, with around 60% esterification, are used in confectionery. Low-methoxy

pectins only form gels in the presence of calcium ions, either added as calcium salts or provided by the calcium phosphate component of milk (see Chapter 4). The junction zones formed closely resemble the so-called “egg-box” structure, considered in more detail when the gel-forming capabilities of alginates are discussed later in this chapter. Amidated pectins, in which the galacturonate carboxyl group ($-\text{COOH}$), is converted to an amide ($-\text{CONH}_2$), are manufactured by treatment of high-methoxy pectins with ammonia. They are sometimes used in the formulation of milk-based dessert products.

The breakdown of pectins is an essential feature of fruit ripening and the enzymes involved occur widely in plant tissues, as well as being secreted by the fungi and bacteria that specialise in attacking and breaking down plant tissues. There are three important classes of enzymes (pectinases) that catalyse pectin breakdown:

1. Pectin methyl esterases, which remove the methanol residues from methylated galacturonate units.
2. Polygalacturonases, which hydrolyse the glycosidic links between unmethylated galacturonate units. There are two types of these enzymes. *Exo*-polygalacturonases release mono- or disaccharides from the non-reducing end of the chain, whereas *endo*-polygalacturonases hydrolyse linkages within the chain and generate short fragments. Polygalacturonases only become active following the action of pectin methyl esterases because they cannot work when the degree of methylation of the pectin molecule as a whole is higher than about 60–70%.
3. Pectin lyases, which break the glycosidic links between methylated galacturonate units by an unusual β -elimination reaction in which a *trans* double bond is inserted between C-4 and C-5, rather than the usual hydrolysis (Figure 3.7).

As ripening takes place, these enzymes will break down the pectin surrounding the cells of the fruit tissue, causing it to soften and become more palatable. Of course, there are many other changes taking place at the same time, including the formation of sugars, pigments and flavour compounds.

Pectinase preparations containing these same enzymes, and others which attack the pectin side-chains, are manufactured commercially using cultures of bacteria

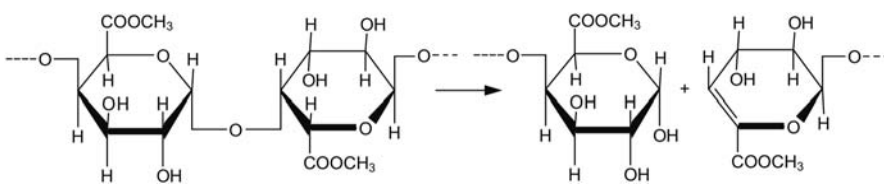


Figure 3.7 The β -elimination reaction catalysed by pectin lyase.

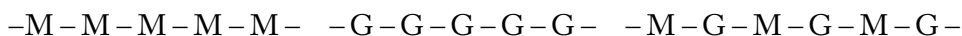
and fungi isolated originally from rotting fruit and vegetables. They are extensively used in wine- and cider-making and the fruit and vegetable juice industry to increase the degree of tissue damage, and therefore the juice yield, when the juice is extracted by pressing. They are also widely used to clarify fruit juices, cider and wines because the large polygalacturonate molecules are very effective at binding protein particles and other cell debris, thereby producing an unwanted haze. Of course, consumers do not expect all fruit juices to be clear, particularly tomato juice. Before tomatoes are fully ripe most of their pectinases remain within the cell membrane, kept away from the pectins of the middle lamella that holds the cells together. When the fruit is crushed, the cell membranes are disrupted and the pectinases are able to reach their substrate. In the case of tomatoes, the ensuing breakdown is extremely rapid. Tomato juice is therefore made in a “hot break” process in which the tomatoes are rapidly heated before, or even during, crushing to inactivate the enzymes.

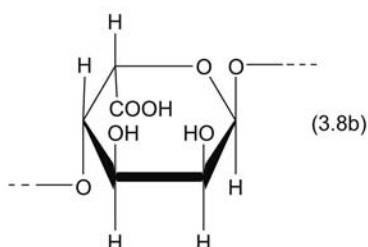
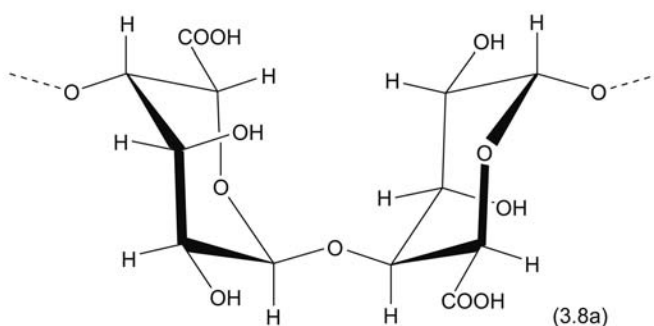
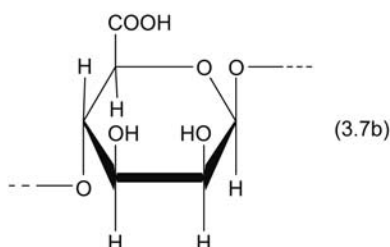
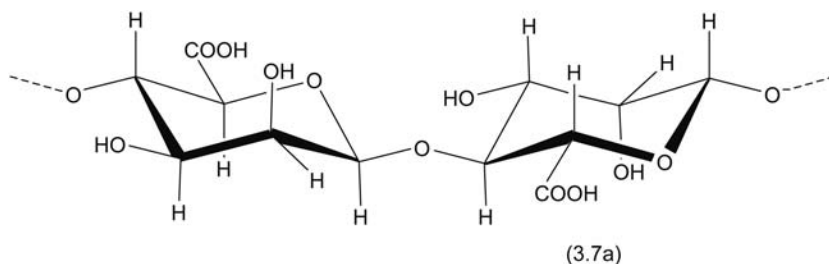
Pectinases can be used to strip off the albedo, or pith, of citrus segments before canning, but a curious variation of this process is to introduce the enzyme under the peel. The peel is punctured and the whole fruit is then placed in a sealed container from which the air is pumped out. As a result, the air in the space under the peel is sucked out, so that when the fruit is transferred to a bath of enzyme solution at atmospheric pressure the enzyme solution is sucked in. An hour or so later the residues of the peel can simply be washed off. Further enzyme treatment then separates the fruit into segments, which, after another good wash, are ready for canning. With fruit such as peaches or apricots, pectinase treatments are replacing the traditional “lye peeling”, in which the peel is dissolved away by immersion in a very strong sodium hydroxide solution (lye).

3.3 Seaweed Polysaccharides

As we have just seen, the pectins have a particular place in the structure of the tissues of higher plants. In the seaweeds, their place is taken either by the alginates (in the brown algae, the Phaeophyceae) or the agars and carrageenans (in the red algae, the Rhodophyceae).

The alginates are linear polymers of two different monosaccharide units: β -D-mannuronic acid (M) (3.7a) and α -L-guluronic acid (G) (3.8a). When their structures are drawn using the Haworth convention (3.7b and 3.8b), these two seemingly rather different monosaccharides are seen in fact to differ only in their configuration at C-5, *i.e.* they are C-5 epimers of one other. This difference leads to the different pyranose ring conformations that are taken up, *C1* by mannuronic acid and *1C* by guluronic acid, the conformations that allow the bulky carboxyl groups to occupy equatorial positions. Both monosaccharide units occur together in the same chains, but linked in three different types of sequence, in each case by 1,4-links.





Alginates from different species of seaweed, and even from different parts of the same plant, differ in the ratios of the two components, as illustrated by the data in Table 3.3. Alternating $-G-M-G-M-G-M-$ sequences contribute about 10–25% of the total.

Alginic acid itself is insoluble, but its alkali metal salts are freely soluble in water. Gels readily form in the presence of Ca^{2+} ions. The junction zones only involve $-G-G-G-G-$ sequences, in an egg-box structure in which a Ca^{2+} ion is complexed by four L-gulonate units, see Figure 3.8. As the proportion of guluronate residues increases, and the corresponding proportion of $-G-G-G-G-$ sequences increases, the gel strength also increases.

Table 3.3 Proportions of guluronate and mannuronate residues in different seaweed species (all types of kelp) and tissues (from K. I. Draget, in *Handbook of Hydrocolloids*, see Further Reading for details).

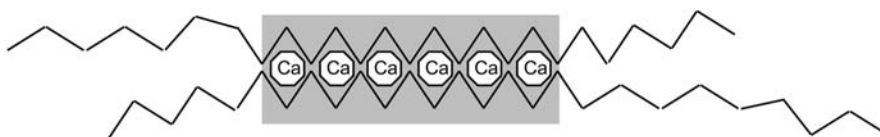
Species	Part	Guluronate : Mannuronate ratio
<i>Laminaria japonica</i> ^a	Unspecified	35 : 65
<i>L. digitata</i> ^b	Unspecified	41 : 59
<i>L. hyperborea</i> ^b	Leaf (frond)	55 : 45
<i>L. hyperborea</i> ^b	Stem (stipe)	68 : 32
<i>Macrocystis pyrifera</i> ^c	Unspecified	39 : 61
<i>Durvillea antarctica</i> ^d	Unspecified	29 : 71

^a*L. japonica*, nowadays classified officially as *Saccharina japonica*, is commercially cultivated in the Far East where it is a popular foodstuff.

^b*L. digitata* and *L. hyperborea* are found particularly on the coasts of the North Atlantic.

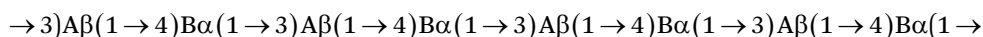
^c*M. pyrifera* is found on the eastern coasts of the Pacific.

^d*D. antarctica* is found on the coasts of countries such as New Zealand and Chile that adjoin the Southern Ocean.

**Figure 3.8** Junction zone of an alginate gel. The junction zone (shaded) consists of two strands of contiguous guluronate units terminated by sequences containing mannuronate residues.

Calcium alginate gels do not melt at temperatures below the boiling point of water and this property gives them a number of applications in food products. If a fruit purée is mixed with sodium alginate and then treated with a calcium-containing solution, various forms of reconstituted fruit can be obtained. For example, if a cherry/alginate purée is added as large droplets to a solution of Ca^{2+} ions, the familiar synthetic cherries popular in the bakery trade can be made. A fairly convincing skin forms on the outside, where the alginate is exposed to the highest concentration of Ca^{2+} ions. The diffusion of Ca^{2+} ions through this skin gives lower concentrations inside, forming a softer jelly-like consistency. Rapid mixing of a fruit/alginate purée with a sparingly soluble calcium salt, such as calcium carbonate or sulfate, followed by appropriate moulding, gives the apple or apricot “pieces” sometimes sold as cheap pie fillings.

The agars and carrageenans^{vii} are a more complex group than the alginates. Most consist of unbranched chains of galactose derivatives, with the generalised structure:



where A and B are residues from two different groups, as indicated in Table 3.4. Different monosaccharides predominate in particular carrageenans, but the distinction between different carrageenans in particular seaweed species is not always clear-cut.

^{vii}The red seaweed *Chondrus crispus* grows abundantly on the rocky Atlantic coasts of the British Isles and Europe, including the Irish coast, where it is known as Irish moss. *Carraigín* is Gaelic for “rock moss”.

Table 3.4 The monosaccharides of agar and carrageenans. The structures of some of the monosaccharide units listed here are shown in Figure 3.9. There is a steady increase in the sulfate content as one goes down the list from agarose to μ -carrageenan, matched by a steady decrease in gelling power; λ - and μ -carrageenans do not gel at all.

		Position	
		A	B
		Major	Minor
Agarose		I	IV
κ -Carrageenan		III	V
ι -Carrageenan		III	VI
λ -Carrageenan		I, II	VIII
μ -Carrageenan		III	VII
I	β -D-Galactose		V, VIII (3.9)
II	β -D-Galactose 2-sulfate		(3.11)
III	β -D-Galactose 4-sulfate		(3.13)
IV	3,6-Anhydro- α -L-galactose		(3.10)
V	3,6-Anhydro- α -D-galactose		
VI	3,6-Anhydro- α -D-galactose 2-sulfate		(3.14)
VII	α -D-Galactose 6-sulfate		
VIII	α -D-Galactose 2,6-disulfate		(3.12)

Commercial carrageenan preparations sold for food use as gelling or thickening agents are blends designed to have properties appropriate to a particular application.

The junction zones which these polysaccharides form are quite unlike the simple egg-boxes we have met so far. Rees's group showed that gelation first involves the formation of double helices of galactan chains wound around each other. The formation of these helices is not sufficient in itself for a gel to form. This only occurs when the rod-like structures go on to associate together to form super-junctions, as shown in Figure 3.10. Examination of carrageenans by X-ray crystallography has shown that it is the intervention in the B-position of residues lacking the 3,6-anhydro bridge (VII and VIII) which disrupts the initial helix formation. As the sulfate content increases, the resulting negative charge on the helix requires the presence of neutralising cations such as K^+ (for κ -carrageenan) or Ca^{2+} (for ι -carrageenan) if the helices are to associate into super-junctions. Agar, with its very low sulfate content, gels well regardless of the cations present. The agar super-junctions are sufficiently large to refract light and are the source of the slight opalescence visible in even the purest agar gels.

Although agar has been used extensively in microbiology for a very long time, the development of food applications for this group of polysaccharides has been slow. They are beginning to be used both as gelling agents in milk-containing desserts and as stabilisers in a wide range of products. Concerns over their safety as food additives based on suggestions that carrageenans could cause intestinal irritation have not been sustained, based largely on the apparent freedom from this problem in Japan, where seaweeds form an important part of the diet. There are studies suggesting that carrageenans degraded by high temperatures in acid conditions are potential carcinogens, and these are not permitted for food use.

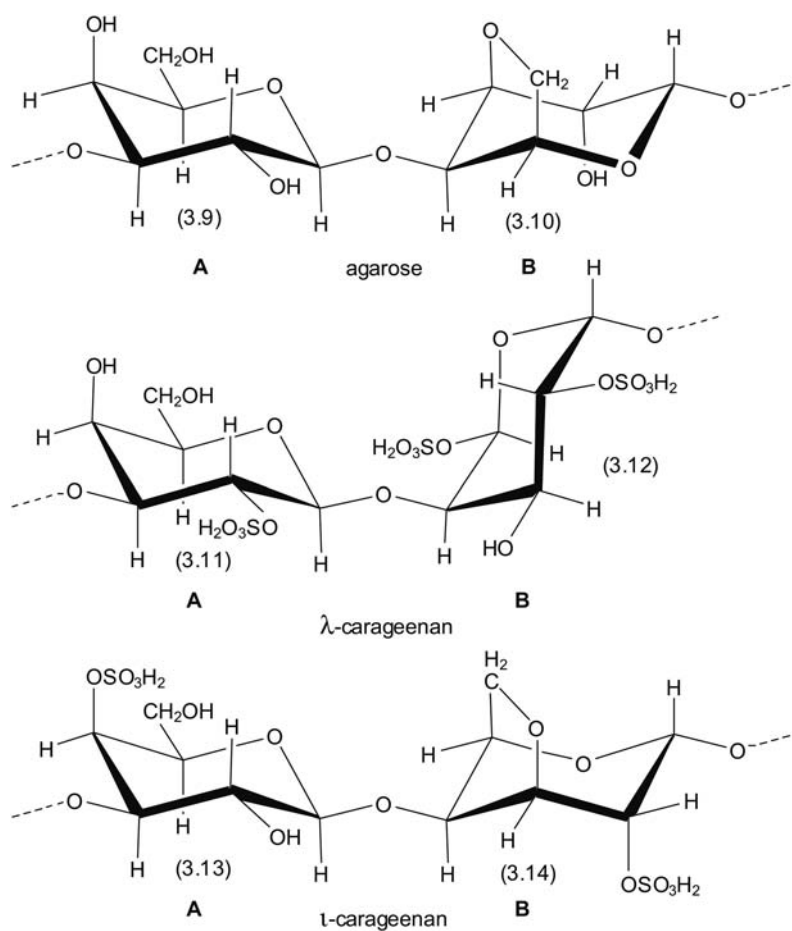


Figure 3.9 Repeating units of agar and carrageenan. The role of the anhydro bridge in maintaining the B residue in the unfavourable conformation (in terms of the axial position of the sulfate groups) is clear in these structural formulae.

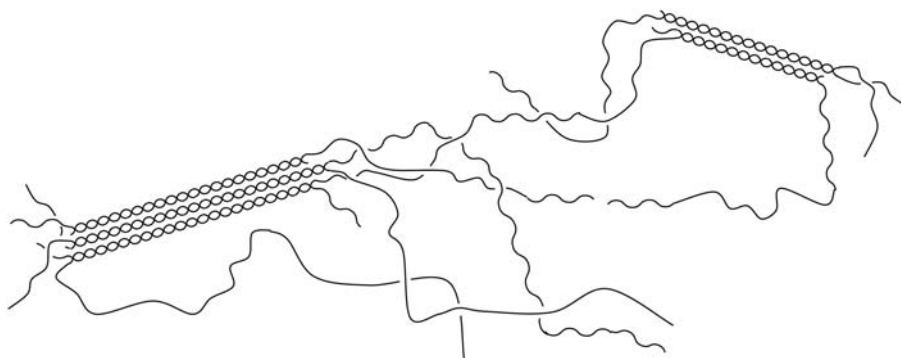


Figure 3.10 Carrageenan or agar super-junctions.

3.4 Cellulose, Hemicelluloses and Fibre

Cellulose and hemicelluloses are major components of the fraction of plant foods, particularly cereals, commonly known as dietary fibre, or “roughage”. The definition of these two terms is rather more problematic than for most food substances because we are dealing with substances without a clear boundary either in terms of their chemistry or their contribution to the function of their plant sources and to human nutrition. It would be useful to attempt to reach a proper understanding of these terms, and some of the others used in this area, before going on to describe potential members of the group in detail.

Besides cellulose and the hemicelluloses, all of the following substances could be described as fibre: lignin, gums, seaweed polysaccharides, pectins, resistant starch and inulin. What these have in common is that they are not broken down in the small intestines of mammals by digestive enzymes. However, most are at least to some degree broken down by the bacteria resident in the large intestine of omnivores such as humans. The colon bacteria go on to ferment the resultant sugars to provide energy for their growth and proliferation. The various fermentation processes give rise to a number of end-products. Besides hydrogen, carbon dioxide and methane, considerable quantities of SCFAs, notably ethanoic, propionic and butyric acids, are produced. Humans obtain little or no energy from them, so the term “unavailable carbohydrate” has often been applied, but this expression has its drawbacks. For example, stachyose is certainly an unavailable carbohydrate, but because it is a sugar it hardly qualifies as fibre. Conversely, lignin should really be included in the fibre category, although it is certainly not a carbohydrate.

The term fibre, like the older term roughage, implies insolubility, but the substances listed here range from totally insoluble cellulose to totally soluble gums. To the analyst, fibre was traditionally the insoluble residue remaining after a defatted plant foodstuff had been extracted, first with boiling dilute acid and then with boiling dilute alkali, a treatment that solubilises almost all plant substances apart from cellulose and lignin. Nowadays, the extractions are carried out using enzymes. Amylases and proteases are used to break down starch and proteins, followed by extraction with 76–80% ethanol. This dissolves the amino acids and glucose derived from the proteins and starch, respectively, as well as removing other low molecular weight substances. The resulting insoluble residue is considered to be close in composition to the unabsorbed residue that passes from the small intestine into the colon during human digestion.

Food composition tables, such as those given in *McCance and Widdowson*, usually quote data obtained by one of two methods. In the slightly earlier one, the Southgate method, the undissolved residue from the enzymic digest is simply dried and weighed. In the more recent method developed by Englyst and Cummings (usually known simply as the Englyst method), the residue is subjected to acid digestion and the resulting monosaccharides are identified and quantified (by GLC or HPLC), and related back to their parent polysaccharides. The Englyst method gives a result for what is referred to as the NSPs, and this method and terminology were officially adopted in the UK for nutritional data. Unfortunately, and with hindsight unsurprisingly, this term meant little or

nothing to consumers, who had their own ideas as to what constituted “dietary fibre”. In 1999, the UK changed its position and joined the rest of Europe in adopting the Association of Official Analytical Chemists International (AOAC International) methodology as the preferred method. This is an updated and refined version of the Southgate method, corresponding to the now internationally accepted definition of dietary fibre developed by the American Association of Cereal Chemists (AAAC) in 2000:

Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.

The notable feature of this definition is that, although it ignores the issue of laboratory analysis, it includes a statement on the physiological activity of dietary fibre in humans. In 2015, the UK Scientific Advisory Committee on Nutrition recommended in their report *Carbohydrates and Health* that the AOAC definition of fibre should be used in the UK (see Further Reading).

Table 3.5 summarises the relationships between these different approaches. In 2007, the Codex Alimentarius Commission, a committee operating under the aegis of the Food and Agriculture Organization of the United Nations and the World Health Organization, published draft guidelines for the application of this definition in food labelling. At the present time, however, unless specified otherwise, data on food labels currently refer only to NSPs. Some data on the fibre contents of a variety of foodstuffs are presented in Table 3.6.

The broad distinction between soluble and insoluble fibre extends beyond its considerable nutritional importance. Insoluble fibre is the subject of the present section; one form of soluble fibre, the pectins, has already been considered, but another, the gums, and the dietary role of soluble fibre generally, will be given further attention in the next section.

The insoluble fibre polysaccharides are subdivided into totally insoluble cellulose and the sparingly soluble hemicelluloses. Cellulose, said to be the most abundant organic chemical on Earth, is an essential component of all plant cell walls. It consists of linear molecules of at least 3000 β 1 $\rightarrow$ 4 linked glucopyranose units. This linkage leads to a flat ribbon arrangement maintained by intramolecular hydrogen bonding along the chain, as shown in Figure 3.11. Within plant tissues, cellulose molecules are aligned to form microfibrils several micrometres in length and about 10–20 nm in diameter. Although doubt still exists as to the exact arrangement of the molecules within the microfibrils, it is clear that the chains pack together in a highly ordered manner maintained by hydrogen bonding between the chains. It is the stability of this ordered structure that gives cellulose both its insolubility in virtually all reagents and also the great strength of the microfibrils. Herbivorous animals, particularly ruminants, are able to utilise cellulose by means of the specialised microorganisms which

Table 3.5 Components of the different fibre classes. The descriptions “soluble fibre” and “insoluble fibre” refer to the earlier usage of these terms rather than suggesting that the fibre is or is not actually soluble. The soluble non-cellulosic polysaccharides include pectins, gums and seaweed polysaccharides.

	Lignin	Cellulose (insoluble)	Hemicelluloses	Soluble noncellulosic polysaccharides	Resistant starch
Southgate	+	+	+	+	+
Englyst (NSP)	—	+	+	+	—
Insoluble fibre	—	+	+	—	—
Soluble fibre	—	—	—	+	—
AAAC	+	+	+	+	+

Table 3.6 Fibre components of different foods^a.

	g per 100 g				
	Cellulose	Other insoluble fibre ^b	Soluble fibre ^c	Lignin	Total
Wheat flour:					
Wholemeal	1.6	6.5	2.8	2.0	12.9
White	0.1	1.7	1.8	Trace	3.6
Oatmeal	0.7	2.3	4.8	~2.0	9.8
Broccoli	1.0	0.5	1.5	Trace	3.0
Cabbage	1.1	0.5	1.5	0.3	3.4
Peas	1.7	0.4	0.8	0.2	3.1
Potato	0.4	0.1	0.7	Trace	1.2
Apple	0.7	0.4	0.5	—	1.6
Gooseberry	0.7	0.8	1.4	—	2.9

^aData quoted by D. A. T. Southgate in *Determination of Food Carbohydrates*, Elsevier Applied Science, London, 2nd edn, 1991.

^bLargely hemicellulose.

^cIncluding pectin.

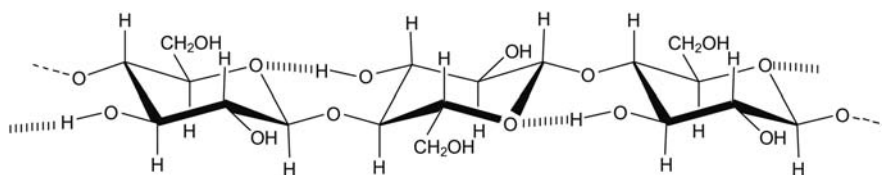


Figure 3.11 Flat ribbon arrangement of the cellulose chain. Hydrogen bonds are shown between the ring oxygen and the C-4 hydroxyl group of the neighbouring glucose residue.

occupy the highly anaerobic sections of their alimentary tracts, notably the rumen. These secrete cellulolytic enzymes that release glucose; they then obtain energy by fermenting the glucose to SCFAs, such as butyric acid, which can be absorbed and utilised by the animal. Cellulose digestion is a slow process, even in these specialised animals, as evidenced by the disproportionately large abdomens of herbivores compared with carnivores.

Purified cellulose and some of its chemical derivatives are widely used as food additives. Cellulose can be obtained from crude wood pulp, but the most usual source for food use are cotton linters, short cotton fibres left adhering to the seed after the longer fibres best suited to textile manufacture have been removed. After washing and drying, the fibres are ground to a fine powder (sometimes referred to as alpha-cellulose) of fragments about 20–100 μm in length. Microcrystalline cellulose is obtained from this by treatment with acid. The acid attacks the amorphous regions of the cellulose microfibrils, releasing small particles from the crystalline regions of the microfibrils.

Powdered and microcrystalline celluloses are insoluble powders that readily disperse in water. The extrusion properties of pasta and puffed snack foods, and the flow properties of batters and the crumb structure of baked goods, can all be improved by the inclusion of cellulose powders. Cellulose can also be synthesised by fermentation with the bacterium *Acetobacter xylinum*.

A variety of chemical derivatives of purified cellulose are manufactured industrially, several of which, listed in Table 3.7, are used as food additives. Methyl cellulose (E461) dissolves readily in cold water, when it has a thickening effect, but its solutions gel at high temperatures. This makes it a valuable additive in burgers and other formed products, such as onion rings, where it helps to maintain their shape during cooking.^{viii} The two derivatives with hydroxypropyl substituents (E463 and E464) share the unusual property of being soluble in cold water, but insoluble in hot water. Hydroxypropyl cellulose has good emulsifying properties (see Chapter 5) and finds applications in whipped dessert products. The applications of hydroxypropyl methylcellulose are similar to those of methyl cellulose in burgers. Although it does have thickening capabilities,

Table 3.7 Cellulose-based food additives. All these are currently permitted for food use in the European Union, UK, USA, Canada, Australia and New Zealand, but the authorities in different countries differ in their view of which of these substances should be regarded as additives, *i.e.* making no direct nutritive contribution, and which should be regarded as ingredients making a direct contribution to the nutritional value of a food product. Similar considerations apply to the chemically modified starches, described in the first of the Special Topics at the end of this chapter.

Derivative	E number	Structure
Microcrystalline cellulose	E460(i)	Cellulose-OH
Powdered cellulose	E460(ii)	Cellulose-OH
Methyl cellulose	E461	Cellulose-O-CH ₃
Ethyl cellulose	E462	Cellulose-O-CH ₂ -CH ₃
Hydroxypropyl cellulose	E463	Cellulose-O-CH(OH)CH ₃
Hydroxypropyl methyl cellulose	E464	Cellulose-O-CH(OH)CH ₃ and Cellulose-O-CH ₃
Methylethyl cellulose	E465	Cellulose-O-CH ₂ -CH ₃ and Cellulose-O-CH ₃
Carboxymethyl cellulose	E466	Cellulose-O-CH ₂ -COOH

^{viii}The viscous, gooey, nature of solutions of methyl cellulose has led to noteworthy applications outside food manufacture, such as the slime produced by the aliens in the film *Alien* and the ectoplasm produced by the ghosts in *Ghostbusters*.

ethyl methyl cellulose is mostly used as a foam stabiliser and emulsifier. In addition to its use as a thickener or stabiliser in fruit-based drinks, *etc.*, it can be used to inhibit excessive ice crystal growth in ice cream.

The hemicelluloses were originally thought to be low molecular weight, and therefore more soluble, precursors of cellulose. Although this idea has long been discarded, we are left with a most unfortunate name to describe this group of structural polysaccharides. They are closely associated with the cellulose of plant cell walls, from which they can be extracted with alkaline solutions (*e.g.* 15% KOH). Three types of hemicellulose are recognised: (1) the xylans; (2) the mannans and glucomannans; and (3) the galactans and arabinogalactans. Although the leaves and stems of vegetables provide a high proportion of the indigestible polysaccharides in our diet, very little is known about the hemicelluloses of such tissues. We know much more about the hemicelluloses of cereal grains, in which the xylans are the dominant group. The essential feature of these xylans is a linear or occasionally branched backbone of $\beta 1 \rightarrow 4$ linked xylopyranose residues with single L-arabinofuranose and D-glucopyranosyluronic acid residues attached to the chain, as indicated in Figure 3.12. There is every reason to suppose that the conformation of the xylan backbone resembles the flat ribbon of cellulose, but, of course, the side-chains will prevent tight packing and thereby allow some solubility.

Xylans are major constituents of the seed coats of cereal grains, which, on milling, constitute the bran. A typical wheat bran composition (on a dry basis) is: lignin^{ix} 8%, cellulose 30%, hemicellulose 25%, starch 10%, sugars 5%, protein 15%, lipids 5%, with inorganic and other substances making up the remainder. Much less hemicellulose (about 5%) is found in the endosperm, which is the basis of white flour.

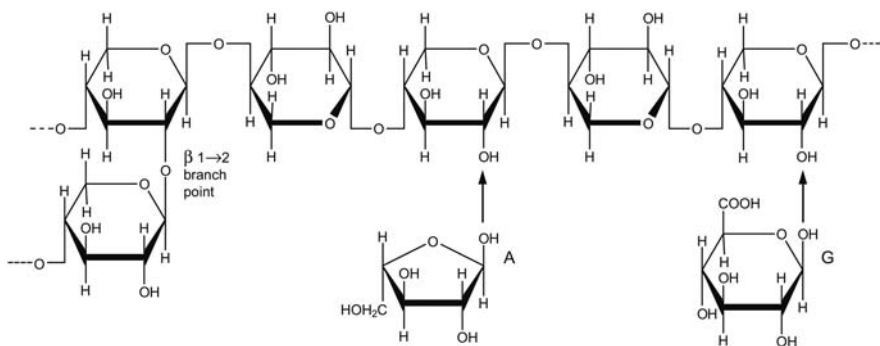
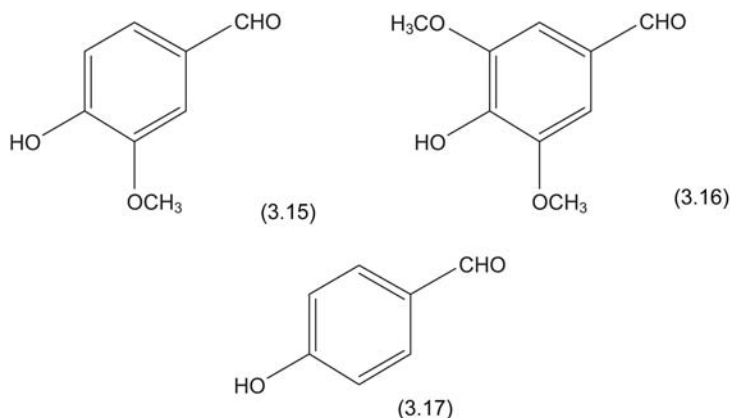


Figure 3.12 Typical structural features of a wheat xylan. The more soluble endosperm xylans are richer in L-arabinose, whereas bran xylans are less soluble, more highly branched and richer in glucuronic acid. The arrows show the points of attachment of any L-arabinose (A) or glucuronic acid (G) residues.

^{ix}Lignin is the characteristic polymer of woody plant tissues. The structure is extremely complex and variable, based on the condensation of a variety of aromatic aldehydes such as vanillin (3.15), syringaldehyde (3.16) and *p*-hydroxybenzaldehyde (3.17).

Nevertheless, various effects of the hemicelluloses on the milling and baking characteristics of particular flours have been noted.



The nutritional benefits of high-fibre diets are strongly promoted in relation to many of the diseases affecting modern urban humans and their highly refined diet. There are two distinct areas of human health where fibre is believed to exert a beneficial influence. The benefits of a high-fibre diet to the functions of the large intestine have long been recognised. Significantly, a lower incidence of a number of bowel disorders, ranging from constipation and diverticulosis to cancer, is associated with high-fibre diets. These benefits appear to be derived from the greater bulk that fibre brings to the bowel contents. In addition to improving the musculature of the bowel wall, this also reduces the time that potential carcinogens derived from our food spend in the bowel. Recent research suggests that a different mechanism may also be important. Some fibre components are broken down and fermented by the bacteria resident in the bowel to give rise to high concentrations of the SCFAs ethanoic, propionic and butyric acid. Butyric acid has been shown to induce the death of bowel tumour cells in a process known as apoptosis.

The possible role of fibre intake in the incidence of other types of cancer is a topic of ongoing, but inconclusive, interest. Interpretation of the results of epidemiological surveys is complicated by the fact that diets that are rich in fibre will almost always include large amounts of fruits and vegetables. It is obviously possible that the health benefits are due to other fruit and vegetable constituents other than the fibre polysaccharides. Separating the two factors in epidemiological studies is extremely difficult.

It is becoming clear that the relationship between dietary fibre intake and the incidence of arterial disease is complex, and that not all forms of fibre are equally effective in lowering levels of different types of blood lipids. However, what does seem to be agreed is that it is soluble rather than insoluble fibre that is the more effective, in particular the β -glucans, to be considered next.

At the boundary between hemicelluloses and gums are the β -glucans, of which the β -glucans in oats are the best known. Oat glucan consists of long chains of glucose units (at least 5000) joined by both $\beta 1 \rightarrow 3$ and $\beta 1 \rightarrow 4$ glycosidic links. As Figure 3.13 shows, the chain is dominated by cellulose-like $\beta 1 \rightarrow 4$ links, but interrupted every third

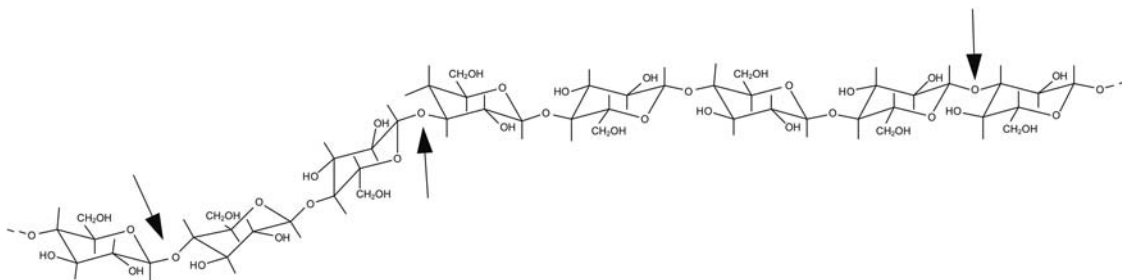
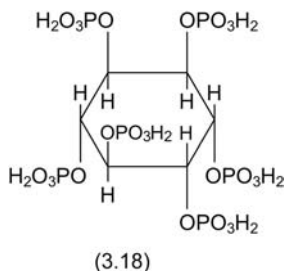


Figure 3.13 Oat β -glucan. The arrows indicate the $\beta 1 \rightarrow 3$ links, the remainder being $\beta 1 \rightarrow 4$.

or fourth glucose unit by a $\beta 1 \rightarrow 3$ linkage. The latter has the effect of disrupting the characteristic ordered cellulose-like structure and imparting solubility in water. Solutions of oat β -glucan are extremely viscous, as are rolled oats cooked to give porridge. This high viscosity, and the related property of water-binding, has led to cereal glucan being investigated as a potential food ingredient because it can stabilise oil-in-water emulsions (see Chapter 4) and, for example, reduce water loss from reduced-fat sausages (see Chapter 5). Other recent applications include its use in non-dairy lactose-free products, such as non-dairy ice cream, yoghurt and cream.

There are strong indications that oat β -glucans are beneficial in relation to heart disease by lowering the level of cholesterol in the blood. The most likely mechanism is that their viscous character impedes the reabsorption of cholesterol and bile acids from the small intestine, thereby placing a drain on the body's cholesterol economy.

One should also be cautious of automatically regarding a high dietary fibre level as beneficial. Phytic acid, inositol hexaphosphate (3.18), is an important component of bran, which is a valued source of dietary fibre. Phytic acid is the phosphate reserve for germinating seeds and its phosphate groups make it particularly effective in complexing divalent cations, implicating it in calcium- and zinc-deficiency diseases in children who consume very little milk and large quantities of bread made from wholemeal flour.



Inulin is another polysaccharide, and one which could be classified as a gum. It is the most important member of a group of plant polysaccharides known as fructans, which are polymers of fructose. Although fructans occur widely in small amounts in leaves, inulin, whose structure is shown in Figure 3.14, is notable for its starch-like role in terms of plant physiology, in the tuberous roots of a number of food plants, especially chicory (*Cichorium intybus*) and Jerusalem artichokes (*Helianthus tuberosus*). These both contain around 18 g inulin per 100 g fresh weight. The tubers of the popular

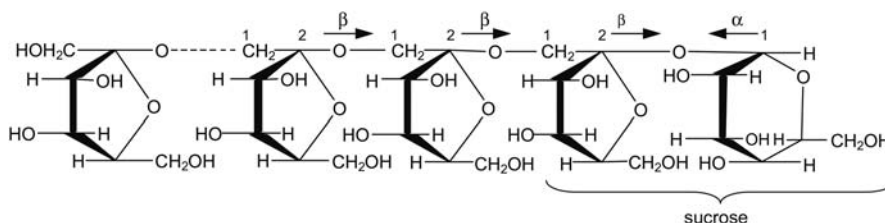


Figure 3.14 The β -(2 $\rightarrow$ 1) linked fructofuranose chain of inulin. A sucrose residue usually provides a non-reducing terminus for what would otherwise be the reducing end of the chain.

garden flower, the dahlia (*Dahlia pinnata*), may contain up to half this amount. Many other food plants, including asparagus, onions, garlic, bananas and cereal flours, such as wheat, contain just a few per cent. The dominance of wheat consumption compared with chicory and Jerusalem artichokes naturally means that wheat is the more usual source of dietary inulin in most diets. Inulin is not a large polymer like starch or cellulose, and only a small proportion of the polymer chains contain >50 fructose units. A significant proportion of these are sufficiently short to qualify as oligosaccharides rather than polysaccharides.

Until recently, inulin was little more than a phytochemical curiosity, but its consumption has now been shown to have a range of effects similar to those of other soluble fibre components. It resists digestion in the human stomach and small intestine, but is hydrolysed and fermented by the bacteria in the colon. The fermentation generates considerable amounts of SCFAs, such as ethanoic, propionic and butyric acids, and promotes the growth of bifidobacteria, important components of the colonic flora. An unfortunate side effect of this fermentation is that it is accompanied by the generation of hydrogen and carbon dioxide, which can cause bloating and discomfort, symptoms often associated with irritable bowel syndrome.

3.5 Gums

There are many substances in cereal products and vegetables that could properly be described as gums. However, the term is usually reserved for a group of fairly well-defined substances obtained as plant exudates or as “flours” made from certain non-cereal seeds. Two other gums secreted by bacteria, xanthan gum and gellan, have also become important food ingredients in recent years.

The distinctive feature of the gums is their great affinity for water and the high viscosity of their aqueous solutions. However, they do not form gels and their solutions always retain their plasticity, even at fairly high concentrations. The reason for this becomes clear when the molecular structures of representative gums are examined. The key structural features of gum tragacanth (an exudate from the tree *Astragalus gum-mifer*) and guar gum (the storage polysaccharide in the endosperm of the seeds of the leguminous shrub *Cyamopsis tetragonoloba*) are illustrated in Figure 3.15. The essential structural feature of all gums, clearly shown in both these examples, is extensive

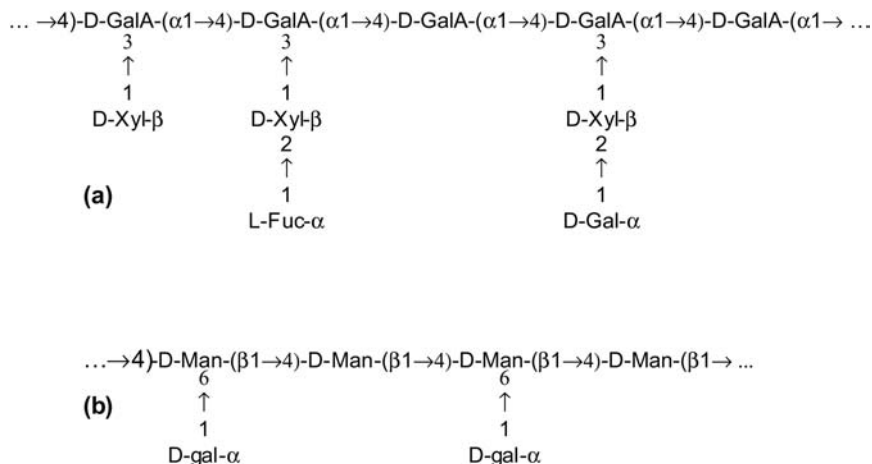
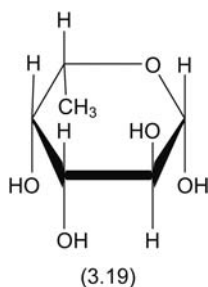


Figure 3.15 (a) Gum tragacanth. Shown here is the structure of its principal component, tragacanthic acid. The backbone of D-galacturonic acid residues has occasional insertions of L-rhamnose. Almost all the backbone residues carry one or other of the D-xylose-based side-chains shown. L-Fuc denotes L-fucose, which is 6-deoxy-L-galactose (3.19). (b) Guar gum. Alternate backbone residues carry single D-galactose residues. Another galactomannan, locust bean gum (from *Ceratonia siliqua*), differs from guar gum only in having a lower frequency of D-galactose side-chains.

branching. This leaves no lengths of ordered backbone available to form the junction zones characteristic of polysaccharide gels. The branches are nevertheless capable of trapping large amounts of water, and interactions between them ensure that even fairly dilute solutions will be viscous. If one examines the solution properties of polysaccharides in the sequence cellulose, amylose, agar, pectin, amylopectin and the gums, there is a clear parallel with the extent of branching and/or the diversity of the monosaccharide components.



In the past, the usual application for gums in food products was as thickening agents, as a substitute for starch. Nowadays, they are often used at fairly low concentrations to assist in the stabilisation of emulsions (see Chapter 4) and to smooth the texture of products such as ice cream. However, the realisation that it is the soluble components that are responsible for the beneficial effects of a high-fibre diet on the incidence of arterial disease has in recent years focused attention on a nutritional role for gums. The favoured (but not universally accepted) explanation for the effect is that blood

lipid concentrations are lowered due to a reduction in the reabsorption of bile acids (see Chapter 4) from the small intestine. The steroidal bile acids are bound to soluble fibre components, which results in them being excreted with the faeces, creating a drain on the body's cholesterol metabolism.

Another valuable effect, particularly associated with guar gum, but clearly involving other soluble fibre substances, concerns the behaviour of starch and sugars in the intestine. The presence of guar gum appears to retard the digestion and absorption of carbohydrates and thereby flattens the peak glucose flow into the bloodstream that normally follows a carbohydrate-rich meal. The effect appears to be purely physical, in that sugars and starch fragments get caught up in the three-dimensional network of fibre molecules, making them less accessible to digestive enzymes and slowing down their diffusion towards the absorptive surface of the small intestine. The application of this effect is believed to have considerable potential in the management of diabetes, particularly type 2 diabetes, the non-insulin-dependent form of the disease.

Xanthan gum is produced by the bacterium *Xanthomonas campestris*. The gum encapsulates the cell and helps the bacteria to stick to the leaves of its favoured hosts, cabbages, as well as helping to protect them from dehydration and other environmental stresses. The gum is manufactured commercially by growing the microorganism in large-scale fermentation processes. Steps are taken to ensure that the final product contains no viable cells of the bacterium. The enormous molecules of the polymer contain between 10 000 and 250 000 sugar units. As shown in Figure 3.16, the molecule has a backbone of glucose units joined by β -1,4 linkages, as in cellulose. However, every fifth glucose unit carries a trisaccharide side-chain that includes one, and sometimes two, carboxyl groups. These charged groups help to ensure the high affinity of xanthan gum for water.

The special properties of xanthan gum solutions result from the ease with which the polymer chains associate together, and also the ease with which these

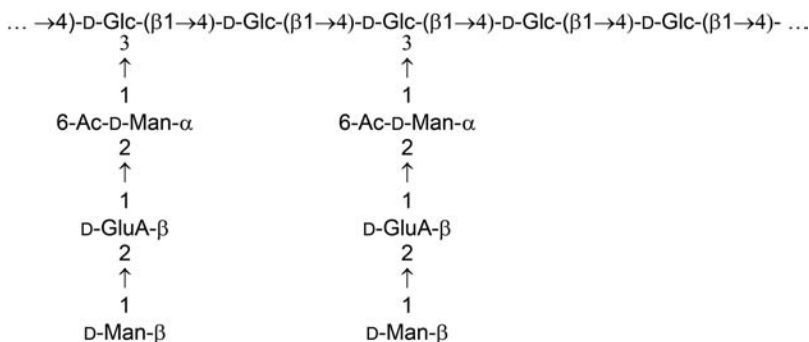


Figure 3.16 Xanthan gum. The trisaccharide side-chains are carried on approximately every other glucose unit of the backbone. The mannose residues next to the backbone have acetyl groups ($\text{CH}_3\text{CO-}$) attached to the C-6 hydroxyl. Approximately half the terminal mannose residues have a pyruvate residue bridged across their C-4 and C-6 hydroxyl groups.

associations are reversibly broken. When a xanthan gum solution (typically in food products at about 0.1–0.3%) is both cool and stationary – that is, not flowing, stirred or shaken – the polymer chains take on a rigid zigzag shape (see Figure 3.12) of sufficient length for them to come together in junction zones (see Section 3.2) and form a gel-like network. What makes these junction zones special is that the slightest hint of physical stress is sufficient to disrupt them. This stress, or shear, can result from any force imposed on the gel. This means that xanthan gum solutions are thixotropic, a property they share with non-drip paint. Such solutions are often described as being susceptible to “shear thinning”. The viscosity of a xanthan gum solution held in a bottle at rest can be sufficiently high to ensure that even quite large suspended particles do not settle out. However, as soon as the bottle is inverted, the solution instantly liquefies and readily flows out of the bottle. The most obvious applications of xanthan gum in food are in salad dressings containing suspended particles of herbs, *etc.*, and in ketchups. The concept of a tomato ketchup that flows easily from the bottle, but does not dribble off one’s chips, has a certain appeal. Xanthan gum is also very effective in stabilising emulsions and it is in this role that it is most often encountered in food products.

Gellan gum comes from the bacterium *Sphingomonas elodea*^{*} and is manufactured by similar processes to those used for xanthan gum. It was cleared for food use in Europe in 1994. Gellan gum (Figure 3.17) has an unbranched polymer chain consisting of a repeating tetrasaccharide unit (–glucose–glucuronic acid–glucose–rhamnose–), with a total length of around half a million monosaccharide units. Individual

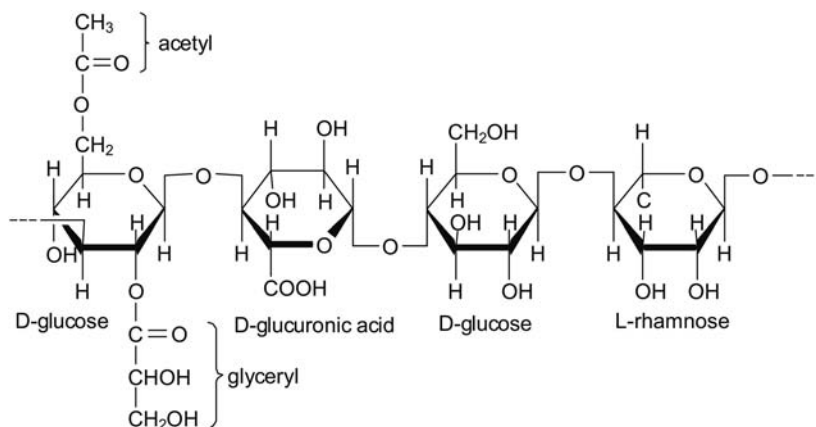


Figure 3.17 The repeating unit of gellan gum. In the native form, shown here, one of the glucose units in each repeating tetrasaccharide is esterified with glyceric acid and approximately half of these same glucose units are also esterified with ethanoic acid. As explained in the text, the gels formed by this “high acyl” form of gellan gum have different properties from those of the “low acyl” form, which have had the glyceryl and acetyl groups removed by treatment with alkali.

^{*}This species used to be classified as a pseudomonad and is still widely referred to as *Pseudomonas elodea*.

polymer chains form double helices around each other. In the native form, referred to as “high acyl”, the first of the glucose units in each repeating tetrasaccharide element is esterified with glyceric acid and approximately half of these glucose units are also esterified with ethanoic acid. Treatment with alkali removes the glyceryl and acetyl groups to give the “low acyl” form. The characteristics of the two types differ in ways that can be exploited in food product formulations, although both will form gels at fairly low concentrations (<0.2%).

High acyl gellan gum solutions set quickly once the temperature falls below 70 °C to give a gel that is soft and elastic. By contrast, low acyl gellan gels require the presence of cations, ideally divalent ions such as Ca^{2+} or Mg^{2+} , and do not set until the temperature decreases to the 30–50 °C range. Their gels are harder and more brittle or, by analogy with descriptions of pastry, short. The divalent cations associate with the carboxyl groups of the glucuronic acid residues, cross-linking the double helical sections to form the junction zones of the gel. Monovalent cations such as Na^+ give a similar result by simply neutralising the acid carboxyl groups and allowing the chains to come together. The levels of sodium and calcium normally found in food systems are usually sufficient to promote gelation.

The low concentration of high acyl gellan gum required for it to form a rigid gel is the basis of one of its most appealing characteristics. Flavours carried by the gel are released in the mouth much more quickly than from other types of gel. Because the gel is also rather brittle, it tends to crumble in the mouth, giving the impression of melting. At much lower concentrations, around 0.03%, gellan gum does not form solid gels, but instead produces thixotropic fluids, which are able to hold fruit particles in suspension. Both these effects were first exploited in some of the creations of one of Europe’s leading chefs, Heston Blumenthal. The various polysaccharide gums (apart from cellulose and starch derivatives) permitted for use as food additives in Europe and elsewhere are listed in Table 3.8. Some of these not otherwise described in the preceding text are:

- Processed eucheuma seaweed is a low-cost alternative to purified carrageenans prepared from *Eucheimia* spp. containing various carrageenan types.
- Locust bean gum (from the seeds of the Mediterranean carob tree, *Ceratonia siliqua*) and tara gum (from the seeds of the South American tara tree, *Cesalpinia spinosa*) are galactomannans structurally related to guar gum.
- Karaya gum, obtained commercially from the Indian tree *Sterculia urens*, is a highly branched polysaccharide dominated by galacturonic acid.
- Gum arabic is a variable and complex mixture of polysaccharides and glycoproteins obtained commercially from *Acacia senegal* and *Acacia seyal* trees found in sub-Saharan Africa.
- Konjac is a glucomannan obtained from the roots of a perennial plant *Amorphophallus konjac*, grown in the Far East.
- Curdlan is a linear β -1,3-glucan that gives elastic gels when heated in water. Like xanthan and gellan gums, it is synthesised by several species of bacteria, notably *Agrobacterium biohar*.

Table 3.8 The gums and non-starch gelling agents permitted for use in the UK, European Union and elsewhere (Y, *i.e.* “Yes”, shows that a substance is permitted). Substances permitted elsewhere, but not in the European Union, are shown with their INS numbers. In most countries, there are conditions of use (*e.g.* specific limits to the range of food products and the amounts that may be used).

Common name	EU or INS No. ^a	EU and UK	USA	Canada	Australia and New Zealand	Japan	South America ^b
Alginic acid	E400	Y	Y	Y	Y		Y
Sodium alginate	E401	Y	Y	Y	Y	Y	Y
Potassium alginate	E402	Y	Y	Y	Y	Y	Y
Ammonium alginate	E403	Y	Y	Y	Y	Y	Y
Calcium alginate	E404	Y	Y	Y	Y	Y	Y
Propylene glycol alginate	E405	Y	Y	Y	Y		Y
Agar	E406	Y	Y	Y	Y		Y
Carrageenan	E407	Y	Y	Y	Y		Y
Processed eucheuma seaweed	E407a	Y	Y		Y		Y
Larch gum (arabinogalactan)				Y	Y		
Locust (carob) bean gum	E410	Y	Y	Y	Y	Y	Y
Guar gum	E412	Y	Y	Y	Y	Y	Y
Gum tragacanth	E413	Y	Y	Y	Y	Y	Y
Acacia gum (gum arabic)	E414	Y	Y	Y	Y	Y	Y
Xanthan gum	E415	Y	Y	Y	Y	Y	Y
Karaya gum	E416	Y	Y	Y	Y	Y	Y
Tara gum	E417	Y			Y	Y	Y
Gellan gum	E418	Y	Y	Y	Y	Y	Y
Curdlan	424		Y				Y
Konjac gum	E425	Y					
Soybean hemicellulose	E426	Y					
Cassia gum	E427					Y	
Pectin	E440	Y	Y	Y	Y	Y	Y

^aThe International Numbering System (INS) numbers for these are given where no E number exists. These are designated by The Codex Alimentarius, a subsidiary organisation of the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO). Possession of an INS number does not automatically imply approval as an additive. INS numbers frequently correspond to E numbers.

^bThe countries to which these headings apply are listed in Appendix II.

3.6 Special Topics

3.6.1 Chemically Modified Starches

The use of waxy starches and pre-gelatinisation widens the range of behavioural characteristics available to food processors, but the most significant changes can be obtained by chemical modification of the starch molecule. Three types of modification are used.

- *Depolymerisation.* Starch granules are treated with hydrochloric acid, followed by neutralisation. Only a very small proportion of the glycosidic links are broken, but the fragmentation of the amylopectin molecules is still sufficient for the granule structure to be lost more easily on heating, and for gelatinisation to be much faster. Known as “thin-boiling” starches, they give stronger, clearer gels and are used in many types of jelly confectionery. Maltodextrins are produced by much more extensive starch hydrolysis, with DP values (degree of polymerisation, the average number of glucose units per molecule) within the range 5–100. The degree of hydrolysis is often expressed as the dextrose equivalent (DE, given by the expression):

$$\text{DE} = 100 \times (\text{Reducing sugar, expressed as glucose}) / (\text{Total carbohydrate})$$

where DE represents the percentage hydrolysis of the glycosidic linkages present. Pure glucose has a DE of 100 and pure maltose 50. Maltodextrin preparations with DE values around 5 (*i.e.* an average of around 20 glucose units per molecule) can be included in a wide range of products, including low-fat margarine, salad dressings and mayonnaise, where they provide some of the organoleptic (mouth feel) properties of fat.

- *Derivatisation.* Esterification of a small proportion (usually <1%) of glucose units with organic acids or phosphates gives “stabilised” starches. Depending on the particular groups attached, it is possible to achieve gels with excellent freeze stability and resistance to retrogradation (acetyl or hydroxypropyl groups), high paste viscosity (phosphates) or emulsifying capabilities (1-octenylsuccinates).
- *Cross-linking.* Fewer than one cross-linkage per 1000 glucose units is sufficient to produce significant changes in starch properties. Cross-links between chains tend to strengthen the granule structure. This gives pastes that are much more resilient in the face of low pH values, extended cooking or physical shear forces than those made from unmodified starch. Not surprisingly, cross-linked starches require much more cooking time to achieve gelatinisation. They are particularly valuable in canned products. Their slow gelatinisation allows the contents of the can to remain liquid during heating, so that heat transfer is not impaired by solidification, but the final product has a very firm texture.

Commercial modified starch products often use both stabilisation and cross-linking in the same product, often applied to a waxy starch rather than the normal type. Some of the chemical reactions involved in derivatisation are shown in Figure 3.18. The extent of these reactions is restricted by food legislation, but is always far too low to have any significant effect on the digestibility and therefore nutritive value. The diversity of applications of the different modified starches results in some being classified in current European legislation as additives, whereas others are regarded as ingredients. The key distinction is that those considered as additives are used in too small a proportion to make a significant contribution to a food product’s nutritional properties. The diversity of international opinions on the status of modified starches is similar to the status of modified celluloses mentioned earlier in this chapter. All the modified starches permitted in Europe are listed in Table 3.9.

3.6.2 Syrups from Starch

The conversion of starch into glucose and other sugars is a major industry. The old-fashioned process based on acid hydrolysis has been almost totally superseded by processes based on enzymic catalysed hydrolysis. The latter processes take advantage of the great specificity of enzymes for certain types of bond in particular substrates. Although enzymes, being proteins, are usually thought of as being inactivated at high temperatures, many enzymes used industrially are now obtained from thermophilic bacteria, which have thermostable enzymes.

In the usual two-phase process, starch, mostly from maize, and cold water are mixed to form a slurry at up to 40% solids content. A thermostable α -amylase from a thermophilic strain of bacillus (*Bacillus licheniformis*, *B. stearothermophilus* or *B. amyloliquefaciens*) is incorporated into the slurry, which is then brought rapidly up to a temperature of 105–110 °C and held within this range for a few minutes. The starch granules gelatinise and the enzyme is able to gain access to the $\alpha 1 \rightarrow 4$ glycosidic links of the amylose and amylopectin. As a result, both polymers are fragmented to oligosaccharides (sometimes referred to as dextrans) averaging around ten glucose units in size. Many of the fragments will include $\alpha 1 \rightarrow 6$ branches derived from the branched structure of amylopectin, but these bonds are not attacked by the α -amylase. Unlike the impossibly stiff starch paste that would have resulted from simply gelatinising a 40% starch slurry, the solution of oligosaccharides is now manageably fluid.

Having gone through the initial liquefaction phase, the oligosaccharides are subjected to further enzymic hydrolysis in the “saccharification” phase of the process. The temperature is brought down to around 55–60 °C and preparations of the amyloglucosidase (otherwise known as glucoamylase) from the fungus, *Aspergillus niger* are added. Amyloglucosidase is remarkable for its ability to catalyse the hydrolysis of both the $\alpha 1 \rightarrow 4$ and $\alpha 1 \rightarrow 6$ glycosidic links and, given time, would break down the oligosaccharides almost entirely to glucose. However, the amyloglucosidase is often supplemented at this stage with the enzyme pullulanase from *Bacillus acidopullulyticus*, which is specific for $\alpha 1 \rightarrow 6$ links. Although the saccharification stage may take as long as 90 hours, the results are spectacular. After suitable clean-up procedures, yields of pure glucose of 95% of the theoretical result are obtained.

This need not be the end of the story. The high-purity glucose syrup may be passed through a reactor in which the enzyme glucose isomerase has been immobilised on solid carrier particles. This enzyme is obtained from a number of different fungi in which its primary role is the interconversion of xylose and xylulose, *i.e.* interconversion of an aldose sugar and its ketose equivalent. This reaction is completely analogous to the interconversion of glucose and fructose and enables syrups with fructose to glucose ratios of 55:45 to be produced. Known as “high-fructose corn syrups” these have a sweetness comparable with invert sugar and are becoming increasingly popular, especially with soft drink manufacturers. In theory, their use in soft drinks should enable their manufacturers to formulate their products with reduced total sugar contents, but there appears to be a growing concern among nutritionists that our children are merely acquiring a taste for ever-increasing degrees of sweetness in their diets.

3.6.3 Glycaemic Index

Although the total dietary intake of carbohydrates is obviously a major factor in the development of obesity, and associated conditions such as diabetes and cardiovascular disease, it has become clear in recent years that the *rate* at which glucose enters the bloodstream following carbohydrate consumption is also very important. Ordinarily, the body responds to the increase in blood glucose concentration after a meal by releasing the hormone insulin from the pancreas into the bloodstream. This activates the transfer of glucose from the blood into the tissues, notably the muscles and liver, where it can be stored as the polymer glycogen, and adipose tissue, where it is stored as fat.

Persistent consumption of foodstuffs that generate glucose in large quantities places the system under strain. One manifestation of this can be the onset of type 2 diabetes, when the tissues become less responsive to insulin. The concept of the glycaemic index (GI) was introduced by Jenkins in 1994 to provide a quantitative measure of the rate of glucose influx into the bloodstream. The GI of a particular foodstuff is normally measured using healthy adult volunteers. Having fasted overnight, a food portion containing 50 g of carbohydrate or a 50 g portion of glucose (as a standard) is consumed and the blood glucose concentration is measured at regular intervals over two hours. Figure 3.19 shows a typical result.

GI data for a selection of foodstuffs are given in Table 3.10. Also included in the table are the corresponding figures for the glycaemic load (GL). Although GI data provide a

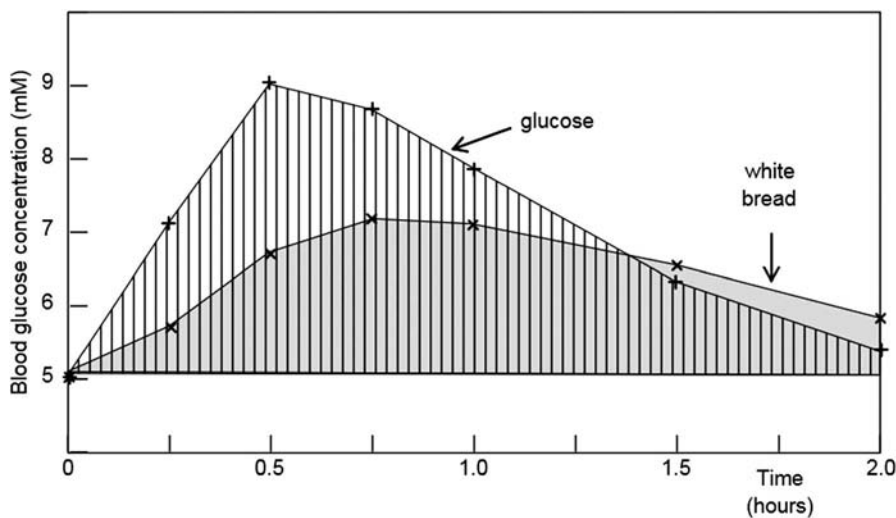


Figure 3.19 Determination of the glycaemic index (GI). The shaded areas show the increase in blood glucose concentration above the subject's baseline fasting level at the start of measurements over a period of two hours. The area generated by a portion of a test foodstuff containing 50 g of carbohydrate is compared with that given by a standard 50 g portion of glucose. Areas are calculated from the sum of the areas of the series of trapeziums formed by the data points and the subject's baseline level. No attempt is made to smooth the curves before calculating the area. The inevitable natural variation between different test subjects means that large numbers of test curves have to be obtained and the results averaged, requiring the services of large numbers of volunteer subjects.

Table 3.10 Glycaemic index (GI) and glycaemic load (GL) for a range of foodstuffs based on data assembled by Kaye Foster-Powell and colleagues (K. Foster-Powell, S. H. A. Holt and J. C. Brand-Miller, *Am. J. Clin. Nutr.*, 2002, **76**, 5). The values recorded in the table should not be treated as precise. A total of 135 different studies involving over 1300 human volunteers contributed to the mean figures given. Different studies on the same foodstuff, each involving tests with considerable numbers of participants, typically gave results varying by up to 5–10% from the mean values shown.

Food	GI	Portion size (g)	GL	Food	GI	Portion size (g)	GL
Cornflakes	81	30	21	Honey	55	25	10
Potato, mashed	77	150	15	Potato crisps	54	50	11
Wholemeal bread	71	30	9	Banana	52	120	12
White bread	70	30	10	Green peas	48	80	3
Sucrose	68	10	7	Orange juice	50	250 ^a	13
Rice, white	64	150	23	Baked beans ^b	48	150	7
Rye crispbread	64	25	11	Spaghetti	44	180	21
Ice-cream	61	50	8	Milk chocolate	43	50	12
Cola	58	250 ^a	15	Apples, raw	38	120	6
Porridge ^c	58	250	13	Chickpeas	28	150	8
Potatoes, boiled	57	150	12	Lentils, red	26	150	5
new							
Rice, brown	55	150	18	Peanuts raw	14	50	1

^aIn mL rather than g.

^bCanned in tomato sauce.

^cMade from rolled oats.

useful characterisation of a foodstuff, they do not in themselves provide any information on the actual glycaemic effect of consuming the foodstuff in a realistic situation outside the laboratory. The concept of the GL was introduced in 1997 to enable the probable elevation of blood glucose from a typical serving of a food to be estimated. The GL is obtained by multiplying the GI by the quantity of available carbohydrate in a standard serving or portion. The consumption of a diet with a relatively high GL figure has come to be associated with a higher probability of type 2 diabetes (non-insulin dependent diabetes, NIDD) and coronary heart disease. Foods are often labelled as high, medium or low GI. To date, the appropriate authorities in different countries around the world have been reluctant define the precise numerical GI values that these terms correspond to (South Africa is one exception). However, the terms high, medium or low GI most often refer to values of 70 and above, 56–69 and below 55, respectively.

Close examination of these data reveals a number of unexpected results, notably for honey, sucrose, chocolate and ice cream, which seem to be less glycaemic than might have been expected. In these cases, a major factor is that the fructose component of the sucrose makes no direct or immediate contribution to blood glucose concentrations. Of course, the fructose contained in these foods cannot be exonerated from its contribution to the calorific content of the diet. It is assumed that the accompanying fat contained in ice cream and chocolate physically slows the rate of sugar absorption from the intestine. In more typical complete meals, in which the glycaemic carbohydrate components are less intimately associated with fat than in ice cream or chocolate, it has

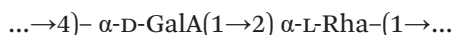
proved possible to predict the glycaemic effect from the GI values and the proportions of individual components.

3.6.4 Further Details of Pectin Structure

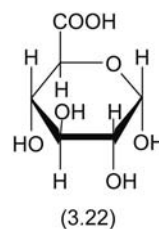
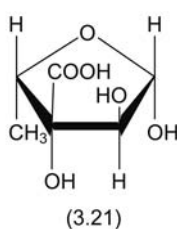
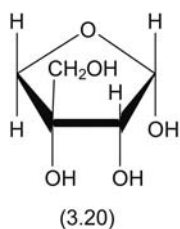
Earlier in this chapter, the essential features of pectin molecules were considered, particularly in relation to pectin's contribution to the structure and texture of foods. Here we give a more detailed account of current views of the molecular structure of pectin.

The currently accepted models have several features in common. The term pectin covers a number, usually three, of different broad types of polysaccharide structures, whose details can vary greatly between plant species and between different tissues within a single species. The difficulty of extracting intact (or supposedly intact) pectin molecules from plant tissues means that there is no absolute certainty about whether the different structures are always combined in the living plant into a single type of molecule, or whether they occur naturally as distinct molecules. At the present time, the first of these alternatives seems to be preferred. The three major types are as follows.

- *Homogalacturonan (HG)*. This is the classical pectin structure, already shown in Figure 3.4, consisting of a linear backbone of α -1,4 linked galacturonic acid residues. HG is the site of the methylation crucial to the gel-forming capability of pectin. A feature of apple pectin, which distinguishes it from citrus pectins, is the presence of regions of the homogalacturonan backbone carrying numbers of D-xylose (3.3) residues, mostly as single residues linked α -1,3 to the galacturonate residues. Xylose-rich regions of the chain will still be classified as "hairy".
- *Rhamnogalacturonan I (RGI)*. This has a linear backbone based on repeating disaccharide units consisting of galacturonic acid and L-rhamnose:



- Various different side-chains are attached to the rhamnose residues, probably in the 4-position. These can include galactans (*i.e.* chains of D-galactose (3.4) residues, only rarely branched, linked α -1,4) and arabinans (3.6), highly branched chains of L-arabinose residues (3.2).
- *Rhamnogalacturonan II (RGII)*. To say the least, this is a most unhelpful name, as RGII rarely contains more than a trace of rhamnose. RGII is based on a homogalacturonan backbone carrying relatively small, but very elaborately branched, side-chains. In addition to galactose and xylose, these can include several otherwise rare sugars, such as D-apiose (3.20), L-fucose, 6-deoxy-L-galactose (3.19), L-aceric acid (3.21) and D-glucuronic acid (3.22).



The assumption that these different types of pectin chain are components of single large polymers still leaves open the issue of how they are connected together. The most generally accepted model, based initially on Pilnik's studies of apple pectin, is shown in Figure 3.20A. This has a central linear backbone mainly consisting of homogalacturonan, with smooth and hairy regions. Hairy regions could be characterised by

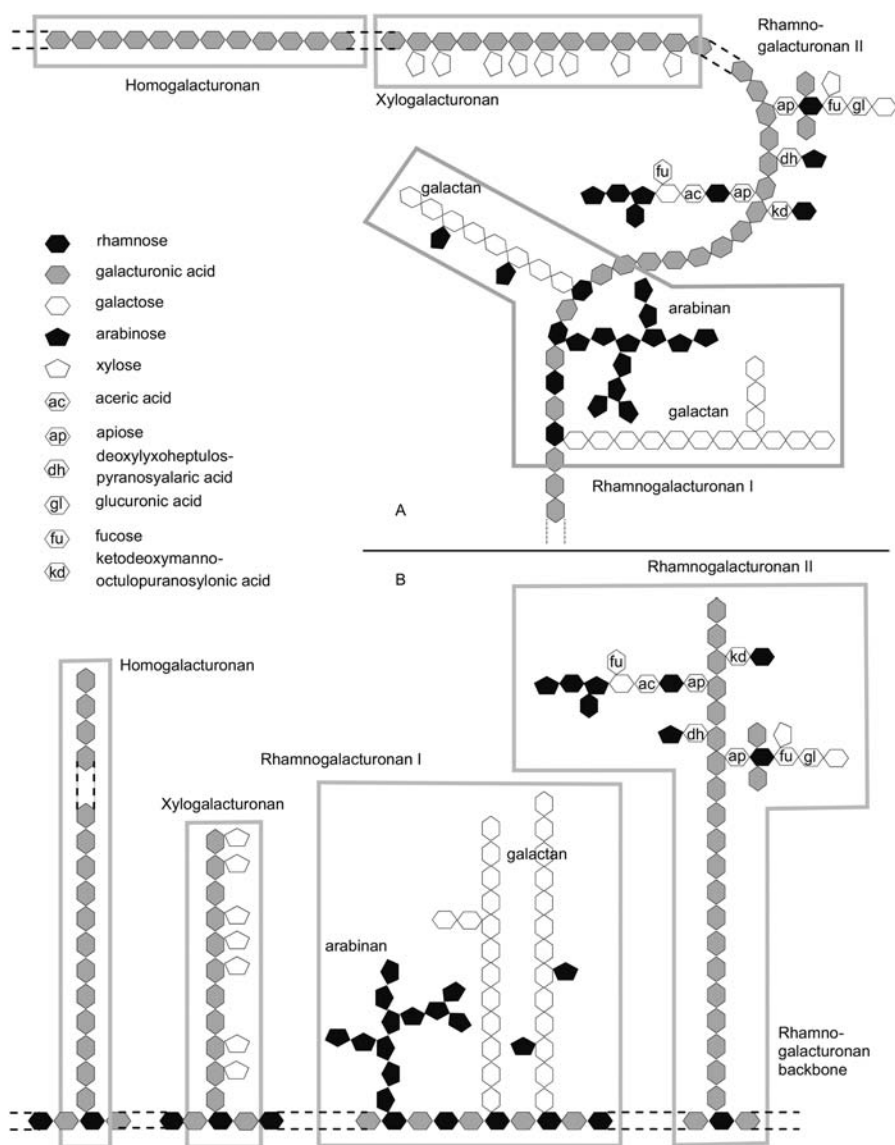
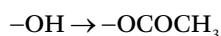


Figure 3.20 Pectin structures, as discussed in the text. (A), based on the work of Pilnik and others since the 1980s, has the extended homogalacturonan backbone carrying RGI and RGII side-chains. The bends shown in the backbone of structure (A) are solely for artistic convenience. (B), based on Vincken's 2003 model, has a backbone of rhamnogalacturonan carrying numerous homogalacturonan chains (only one is shown here), together with various other branched chains, including those associated with RGI and RGII.

the presence of xylose substituents or by concentrations of branches leading to the formation of an RGII region. The galacturonan backbone is interrupted at intervals by a region of alternating rhamnose and galacturonate residues, forming an RGI region. An alternative to this arrangement, shown in Figure 3.20B, was proposed by Vincken in 2003 (see G. T. Willats *et al.*, in Recent Reviews at the end of this chapter). In this, RGI forms the backbone of the pectin molecule, carrying a variety of different types of side-chain, including simple homogalacturonan and various RGII chains. The location of regions of homogalacturonan extensively substituted with the xylose residues (xylogalacturonan) particularly associated with apple pectin remains uncertain. As shown in Figure 3.20B, these chains may be separately joined to the backbone, or they may simply represent regions of the homogalacturonan chain as shown in Figure 3.20A.

No attempt has been made in Figure 3.20 to illustrate the methylation of the galacturonate residues. A significant degree of acetylation, *i.e.* conversion of either the 2- or 3-hydroxyl group of galacturonic acid residues to an acetyl group:



is found in some pectins, notably that from sugar beet pulp. The residue after the sugar has been extracted from sugar beet is becoming another important commercial source of pectin.

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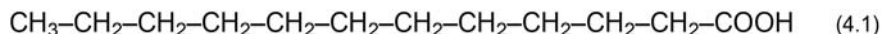
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4 Lipids

The term lipid refers to a group of substances that is even less clearly defined than the carbohydrates. Generally speaking, the term denotes a heterogeneous group of substances associated with living systems, such as the paraffins (hexane, *etc.*), diethyl ether and chloroform, that have the common properties of insolubility in water and solubility in non-polar solvents. Included in this group are the oils and fatsⁱ of our diet, together with the phospholipids associated with the membranes of cells and other structures. Although food materials such as oils and fats clearly consist of very little other than lipids, their presence in a variety of other foods is less obvious, as indicated by the data in Table 4.1. The predominant lipids in all these foods are esters of long-chain fatty acids, but there are many other lipids that lack this structural feature. These include the steroids and terpenes, but, with the exception of cholesterol and its long-chain fatty acid esters, in cases where these substances do have significance in food, they will be found under the headings of vitamins, pigments or flavour compounds.

4.1 Fatty Acids: Structure and Distribution

Monocarboxylic fatty acids (*i.e.* fatty acids with only one carboxyl group) are structural components common to most of the lipids of interest to food chemists and, because many of the properties of food lipids can be accounted for in terms of their component fatty acids, they will be considered here in some detail. Almost without exception, the fatty acids which occur in foodstuffs contain an even number of carbon atoms in an unbranched chain, *e.g.* lauric (or dodecanoic) acid (4.1).



The fact that the hydrocarbon chains of most fatty acids have even, rather than odd, numbers of carbon atoms is the inevitable consequence of the biochemical mechanism of their synthesis. In all types of organisms, this process starts with a two-carbon unit

ⁱThere is no formal distinction between the dietary *oils* and the dietary *fats*. However, the former tend to be liquid at room temperature and the latter solid.

Table 4.1 The total fat content of a variety of foods consumed in the UK. This data should be regarded as typical values for particular foods rather than absolute values to which all samples of the food comply. The definition of fat used here covers all lipids, although polar lipids are only quantitatively significant in the case of egg yolk and products containing egg. These data are derived from McCance and Widdowson's 'Composition of Foods Integrated Dataset' on the nutrient content of the UK food supply, <https://www.gov.uk/government/publications/composition-of-foods-integrated-dataset-cofid> (last accessed in 2020). This site provides access to a database of the nutrient composition of nearly 3000 different foods, including both raw materials and food products. It can also be expected to provide indirect access to updated data published in the future.

Food	Total fat (%)	Food	Total fat (%)
Wholemeal flour	2.0	Lard	99.0
White bread	2.1	Vegetable oils	99.9
Shortbread	29.0	Bacon, streaky, grilled	26.9
Cake (sponge)	26.4	Pork sausage, grilled	24.8
Flaky pastry	37.4	Beef lean, sirloin, roasted	6.5
Skimmed milk (cow's)	0.3	Beef burger, raw	15.2
Whole milk (cow's)	3.6	Lamb chop, grilled	26.9
Human milk	4.1	Chicken, roasted, breast	3.6
Soya milk	1.6	Turkey breast, roast	2.0
Clotted cream	63.5	Cod, raw	0.6
Cheese (Brie)	29.1	Cod, battered and fried	14.7
Cheese (Cheddar)	34.9	Mackerel, smoked	24.1
Dairy ice cream	8.2	Salmon, smoked	8.8
Egg yolk (chicken)	31.3	Taramasalata	52.9
Egg white	Trace	Brazil nuts	68.2
Scotch egg	16.0	Peanuts, dry roasted	49.8
Butter	82.2	Plain chocolate	28.0
Low-fat spread	38.9	Milk chocolate	31.1

(an acetyl group, $\text{CH}_3\text{CO}-$), to which further acetyl groups are added in turn, with conversion to $-\text{CH}_2\text{CH}_2-$ units, to lengthen the chain. In addition to saturated fatty acids, of which lauric acid is an example, unsaturated fatty acids with one, two or sometimes as many as six double bonds are common. The double bonds in unsaturated fatty acids have two possible conformations, *cis* or *trans*, but those occurring naturally are almost invariably *cis*. As shown in Figure 4.1, the conformation of fatty acid double bonds has significant implications for their physical properties, as will be explained later.

When a fatty acid has two or more double bonds, referred to as a polyunsaturated fatty acid (PUFA), the bonds are normally positioned with two single bonds between them:



an arrangement known as methylene interrupted conjugation. The term "conjugated" is applied when the carbon-carbon bonds are alternately single and double along the chain:



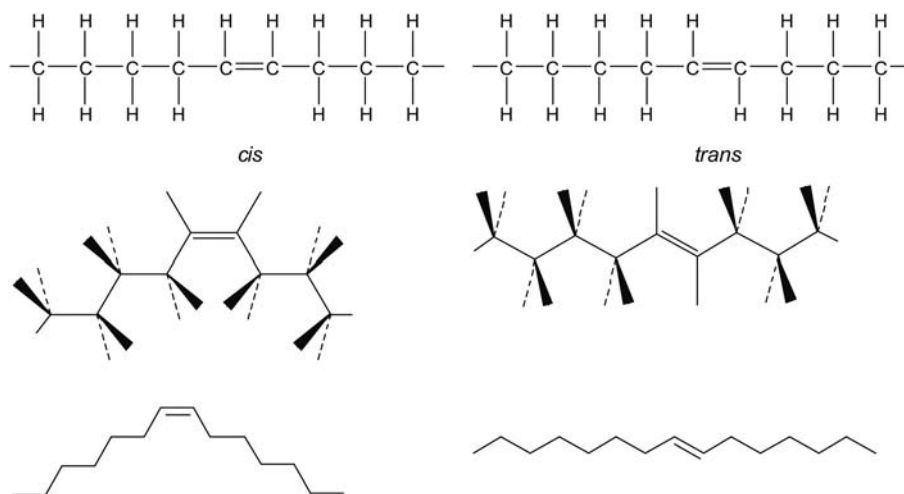


Figure 4.1 *Cis* and *trans* double bonds and their effects on the conformation of the fatty acid chain. *Cis* and *trans* are Latin for “on this side” and “on the opposite side”, respectively. Unlike single bonds, double bonds cannot rotate, so the four atoms involved in the double bond, two carbon atoms and two hydrogen atoms, remain fixed in the same plane.

Despite their comparative rarity, conjugated fatty acids are not without food significance and are considered in detail in Section 4.1.1.

It is worthwhile mastering both the trivial, or common, names and the systematic names of the principal fatty acids.ⁱⁱ

The structure of a fatty acid can be indicated in a convenient shorthand form giving the total number of carbon atoms followed by a colon and the number of double bonds; the positions of the double bonds are shown after the symbol Δ . For example, linoleic acid, shown in Figure 4.2, would be written simply as 18:2 Δ ^{9,12} or sometimes 18:2 Δ ^{9,12}. In recent years, interest has grown in the nutritional significance of PUFAs. These fatty acids are often classified by the relationship of their double bonds to the terminal methyl group, often referred to as the ω -carbon atom. Thus, in the case of linoleic acid, the double bond furthest from the carboxyl group is between the sixth and seventh carbon atoms counting from the ω -carbon atom, so that linoleic acid is classified as an *omega-6* fatty acid, whereas α -linolenic acid (18:3 Δ ^{9,12,15}) is an *omega-3* fatty acid. Table 4.2 gives the names and structures of a number of the fatty acids commonly encountered in food lipids.

The oils and fats are obviously the lipids of most interest to the food chemist. These consist largely of mixtures of triglycerides, *i.e.* esters of the trihydricⁱⁱⁱ alcohol glycerol

ⁱⁱReaders may find pronunciation of the elaborate systematic names of some of the PUFAs difficult, an issue successfully addressed, using the example ‘ante-iso-*trans*-oleic acid’, in the epic poem “Hiawatha’s Lipid” by Hugh Sinclair (*Am. J. Clin. Nutr.* 1958, 6, 584). Readers with an interest in analytical methodology may also enjoy these lines from this poem: ... Then he used the latest method,/Gas-chromatographic method/Introduced by James and Martin,/Showing peaks upon the paper/Like the Rockies at the sunset,/Like the mole-hills in the prairies ...

ⁱⁱⁱTrihydric, having three hydroxyl groups.

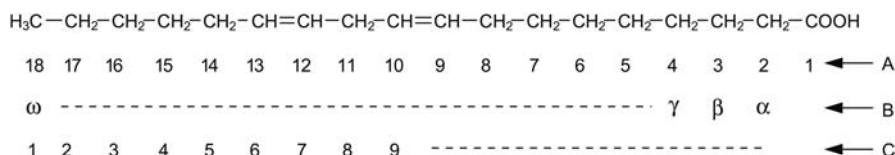


Figure 4.2 Numbering of the atoms in fatty acids, illustrated by linoleic acid. The most common system (A), and the one adopted to define the positions of double bonds in Table 4.2, simply numbers the carbon atoms starting from the carboxyl group (1) to the terminal methyl group (18) in the example shown. Alternatively, Greek letters may be used to label successive CH_2 groups, starting at the one adjoining the carboxyl group, as in (B). This system is rarely used beyond the γ carbon, except that the terminal methyl can be designated ω (omega) to indicate that it is the final one regardless of the length of the chain. In some considerations of fatty acid metabolism, notably essential fatty acids, the carbon atoms are counted from the methyl terminus (C), see Figure 4.3.

Table 4.2 The fatty acids commonly found in foodstuffs. The meaning of the term *cis* and its opposite, *trans*, in the names of unsaturated fatty acids is explained in Figure 4.1. In most cases, the common names are derived from the name (mostly Latin, *L*, but also Greek, *Gk*, or otherwise English) of a characteristic source.

Systematic name	Common name	Structure
<i>Saturated acids</i>		
<i>n</i> -Butanoic	Butyric (<i>L. butyrum</i> = butter)	4:0
<i>n</i> -Hexanoic	Caproic (<i>L. caper</i> = goat)	6:0
<i>n</i> -Octanoic	Caprylic (<i>L. caper</i> = goat)	8:0
<i>n</i> -Decanoic	Capric (<i>L. caper</i> = goat)	10:0
<i>n</i> -Dodecanoic	Lauric (<i>L. laurea</i> = laurel)	12:0
<i>n</i> -Tetradecanoic	Myristic (<i>L. myristica</i> = nutmeg)	14:0
<i>n</i> -Hexadecanoic	Palmitic (palm oil)	16:0
<i>n</i> -Octadecanoic	Stearic (<i>Gk. stear</i> = tallow)	18:0
<i>n</i> -Eicosanoic	Arachidic (<i>L. arachis</i> = peanut)	20:0
<i>n</i> -Docosanoic	Behenic (ben oil, <i>Moringa oliefera</i>)	22:0
<i>Unsaturated acids</i>		
<i>cis</i> -9-Hexadecenoic	Palmitoleic	16:1 Δ 9
<i>cis</i> -9-Octadecenoic	Oleic (<i>L. oleum</i> = oil)	18:1 Δ 9
<i>cis, cis</i> -9,12-Octadecadienoic	Linoleic (<i>L. linum</i> = flax, + oleic)	18:2 Δ 9,12
^a all <i>cis</i> -9,12,15-Octadecatrienoic	α -Linolenic	18:3 Δ 9,12,15
all <i>cis</i> -6,9,12-Octadecatrienoic	γ -Linolenic	18:3 Δ 6,9,12
all <i>cis</i> -5,8,11,14-Eicosatetraenoic	Arachidonic (<i>L. arachis</i> = peanut)	20:4 Δ 5,8,11,14
all <i>cis</i> -5,8,11,14,17-Eicosapentaenoic	EPA	20:5 Δ 5,8,11,14,17
all <i>cis</i> -7,10,13,16,19-Docosapentaenoic	Clupanodonic (<i>L. clupea</i> = herring)	22:5 Δ 7,10,13,16,19
all <i>cis</i> -7,10,13,16,19-Docosaheptaenoic	DHA	22:6 Δ 4 Δ 7,10,13,16,19

^a“all” indicates that all the double bonds are *cis*.

(propane-1,2,3-triol) (4.2), and three fatty acid residues (not necessarily identical) and sometimes referred to as triacylglycerols. The three hydroxyl groups are usually identified by the numbers 1, 2 and 3, but sometimes the outer hydroxyl groups, *i.e.* 1 and 3, are referred to as 1 and 1', respectively, to emphasise the point that, under most circumstances, they cannot be distinguished from one another. Another terminological point

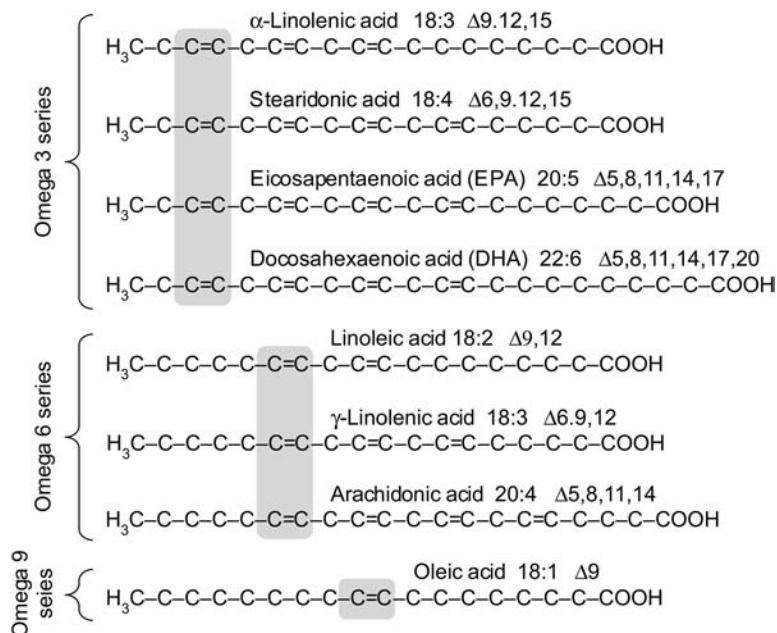
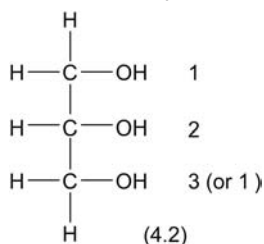


Figure 4.3 The ω -3, ω -6 and ω -9 series of fatty acids. The chemical structures have been simplified so that only the backbone carbon atoms, linked by either single or double bonds, are shown. The positions of double bonds are included in names of the fatty acids in the conventional way, e.g. Δ 9,12,15, as explained in Figure 4.1. The three series differ in the position of the double bond furthest from the carboxyl group, numbered relative to the ω -carbon, the terminal methyl, $-\text{CH}_3$. The structural relationships between the members of the three series are emphasised by the shading of these double bonds. Some authorities use the letter n in place of the Greek letter ω , but, in recent years, the popular press has adopted the term “omega fatty acid” to describe supposedly beneficial PUFAs without discriminating between ω -3 and ω -6. The manufacturers of cosmetic creams *etc* seem to have a similar fondness for “omega”-based formulations.

is that “simple” triglyceride molecules have three identical fatty acid residues, whereas “mixed” triglycerides have more than one fatty acid.

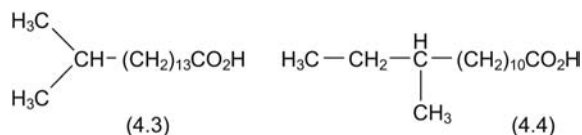


Naturally occurring fats are always mixtures of “simple” and “mixed” triglycerides. It is important to remember that evolution has equipped different species with desirable patterns of physical properties for the lipids of, say, their cell membranes or adipose tissue, by utilising a mixture of different molecular species rather than a single molecule with precisely the right properties, as is usually the case with proteins or carbohydrates.

The relative abundance and importance of the various fatty acids can be appreciated from Table 4.3, in which the molar proportions of the fatty acids in a number of fats and oils are presented. The relationship between the fatty acid composition of a fat and its melting properties will be apparent from a comparison of the data in Table 4.3 with that in Table 4.4, which gives the melting points of the most important fatty acids. The lower melting temperature characteristic of oils is associated with either a high content of unsaturated fatty acids (*e.g.* corn oil and olive oil) or a high proportion of short-chain fatty acids (*e.g.* milk fat and coconut oil).

The insertion of a *cis* double bond has a dramatic effect on the shape of the molecule, introducing a kink of about 42° into the otherwise straight hydrocarbon chain (Figure 4.1). The insertion of a *trans* double bond has very little effect on the conformation of the chain and has therefore very little effect on the melting temperature. For example, elaidic acid, the *trans* isomer of oleic acid, has similar physical properties to stearic acid. Although some *trans* fatty acids do occur in nature, their primary importance to us is as by-products of the hydrogenation process in margarine manufacture. They are considered in detail in Section 4.3.1.

The milk fats of ruminants are characterised by high proportions of short-chain fatty acids. These are derived from the anaerobic fermentation of carbohydrates, such as cellulose, by microorganisms in the rumen. The branched-chain fatty acids that are also found in ruminant milk fats are also by-products of rumen metabolism. Most of these branched fatty acids belong to either the *iso* or *anteiso* series, depending on the position of the additional methyl group. For example, the milk fat of both cows and goats contains around 0.5% of 15-methylhexadecanoic acid (4.3), an *iso* fatty acid, alongside a similar proportion of the *anteiso* fatty acid, 12-methyltetradecanoic acid (4.4).



Human milk fat, like that of other non-ruminant species such as the pig, is particularly rich in linoleic acid. Although no direct benefits of this to human infants have yet to be identified, it will be surprising if there are none, in view of the importance of linoleic acid in human nutrition (see later in this chapter).

Cod liver oil is most familiar to us as a source of vitamin D, but fish oils are particularly interesting for the diversity of the long-chain, highly unsaturated fatty acids they contain. The special significance of these fatty acids for human diets is discussed in detail later in this chapter.

A number of fatty acids of minor importance, but unusual chemical structures, occur in particular groups or species of plants. For example, petroselenic acid (18:1 Δ^6 *cis*) is found in the seed oils of celery, parsley and carrots. Ricinoleic acid (4.5) makes up about 90% of the fatty acids of castor oil.

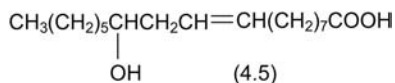


Table 4.3 Fatty acid composition of fats and oils. The figures are typical values expressed in mol%. There is often considerable variation in composition between different samples of the same fat, due, for example, to variations in the animal's diet or the conditions during plant growth. Trace components representing <0.5% have been ignored.

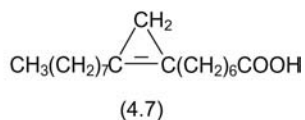
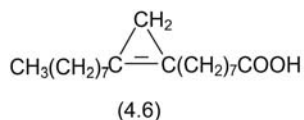
Fatty acid	Beef fat	Lard	Goose fat	Cow's milk fat	Human milk fat	Herring oil	Maize (corn) oil	Cocoa butter	Coconut oil	Almond oil	Palm oil	Olive oil
4:0				9								
6:0				5								
8:0				2					12			
10:0				4	1				8			
12:0				3	3				49			
14:0	4	2	1	10	5	7			16		1	
14:1	1			2	1							
15:– ^a	2			1	1							
16:0	28	26	22	23	26	13	14	29	7	5	48	11
16:1	5	4	3	2	5	9						1
17:– ^a	1			1	1							
18:0	20	15	6	12	7	1	2	35	2		6	3
18:1	34	44	57	23	37	12	34	32	5	68	34	79
18:2	3	9	9	2	11	2	48	3	1	25	11	5
18:3	2		2	1	1		1					1
20:0							1	1				
20:1						19						
20:5						8						
22:1						25						
22:5						2						
22:6						2						

^aThe sum of straight- and branched-chain, saturated and unsaturated fatty acids.

Table 4.4 Melting points of fatty acids.

Fatty acid	Melting point (°C)	Fatty acid	Melting point (°C)
Butyric	-7.9	Elaidic	43.7
Caproic	-3.4	Oleic	10.5
Caprylic	16.7	Linoleic	-5.0
Capric	31.6	α -Linolenic	-11.0
Lauric	44.2	Arachidonic	-49.5
Myristic	54.1		
Palmitic	62.7		
Stearic	69.6		
Arachidic	75.4		

Fatty acids containing cyclopropene rings are mostly associated with bacteria, but traces of sterculic acid (4.6) and malvalic acid (4.7) are found in cottonseed oil. These are toxic to non-ruminants and the residual oil in cottonseed meal is sufficient to produce an adverse effect in poultry fed exclusively on this meal. It must be assumed that the low levels humans have consumed for many years in salad dressings and margarine manufactured from cottonseed oil have had no deleterious effect.



Erucic acid, *cis*-13-docosenoic acid (22:1 Δ 13), is a characteristic component of rapeseed oil, extracted from the seeds of the genus *Brassica*, notably varieties of *B. napus* and *B. campestris*, which are known as rape (“*rapum*” is Latin for turnip) or rapeseed. Rapeseed oil was not originally used for human consumption, but was highly valued as a lubricant for the steam engines that powered ships. However, diesel engines began to dominate in the 1940s and the market for rapeseed oil was taken over by the oil and gas industry. Rapeseed grew particularly well in Western Canada and, in the 30 years that followed, Canadian plant-breeders were able to develop rapeseed varieties that gave an oil (which came to be known as “canola oil”) with a massively reduced erucic acid content, down from <40% to >0.5%. It also had the nutritional benefit of a high oleic acid content. Although excellent for many food applications (*e.g.* as a salad oil), the original canola oil’s high linolenic acid content (>10%) made it prone to oxidative rancidity and therefore unsuitable for frying. More recently, plant-breeders have been able to develop rape varieties giving canola oil with a linolenic acid content of about 3%. An additional benefit of these varieties is that the seed meal, the residue from oil extraction, has greatly reduced levels of glucosinolates. These are the characteristic flavour compounds of the genus *Brassica* (see Chapter 7, Section 7.1.6), which are particularly distasteful to farm animals.

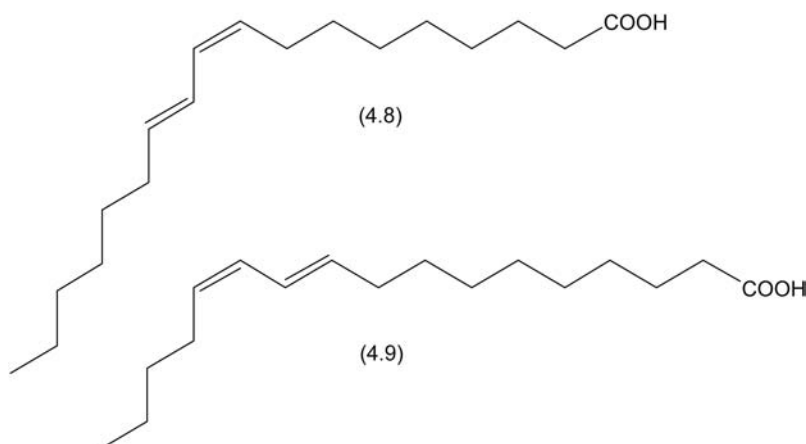
Bacteria, especially those that occupy the rumen of cattle, synthesise a considerable variety of fatty acids that do not fit the patterns associated with most of the higher animals and plants. Small amounts of some of these fatty acids eventually reach the depot

and milk fats of these animals and, ultimately, traces find their way into the human diet. Elaidic acid has already been mentioned; vaccenic acid, *cis*-11-octadecenoic acid, is another.

4.1.1 Conjugated Linoleic Acids

Of all the naturally occurring fatty acids containing *trans* double bonds, the conjugated linoleic acids (CLAs) are among the most interesting because a number potentially beneficial effects on human health have been attributed to them.^{iv}

The two double bonds in CLAs are not in their usual positions, but are conjugated (see Section 4.1), starting at carbons 9, 10 or 11, and most commonly with at least one of the two bonds in the *trans* configuration. The most abundant CLAs are 18:2 Δ *cis*9,*trans*11 (4.8) and 18:2 Δ *trans*10,*cis*12 (4.9). The CLA content of dairy products based on cow's milk is around 5–10 mg g⁻¹ total fat. Similar levels are found in the depot fat and milk of other ruminants, but much lower levels are found in non-ruminants (monogastric organisms), including horses, pigs and humans. CLAs arise as by-products of the hydrogenation of PUFAs by specialist rumen bacteria such as *Butyrovibrio fibrisolvens*. Vegetable oils and seafood contain much lower levels of CLAs, the most abundant being the *cis*9,*trans*11 type, which have been shown to be effective anti-mutagens^v and antioxidants (see Section 4.3.3). In laboratory experiments with rodents, elevated dietary levels of CLAs (five to ten times commonly found in human diets) have proved to be effective anti-tumor agents. Details of the proposed mechanisms of these effects are outside the scope of this book. Research in this area is some way from the stage of clinical studies with humans, but this has not prevented CLAs (prepared in laboratories by chemical isomerisation) being enthusiastically taken up as dietary supplements.



^{iv}There are suggestions that CLAs may have roles in the treatment of various cancers, obesity and atherosclerosis, but, to date, their efficacy and safety in preclinical and human trials is not well established; see L. J. den Hartigh Conjugated linoleic acid effects on cancer, obesity, and atherosclerosis: a review of pre-clinical and human trials with current perspectives, *Nutrients*, 2019, **11**, 370.

^vUsing the well-known Ames test for mutagens and potential carcinogens.

4.2 Fatty Acids and Health

Fatty acids in the form of the triglycerides of dietary fats and oils provide a major proportion of our energy requirements. However, food chemists need to understand that our relationship with the fats in our diet is far more complex than this simple statement might imply. We all recognise that when we consume more energy than we need, whether in the form of fats, carbohydrates, proteins or alcohol, the unwelcome burden of superfluous adipose tissue soon appears, ultimately leading to obesity and other health problems, including heart disease and diabetes. The issue is complicated by the observation that some of the fatty acids found in the fats and oils we consume are more likely to lead to health problems than others. We also recognise that certain fatty acids, not necessarily particularly abundant in our normal diets, are essential for health. Without claiming to be qualified nutritionists or dietitians, food chemists need to appreciate the health implications of the food products they may be involved with.

The involvement of dietary fatty acids in atherosclerosis, particularly heart disease, is a complex issue. Nutritional guidelines published by medical and public health authorities focus on reducing the contribution fats make to the total energy content of the diet and on lowering the proportion of saturated fatty acids in dietary fat. However, it is becoming increasingly clear that the apparently simple relationships (which formed the scientific basis of these guidelines) between dietary lipids, blood cholesterol levels and the risk of coronary heart disease (CHD), are not simple at all. A diverse range of other risk factors and refinements are being identified that help to explain the apparent inconsistencies of the so-called “lipid hypothesis” of CHD. Although some of these risk factors have obvious links to basic aspects of human physiology and biochemistry, others, such as ethnicity, type of employment, smoking and medical aspects of family history do not. It should not be overlooked that good, and bad, dietary habits are often passed down from one generation to the next,

Thus, when we consider the fatty acids in our diet, it is now believed important to discriminate between saturated fatty acids of different chain lengths, as well as those that are mono- or polyunsaturated. In recent years, myristic, lauric and palmitic acids have been regarded as the most effective at increasing plasma cholesterol levels, whereas it has been suggested that stearic acid may be much less effective. However, because many fats that are relatively rich in stearic acid also contain palmitic acid, it is difficult to draw firm conclusions from population studies. An additional issue is that, regardless of their nutritional advantages, oils rich in unsaturated fatty acids are of limited value in many situations where harder spreading fats, rich in saturated fatty acids, are normally used, such as pastry- and cake-making. Many snack foods are necessarily fried at high temperatures, which necessitates the use of more saturated fats.

The evidence that changing one's diet – for example, by replacing butter with margarine – actually lowers the risk of CHD by a significant amount is also less clear-cut than the advertisements for margarine “rich in polyunsaturates” might imply. The account of *trans* acids in the following section shows that the benefits of margarine have not always been all that they appear to be. Similarly, the fatty acid compositions presented in Table 4.3 show that animal fats can be highly unsaturated (*e.g.* fish oils)

and plant fats can be the reverse (*e.g.* coconut oil). The observation that the level of cholesterol in the diet may have little influence on the level of cholesterol in the blood is also widely ignored by the advertisers of products derived from plant oils.

The observation that the occurrence of CHD is linked to the peroxidation (*i.e.* oxidation involving the formation of peroxides) of blood lipids has drawn attention to the possible role of the “antioxidant vitamins” (A, E and C), in addition to other naturally occurring antioxidants, and hence fruit and green vegetable consumption. The function of antioxidants, substances that interfere in the oxidation of unsaturated fatty acids, is discussed in detail later in this chapter. There are now indications that many of the differences in the incidence of CHD between population groups (*e.g.* between the north and south of the UK), which have defied explanation in terms of dietary fat consumption, can be correlated with the intake of fruit and green vegetables.

It is now well understood that certain dietary fatty acids have a more specific function in human nutrition. Rats fed on a totally fat-free diet show a wide range of acute symptoms affecting the skin, vascular system, reproductive organs and lipid metabolism. Although no corresponding disease state has been recorded in human patients, similar types of skin disorder have been observed in children subjected to a totally fat-free diet. The symptoms in rats can be eliminated by feeding linoleic or arachidonic acids. As a consequence, arachidonic acid became known for a time as vitamin F. It is generally accepted that 2–10 g of linoleic acid (Figure 4.3) per day is sufficient to meet the requirements of an adult human.

About 25 years after the original identification of linoleic and arachidonic acids as “essential fatty acids” (EFAs) in the 1930s, they were recognised as precursors of a group of substances, the prostaglandins, that had first been isolated from prostate glands (a particularly rich source for laboratory studies, hence the name) in 1935. The prostaglandins are often described as hormones, but this is not technically correct because they are synthesised in a wide range of tissues where they exert their effects locally rather than after transport to a target tissue or organ. Their effects vary widely and are specific to the tissue that generated them, often in the mediation or facilitation of the action of other hormones originally secreted elsewhere in the body. The prostaglandins are, in fact, only one class of a group of biologically active substances known as eicosanoids, which also include the thromboxanes and leukotrienes. The list of physiological activities in which prostaglandins are known to be involved continues to grow, but they are best known for their role in inflammation and the contraction of smooth muscles. Thromboxanes have a role in the aggregation of platelets, part of the process of the formation of blood clots.

The conversion of PUFAs into eicosanoids is a complex process, but the potential structural relationships between precursors and end-products are illustrated by the example of arachidonic acid in Figure 4.4.

Animals, including humans, are equipped to synthesise saturated fatty acids up to 18 or so carbon atoms in chain length, but are limited in their ability to then include double bonds. Animals are usually only able to insert a double bond between C-9 and C-10. This means that animals *cannot* synthesise linoleic acid or linolenic acid, although

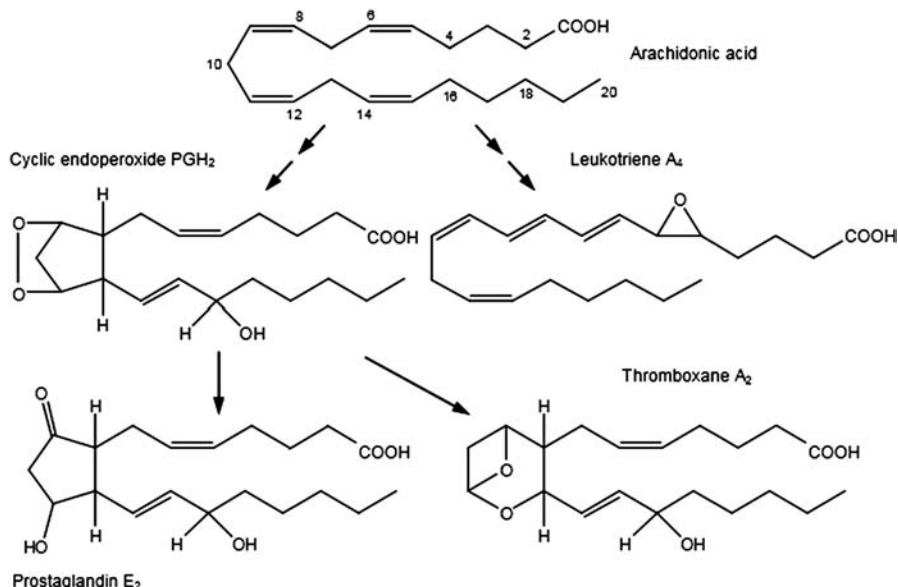


Figure 4.4 Biosynthesis of eicosanoids from arachidonic acid. The numbering of arachidonic acid carbon atoms will facilitate comparison with the simplified structure in Figure 4.3. The F series prostaglandins differ in that both oxygen atoms attached to the ring are present as hydroxyl groups. This pathway also shows why the position of the double bonds in essential fatty acids is critical. Similar reactions lead to eicosanoids based on ω -3 fatty acids.

many animal species, including humans, but not cats,^{vi} are able to convert linoleic acid to arachidonic acid. Thus, although arachidonic acid and other long-chain PUFAs are not found in plants, humans can still obtain some of them from dietary linoleic acid, abundant in plant and animal sources.

Linoleic acid, the first of the ω -6 fatty acids, is abundant in human diets, omnivorous or vegetarian, as long as sensible amounts of fats or oils are included (see Table 4.3). Humans, like most other animals (except cats) are able to convert linoleic acid to arachidonic acid. The product of the first reaction in the sequence, γ -linolenic acid (18:3 Δ 6,9,12) is found in only trace amounts in most species, except that it replaces the α -analogue in a few species of higher plants, notably evening primrose (*Oenothera* spp.) and borage (*Borago officinalis*), and the *Phycomycete* fungi, the class which includes the familiar mould genera, *Mucor* and *Rhizopus*. The theoretical justification for the quasi-pharmaceutical use of evening primrose oil is that our natural synthesis of arachidonic acid may need some encouragement and might benefit from an enhanced supply of γ -linolenic acid. There have, however, been some indications that large increases in

^{vi}In the course of evolution, domestic cats (and, by inference, the domestic cat's larger, wild cousins) have lost, but not missed, the ability to convert linoleic acid into arachidonic acid. Being carnivores, cats have always been able to obtain sufficient arachidonic acid from their diet. This could, however, become a serious problem for pet cats whose owners feed them a vegetarian or vegan diet, although most of these cats can still obtain sufficient arachidonic acid by hunting and eating small mammals, birds, etc, or from the supplements added to commercial vegetarian and vegan cat foods.

linoleic acid intake – for example, by the consumption of products rich in safflower oil – may actually increase the risk of cardiovascular disease rather than reduce it.^{vii}

The dietary supply of precursors for the eicosanoids derived from ω -3 fatty acids is more problematic. The ω -3 fatty acid α -linolenic acid (18:3 Δ 9,12,15) occurs in small amounts (about 1%) in many animal and plant fats and in large amounts (>50%) in a few plant oils, notably linseed oil (sometimes known as flaxseed oil), *Linum usitatissimum*, and chia seed oil (*Salvia hispanica*). Unfortunately, α -linolenic acid is a poor source of eicosanoid precursors. The first stage in the pathway to *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-7,10,13,16,19-docosahexaenoic acid (DHA), the oxidation of α -linolenic acid to stearidonic acid catalysed by the enzyme Δ -6-desaturase, is very slow. The normal activity for this enzyme is the conversion of linoleic acid to γ -linolenic acid. Unicellular algae also synthesise long-chain members of the ω -3 series of fatty acids. Commercially cultured algae provide a useful, if expensive, source of EPA and DHA supplements suitable for vegetarians and vegans, but the real relevance of algal synthesis is that, directly or indirectly, fish and marine mammals are consumers of algae. The tissues of fatty fish (*e.g.* herring, mackerel, salmon and trout) can provide considerable quantities of ω -3 fatty acids in human diets. A single serving of salmon or mackerel can provide a total of 1.5–3.5 g of EPA and DHA. Unfortunately, the average consumption of fatty fish by adults in Europe and North America provides <0.25 g per day, with a considerable proportion of these populations actually consuming much less.

The significance of oily fish and its fatty acids was first realised in the 1970s when studies of the Inuit population of Greenland revealed exceptionally low levels of cardiovascular disease compared with people mainland Denmark. At the time of the study, the diet of the Inuit population had a very high fat content and a correspondingly low carbohydrate content and was dominated by fish, seal meat (seal oil contains about 10% EPA) and whale meat. Typical adult daily intakes of around 400 g of fish and marine mammal meat provided about 14 g of ω -3 fatty acids. Subsequent analyses have showed correspondingly high serum concentrations of EPA and DHA. Since that time, the health benefits of diets high in ω -3 PUFAs have become better understood and now include improvements in inflammatory diseases, hypertension, diabetic retinopathy and neurological disorders. Although the mechanisms of these effects are not understood in detail, a common feature appears to be the balance between the activities of ω -3 and ω -6 eicosanoids.

Bearing in mind the improbability of persuading the urbanised populations of world to consume the quantities of seal and whale meat once normal for inhabitants of the polar regions and, of course, the limitations of these resources, we must look for alternative sources of ω -3 fatty acids. Unfortunately, the fish oil capsules many people consume daily rarely contain more than 150 mg of EPA and DHA in total, and are unacceptable to vegetarians and vegans. Fish do not synthesise EPA or DHA themselves, but consume them directly, or indirectly *via* marine invertebrates, from unicellular algae. The industrial-scale cultivation of various species of these so-called microalgae, *e.g.* *Porphyridium cruentum* and *Dunaliella bardawii*, is now being examined as an acceptable

^{vii}C. E. Ramsden, *et al.*, Use of dietary linoleic acid for secondary prevention of CHD and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis, *BMJ*, 2013, 346, e8707.

source of these fatty acids. The oil extracted from the filamentous marine alga *Spirulina*, better known as a source of a blue food colour (see Chapter 6, 6.11.3 (iii)), is also currently marketed as a source of ω -3 fatty acids. Another promising approach is the development, using genetic modification, of variants of canola oil and soybean oil containing up to 23 and 30%, respectively, stearidonic acid. Seed oils from two members of the borage family (Boraginaceae) have been shown to have useful levels of stearidonic acid. This fatty acid constitutes >10% of the fatty acids in refined echium oil, extracted from the seeds of purple viper's bugloss (*Echium plantaginum*). Echium oil is approved for use in the European Union in a number of food products. A similar oil (which gained European Union approval in 2015) from *Buglossoides arvensis* (the common name in the USA is corn gromwell) contains 15–30% stearidonic acid.

4.3 Reactions of Unsaturated Fatty Acids

Unsaturated fatty acids take part in a number of chemical reactions important to the food scientist; the hydrocarbon chain of the saturated fatty acids is essentially chemically inert under the conditions encountered in food. These reactions do not involve the carboxyl group and, because most occur regardless of whether or not the fatty acid is esterified with glycerol, it is convenient to deal with them at this point rather than when the properties of the fats and oils are considered.

Halogens react readily with the double bonds of fatty acids and the stoichiometry of the reaction has been utilised for many years as an indication of the proportion of unsaturated fatty acids in a fat. The iodine number of a fat (*i.e.* the number of grams of iodine that react with 100 g of fat) is still quoted in the older literature as a measure of the overall degree of unsaturation. However, for most purposes, the iodine number has been superseded by gas chromatographic analysis of the volatile methyl esters of the fatty acids obtained by saponification and methylation of the fat. This can provide complete data on the fatty acid composition of an unknown fat.

4.3.1 Hydrogenation, Margarine and *trans* Fatty Acids

In the presence of hydrogen and a suitable catalyst, the double bonds of unsaturated fatty acids are readily hydrogenated, a reaction at the heart of modern margarine manufacture.^{viii} The aim of hydrogenation is to convert a liquid vegetable or fish oil into a fat with a butter-like consistency by reducing the degree of unsaturation in its component fatty acids. At the present time, soybean and palm oils, which together account for about 60% of worldwide consumption of fats and oils, are the major raw materials used in the manufacture of margarine and other hydrogenated fats.

After preliminary purification to remove polar lipids and other substances, which tend to poison the catalyst, the oils are exposed to hydrogen gas at high pressures and

^{viii}The first margarine was invented by a French chemist, Hippolyte Mège Mouriès, in 1869 under the name “beurre économique”. It was a mixture of milk, chopped cow's udder and a low-melting fraction of beef fat. The later name, margarine, was derived from the Greek *margaritas*, meaning “pearl-like”.

temperatures (2–10 atm, 160–220 °C) in the presence of 0.01–0.2% of a finely divided nickel catalyst. Under these conditions, the reduction of the double bonds is fairly selective. Those furthest from the ester link of the triglycerides and those belonging to the most highly unsaturated fatty acid residues are the most reactive. This selectivity fortunately results in the trienoic acids (*i.e.* having three double bonds) being converted to dienoic acids (*i.e.* having two double bonds) and dienoic to enoic acids (*i.e.* having one double bond), rather than producing an accumulation of fully saturated acids. The margarine manufacturer is, of course, able to control the degree of hydrogenation to give the desired characteristics to the particular finished product.

The hydrogenation reactions are by no means as straightforward as one might imagine. (The first of the Special Topics at the end of this chapter gives additional details.) Isomerisation of *cis* to *trans* fatty acids occurs under the extreme conditions of the hydrogenation process, *trans* being the thermodynamically preferred configuration of the double bond. Double bonds also tend to migrate during the process, so, for instance, a fatty acid chain starting with a *cis* double bond at C-9 might end up with either a *cis* or *trans* double bond anywhere between C-4 and C-16.

Until the late 1990s, the total consumption of *trans* fatty acids was fairly high. The average intake figure for UK adults was around 5 g per day, corresponding to >2% of the total energy content of the diet, and individual consumption of >12 g per day was not uncommon. The total amount of *trans* fatty acids ranged from 5% in lightly hydrogenated vegetable oils, to >40% in commercial frying fats. The contribution of different foods to *trans* acid intake differed widely between individual consumers. Spreading fats (margarine, but not butter in this context) contributed about one-third and shortening fats, used in pastry and biscuits, contributed a similar amount. Although the *trans* acid content of animal fats is low,^{ix} dairy products and meat can provide around one-tenth of total consumption. A typical fast food meal comprising a cheeseburger, French fries and a milk shake, with a pastry for dessert, could have supplied almost 4 g of *trans* acids.

In spite of their major presence in our diet, the metabolism and possible health effects of *trans* acids did not receive much attention and no clearly defined toxic effect came to light. Humans are equipped with appropriate enzymes to metabolise at least some *trans* acids, but it remains to be shown how we can cope with some of the more exotic positional isomers. Epidemiological studies suggested a correlation between mortality from arterial disease and the consumption of hydrogenated fats, but did not focus specifically on their *trans* fatty acid content. One possible explanation is that the human body reacts to *trans* unsaturated fatty acids as if they were saturated fats. However, it is now recognised that dietary *trans* fatty acids tend to increase levels of low-density lipoprotein (LDL) cholesterol and reduce levels of high-density lipoprotein (HDL) cholesterol. Elevated levels of LDL cholesterol are specifically associated with the incidence of atherosclerosis and, in this respect, *trans* unsaturated fatty acids should be regarded as being worse for health than saturated fatty acids. It has thus become desirable to

^{ix}The fats of ruminants, including the intramuscular and milk fats, contain traces of these fatty acids, which, like the short-chain and branched-chain fatty acids considered earlier in this chapter, are by-products of the hydrogenation reactions carried out by bacteria in the rumen.

exclude *trans* fatty acids from the total of monounsaturated fatty acids and PUFAs when analyses of margarines, *etc.*, are quoted on labels. Since 2006, legislation in the USA requires that the *trans* fatty acid content of food products containing >0.5 g per serving must be declared on the label. Unfortunately, there appear to be no plan at present for similar legislation in Europe. The measurement of the *trans* acid contents of food products is relatively easy. In addition to gas-liquid chromatography, they may be detected by their characteristic infrared absorption bands (965–990 cm^{-1}).

The *trans* acid problem has proved to be short-lived. By 1999, data from the oils and fats industry showed that very few hydrogenated fat products, all of which were short-enings (in food industry parlance “white fats”), had *trans* acid contents as high as 10%. Most, including a wide range of margarines and low-fat spreads (“yellow fats”), now contain 1% or less. According to the European Food Safety Authority, the intake of *trans* fatty acids in most European Union member states was already below the World Health Organization recommendation maximum of 1% of total energy by 2004. Data published in 2007 suggested that, at least in the UK, the contribution of *trans* fatty acids to total energy consumption had fallen from its 1980s levels of >2% to 1.5% or less. Similarly, in the USA in 2012, the Food and Drug Administration reported an average intake of 1.3 g per person per day, substantially lower than a decade earlier. These reductions have been achieved by means of an adaptation of the interesterification process, which is described in detail later in this chapter.

4.3.2 Rancidity

Rancidity is a familiar indication of the deterioration of fats and oils, leading to unpleasant smells. There are two distinct mechanisms by which this can arise: lipolytic rancidity and oxidative rancidity.

Lipolytic rancidity can be a problem in dairy fats, particularly butter. It results when lipases secreted by microbial flora catalyse the hydrolysis of the triglycerides in the fat, liberating short-chain fatty acids (see Table 4.3). Although low concentrations of these acids make an important contribution to the desirable flavour of butter, too much can produce flavours resembling over-ripe cheese. These enzymic reactions do, in fact, have an essential role in cheese-making. Lipases from the moulds that develop as the cheese matures liberate butyric acid, caproic acid, *etc.*, which constitute a major element of the flavour of cheese. Proteolytic enzymes (proteases) from the same moulds liberate peptides and free amino acids (see Chapter 5) from milk proteins, which provide the other major component of cheese flavour. For many of us, Stilton, Edam, Camembert and Gorgonzola, and their innumerable country cousins, are what we understand as cheese – the yellow plastic slice in the cheeseburger is not. Nevertheless, we have to accept that vast numbers of cheeseburgers do get sold every day and, for such a large market, any reduction in the maturation period of cheese makes financial sense. So-called “accelerated cheese ripening” preparations, containing both lipases and proteases, are used extensively in the manufacture of cheese for cheeseburgers, *etc.*

Oxidative rancidity, which has no redeeming features, affects fats and oils and the fatty parts of meat and fish. It is the result of the autoxidation of unsaturated fatty acids. The course of the reaction has been studied in free fatty acids, but there is no

reason to suppose that the behaviour of fatty acids esterified in the form of triglycerides is significantly different.

This sequence of reactions is traditionally presented in the three stages: initiation, propagation and termination (shown in Figure 4.5). The initiation reactions give rise to small numbers of highly reactive fatty acid molecules containing unpaired electrons (*i.e.* free radicals). These are shown as $R^\bullet$ in Figure 4.5a. The dot ($\bullet$) shows the presence of an unpaired electron and R denotes the remainder of the molecule. Most free radicals are short-lived and highly reactive as they seek a partner for their unpaired electron.

In the propagation reactions, atmospheric oxygen reacts with $R^\bullet$ to generate peroxy radicals, $ROO^\bullet$ (Figure 4.5b). These are also highly reactive and react with other unsaturated fatty acids to give hydroperoxides, $ROOH$, regenerating free radicals, $R^\bullet$ (Figure 4.5c). A hydroperoxide group should not be confused with a carboxyl group. In a hydroperoxide, the hydroxyl group $-OH$ is attached directly to the other oxygen atom, not to a carbonyl carbon as in a carboxyl group.

This free radical can then repeat the process, forming a chain reaction (Figure 4.5b), but the hydroperoxide is able to break down to give other free radicals (Figure 4.5d). These can also behave in a similar way to $ROO^\bullet$. The result is that increasing numbers of free radicals accumulate in the fat and absorb considerable quantities of oxygen from the air. Eventually, the concentration of free radicals reaches the point when they react with each other to produce stable end-products (Figure 4.5e). These are known as termination reactions.

There used to be great uncertainty about the nature of the reactions that give rise to the first free radical species ($X^\bullet$) that initiates the autoxidation sequence. It is now accepted that the initiation reaction involves a short-lived, but highly reactive, high-energy form of oxygen known as singlet oxygen, $^1O_2$. Singlet oxygen molecules arise from the normal low-energy ground state oxygen (triplet oxygen, $^3O_2$) in a number of reactions, but most relevant to food systems are those in which oxygen, in the presence of light, reacts with pigments such as chlorophyll, riboflavin (very important in the deterioration of fresh milk) and haem. The second of the Special Topics at the end of this chapter provides more details of the nature of singlet and triplet oxygen. As shown in Figure 4.6, singlet oxygen reacts with the double bond of an unsaturated fatty acid. In this reaction, the double bond changes its configuration from *cis* to *trans* and, at the same time, it moves along the chain.

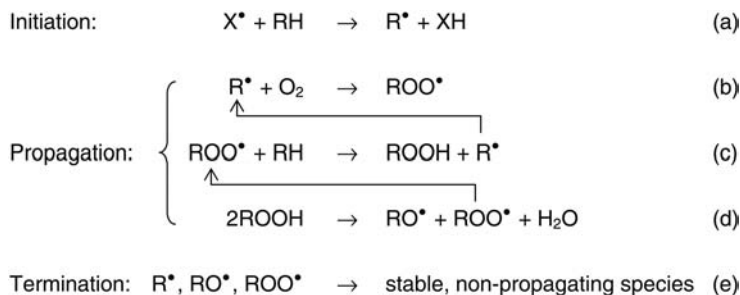


Figure 4.5 Outline of the reactions involved in the autoxidation of unsaturated fatty acids. The various stages, (a) to (e), are discussed in the text.

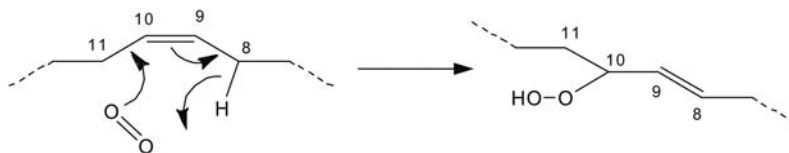


Figure 4.6 Reaction between a singlet oxygen molecule and a double bond to form a hydroperoxide. The curly arrows show serious organic chemists how the electrons migrate during the course of the reaction, but they can be ignored by the rest of us.

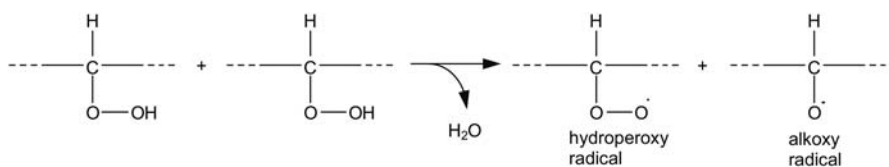


Figure 4.7 Breakdown of hydroperoxides.

Autoxidation proper begins as these first hydroperoxides decompose into hydroxyloxy and alkoxy radicals (Figure 4.7). These are sufficiently reactive to abstract $\text{H}^\bullet$ (*i.e.* a hydrogen atom, or more correctly, a hydrogen radical) from particularly vulnerable sites in monoenoic (*e.g.* oleic) and polyenoic (*e.g.* linoleic) fatty acid residues.

The most vulnerable sites are the CH_2 groups adjacent to double bonds, known as α -methylene groups. A hydrogen atom of an α -methylene group is particularly labile (*i.e.* easily abstracted). Not surprisingly, the most vulnerable α -methylene groups are those lying between neighbouring double bonds in a PUFA, *e.g.* number 11 in linoleic acid, as shown in Figure 4.8a and b. Once the hydrogen radical has been abstracted, the unpaired electron can migrate, leading to the formation of *trans* double bonds. Reaction with oxygen then leads (Figure 4.8c) to the formation of hydroxyloxy radicals. These abstract hydrogen from other fatty acids, forming hydroperoxides and continuing the chain reaction. Oleic acid gives rise to 8-, 9-, 10- and 11-hydroperoxides in roughly equal amounts. Linoleic acid, which reacts with oxygen ten times faster than oleic acid, gives only 9- and 13-hydroperoxides because the stability of the conjugated diene system favours attack by oxygen at the ends of the system rather than in the middle.

As hydroperoxides accumulate in the autoxidising fat, considerable quantities of oxygen are absorbed from the atmosphere, but, as the chain reaction proceeds, the build-up of their breakdown products becomes increasingly important. As shown in Figure 4.9, the alkoxy radicals give rise to aldehydes, ketones and alcohols, in addition to a continuing supply of free radicals that maintain the chain reaction.

Transition metal cations (inevitable trace contaminants in oils that have been processed, stored or utilised in metal vessels) are important catalysts of hydroperoxide breakdown:

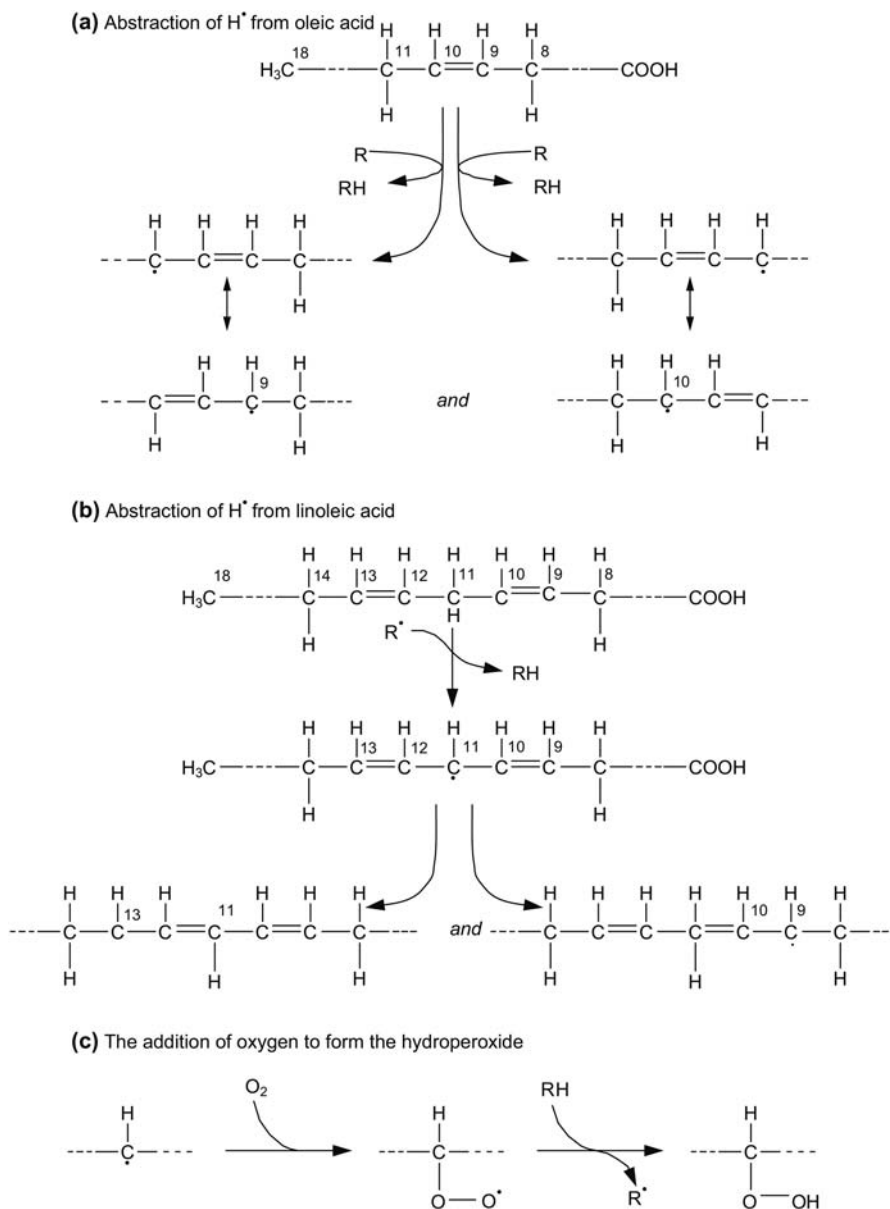
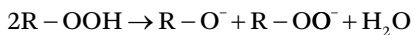
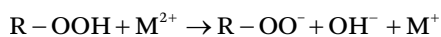
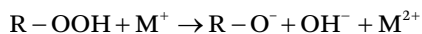


Figure 4.8 Formation of hydroperoxides in the propagation phase of autoxidation.



Aldehydes arising from cleavage of the carbon chain on either side of the alkoxy radical are the source of the characteristic odour of rancid fats. For example, the

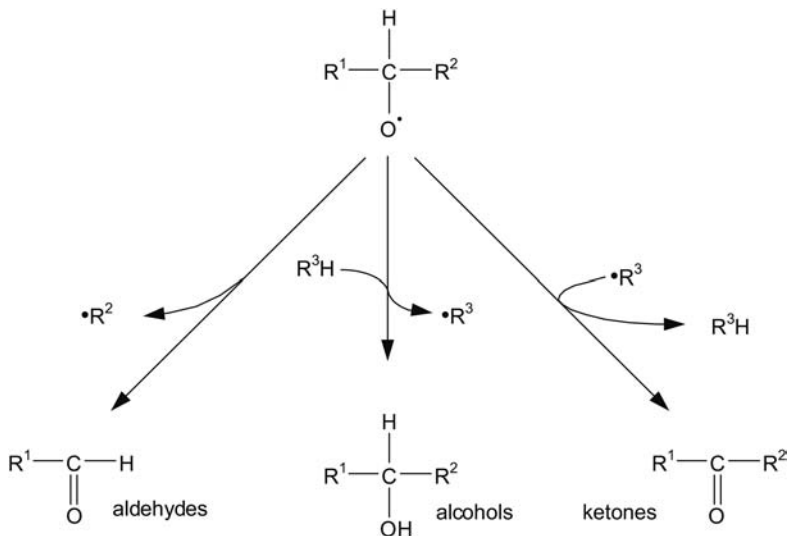


Figure 4.9 Formation of stable end-products from alkoxy radicals.

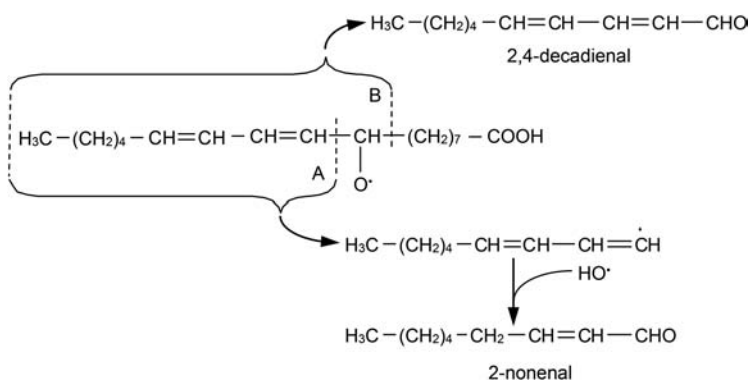
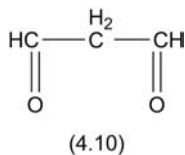


Figure 4.10 Formation of unsaturated aldehydes during autoxidation. Cleavage can occur on either side of the alkoxy group, at A or B.

9-hydroperoxide from linoleic acid will give either 2-nonenal or 2,4-decadienal (Figure 4.10).

One particular compound, malonaldehyde (4.10), results from cleavage at both ends of a diene system. It forms a pink colour with thiobarbituric acid, a reaction that forms the basis of a useful method for assessing the deterioration of fats. Other tests include measurements of the carbonyl content using 2,4-dinitrophenylhydrazine, the hydroperoxide content by reaction with iodine, and the characteristic absorbance of conjugated dienes and trienes at 230 and 270 nm, respectively.



The use of tests such as these, as well as monitoring the oxygen uptake, has shown that the course of oxidation of a fat is marked by an induction period of slow oxygen uptake while the level of initiating free radicals builds up, followed by a period of rapid oxidation. A pronounced rancid odour is not usually detectable until the rapid oxidation phase is well established, although some oils can develop off-flavours and odours even when little oxidation has occurred. Soya bean oil is particularly susceptible to this phenomenon, termed reversion, apparently due to its content of the highly labile linoleic and linolenic acids.^x

Among the compounds that have been implicated in the “flavour” of reverted soya bean oil are *cis*-3-hexenal, diacetyl (butanedione), 2,3-pentanedione and 2,4-pentadienal. Reduction in the linolenic acid content of seed oils has been an important objective for plant-breeders.

Fats and oils exposed to the atmosphere and to heating over a long period of time exhibit the final stage of the oxidation sequence, polymerisation. The highly unsaturated oils used in paint (*e.g.* linseed oil) show the phenomenon even more readily. The cross-linking reactions can be of various types (Figure 4.11).

Polymerisation reactions are particularly significant in frying oils, where prolonged use leads to high molecular weight compounds that cause foaming and increased viscosity. Heavily used batches of oils are sometimes found to contain as much as 25% polymerised material. The chain reaction characteristic of autoxidation reactions is the reason why a half-empty bottle of oil approaching the end of its useful life should be discarded in its entirety rather than topped up with fresh oil.

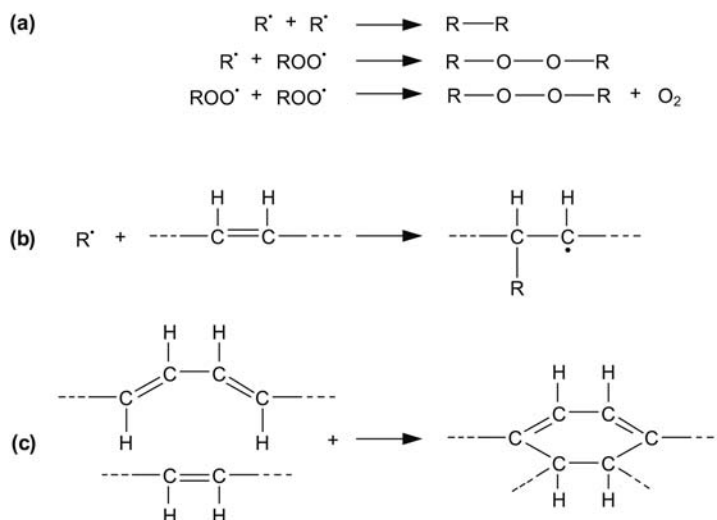


Figure 4.11 Cross-linking reactions in the formation of end-products. Free radicals may react directly together (a) or with other alkenic systems (b). Diels-Alder type reactions also occur, to give cyclic structures (c).

^xSoya bean varieties have been developed, *via* genetic engineering, in which the proportions of linoleic acid have been reduced from 56% to 2% and linolenic acid from 8% to 2%.

The possibility that lipid oxidation products are toxic to humans remains unresolved. The most important source in the human diet is deep-fried foods, although fatty fish and their oils may be significant in some diets. To produce measurable levels of adverse effects, experiments with animals use much larger amounts (in relation to body weight and life span) than would be encountered by humans. Feeding these large amounts of highly oxidised fats induces a wide range of symptoms, most of them corresponding to vitamin E deficiency. It is assumed that the high intake of oxidised fats overwhelms the body's natural antioxidant systems (see Section 4.2).

In recent years attention has been focused on the potentially harmful involvement of lipid oxidation products in arterial disease, particularly those derived from cholesterol. The mechanisms of these effects are complex and beyond the scope of this book. As is so often the case, the link between experimental results using laboratory animals and the incidence of human disease is tenuous and vulnerable to criticism from scientists and others who may have different priorities. Suggestions, not wholly endorsed by other workers, have been made that the elevated levels of cholesterol oxides in clarified butter (ghee) used in cooking may account for the high level of atherosclerosis in British Asian communities. Cholesterol oxides have also been recorded at 50 ppm in French fries from fast food outlets.

It is commonly assumed that at least some of the oxidation products of fats may be carcinogenic; indeed, some have been definitely shown to be mutagenic. The observation that many antioxidants have some anticarcinogenic activity supports this view. However, there is currently little evidence to suggest that oxidised fats cause cancer in humans. Perhaps the adverse effects of a diet over-rich in fats swamp these more subtle influences.

4.3.3 Antioxidants

Although the development of rancidity in bulk fats and oils can be retarded by careful processing procedures avoiding high temperatures, metal contamination, *etc.*, such measures are never wholly effective. Fatty foods, such as biscuits and pastries, are particularly susceptible to rancidity because their structure necessarily exposes the maximum surface of the fat to the atmosphere. The shelf life of these types of foods (on the customer's shelf as well as the shopkeeper's) can be massively extended by the inclusion of antioxidants in many of the fats we buy, particularly in lard. Antioxidants are substances used as food additives to retard autoxidation reactions. Some are wholly synthetic products of the chemical industry, but antioxidants of natural origin are becoming more important as consumers and the food industry seek out what are perceived to be healthier alternatives. As mentioned earlier in this chapter, the involvement of lipid peroxidation reactions in arterial disease has focused attention on the role of antioxidants as dietary components in their own right, not merely as protectors of fats prior to consumption.

There are two well-recognised mechanisms of antioxidant action. In the best-known mechanism, particularly associated with synthetic antioxidants such as BHA and BHT (see Figure 4.12), the antioxidant blocks the propagation phase of the autoxidation process (Figure 4.13).

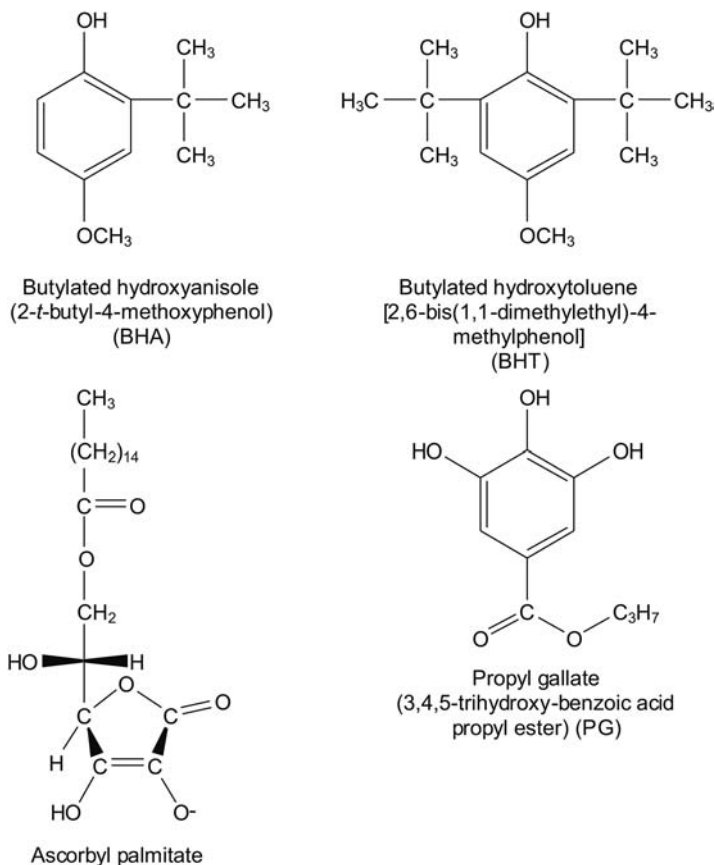


Figure 4.12 Chemical structures of the best-known synthetic antioxidants used in food.

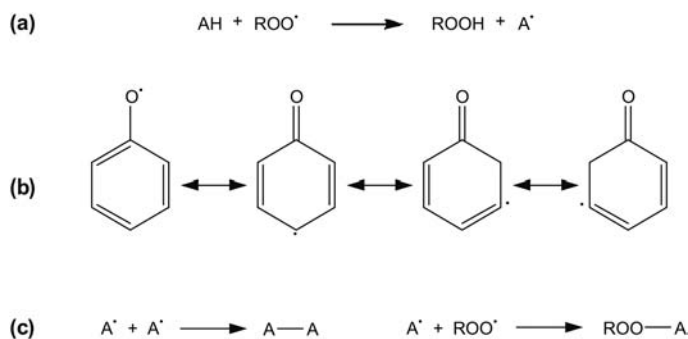
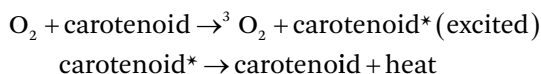


Figure 4.13 Free radical stabilisation by resonance. (a) A free radical intermediate in the lipid autoxidation reaction is stabilised when an antioxidant molecule, AH, donates a hydrogen atom; (b) the antioxidant free radical is stabilised by resonance and is consequently insufficiently reactive to propagate the autoxidation sequence; and (c) antioxidant radicals inter-react to terminate the process.

Many other antioxidants, most famously β -carotene, lycopene and other carotenoids (see Chapter 6), operate by a singlet-quenching mechanism. These molecules react with singlet oxygen, $^1\text{O}_2$, returning it to the relatively unreactive triplet state:



A number of highly reactive free radical forms of oxygen are important in biological systems, such as the superoxide anion, O_2^- (an oxygen molecule carrying an extra unpaired electron, giving it a negative charge), and the hydroxyl radical, $\cdot\text{OH}$ (a hydroxyl ion lacking an electron). These arise as by-products of enzyme and other reactions involving oxygen (see Figure 5.23) and it appears likely that singlet-quenching antioxidants also react with them.

Antioxidants do not reduce the ultimate degree of rancidity; they merely extend the induction period in rough proportion to their concentration. Thus the inclusion of 0.1% BHA in the lard used to make pastry could be expected to add at least a month to the period, otherwise only a few days, before obvious signs of rancidity develop. The three entirely synthetic antioxidants shown in Figure 4.12 (BHA, BHT and PG) are normally added to fats at lower levels than this, usually up to about 200 ppm. There is some concern about the safety of synthetic antioxidants: some laboratory studies with animals have hinted at carcinogenicity. Although this issue is far from resolved, there is growing pressure for their withdrawal. However, it seems likely that they will not be banned completely until the carcinogenicity question is clarified by more research and more progress is made towards their replacement with more natural antioxidants, such as the tocopherols and fatty acid derivatives of ascorbic acid.

Tocopherols occur in most plant tissues, as much as 0.1% in vegetable oils. Animals require tocopherols in their diet, as vitamin E, and these are therefore discussed more fully in Chapter 8. Two other vitamins, ascorbic acid and retinol, also have antioxidant activity, as discussed in Chapter 8. There are indications from both epidemiological studies and the results of experimental dietary supplementation that the so-called antioxidant vitamins can have a collective beneficial effect on arterial disease. The explanation for this effect is that the progress of atherosclerosis involves the peroxidation of the lipid components of lipoproteins. It remains to be seen whether the dramatic differences in the incidence of heart disease between different populations will prove to be at least as much a function of vitamin intake as it is of fat consumption.

Some other sources of antioxidants are beginning to attract attention, notably tea and spices. In the Far East, the consumption of green (*i.e.* unfermented, see Chapter 6, Section 6.5.1) tea has long been associated with good health. Catechin (see Figure 6.12), one of the principal polyphenolic compounds in green tea, has been shown in laboratory experiments to be a most effective free radical scavenger and inhibitor of lipid peroxidation. Theaflavins (6.29), also polyphenolic compounds and the major red pigments in black (*i.e.* fermented) tea, are also very effective free radical scavengers. It remains to be seen whether these activities translate from the laboratory to normal human nutrition. The same reservations must apply to the enthusiasm currently being shown for the polyphenolic anthocyanin pigments found in red and purple species of fruit. These will be considered in detail in Chapter 6.

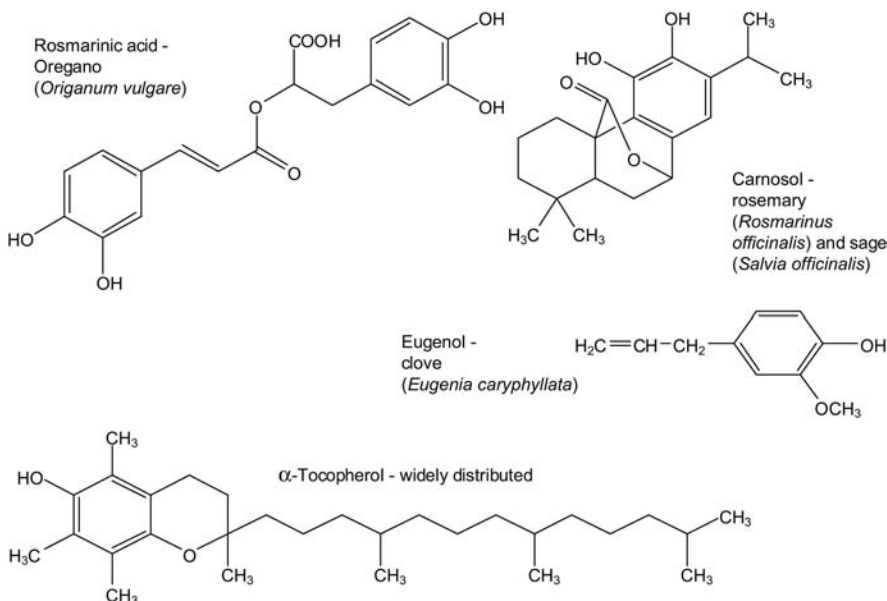


Figure 4.14 Some naturally occurring antioxidants.

Spices have traditionally been used to add to the flavour of foods or to mask off-flavours, but modern research has shown that many spices are rich in antioxidants that have potential for use in curbing rancidity. One, rosmarinic acid (see Figure 4.14), is now approved as an antioxidant additive (E392) for use in the European Union. Some naturally occurring antioxidants are illustrated in Figure 4.14. Their use poses a number of practical problems, including the high costs of producing them in useful amounts and, in many cases, flavours that, while desirable in some foods, will be unwelcome in others. Rosemary extracts are already finding applications in meat products, where their antioxidant activity can be regarded as a beneficial side-effect alongside their principal flavouring function. Rosemary extracts are also valued for food products to be labelled as “organic”, where synthetic antioxidants such as BHT cannot be used. In these applications, the rosemary extract itself must, of course, also qualify as organic. The synthetic antioxidants currently permitted for use in the European Union and elsewhere are listed in Table 4.5.

4.4 Triglycerides

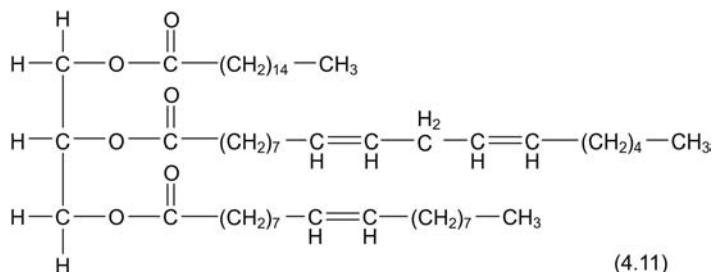
Having considered the properties of the fats and oils from the point of view of the chemistry of their component fatty acids, we can now examine them in terms of their component triglycerides. The first descriptions of the glyceride structure of fats assumed that all their component triglycerides were simple compounds – that is, a fat containing palmitic, stearic and oleic acids would be a mixture of the three triglyceride species tripalmitin, tristearin and triolein. The first attempts to separate the component glycerides of fats used the laborious process of fractional crystallisation from acetone (propanone)

Table 4.5 Antioxidants permitted for use in the European Union and elsewhere (Y, *i.e.* “Yes”, shows that a substance is permitted). Substances permitted elsewhere, but not in the European Union, are shown with their INS numbers. The internet sources for the legislative information in this table are listed in Appendix II. In most countries, there are conditions of use (*e.g.* specific limits to the range of food products and the amounts that may be used).

Antioxidant	EU or INS No.	EU and UK	USA	Canada	Australia and New Zealand	Japan	South America ^a
Ascorbic acid	E300	Y	Y	Y	Y	Y	Y
Sodium ascorbate	E301	Y	Y	Y	Y	Y	Y
Calcium ascorbate	E302	Y	Y	Y	Y	Y	Y
Potassium ascorbate	303				Y		Y
Fatty acid esters of ascorbic acid	E304	Y	}	Y	Y		Y
Tocopherols (mixed or unspecified)	E306	Y		Y	Y	Y	Y
α -Tocopherol	E307	Y			Y	Y	Y
γ -Tocopherol	E308	Y			Y		
δ -Tocopherol	E309	Y			Y		
Propyl gallate	E310	Y	Y			Y	Y
Octyl gallate	E311	Y					Y
Dodecyl gallate	E312	Y			Y		Y
Guaiac resin	314						
Erythorbic acid	E315	Y	Y	Y	Y	Y	Y
Sodium erythorbate	E316	Y		Y	Y	Y	Y
Tertiary butyl hydroquinone	319						Y
Butylated hydroxyanisole (BHA)	E320	Y	Y	Y	Y	Y	Y
Butylated hydroxytoluene (BHT)	E321	Y	Y	Y	Y	Y	Y

^aThe Mercosur group: Argentina, Brazil, Paraguay and Uruguay.

solution at low temperatures. This made it clear that much greater numbers of species of triglycerides were present than expected from this simple concept. Fats and oils became recognised as clearly defined mixtures of mixed [*e.g.* 1-palmityl, 2-linoleyl and 3-oleyl glycerol (4.11)] and simple triglycerides.



If we reconsider our simple fat consisting of only three fatty acids, there should be 27 possible triglycerides. However, as the 1- and 3-positions of the glycerol molecule are usually treated as indistinguishable, the total comes down to 18. This range of possibilities is illustrated in Table 4.6. It is important to note that isomeric pairs of triglycerides, such as POP and PPO, must be differentiated. Such an abundance of data for just one fat forces us to simplify triglyceride composition data when we are comparing different fats (*e.g.* Figure 4.15). The most striking feature of Figure 4.15 is that fats of fairly similar fatty acid composition, such as lard and cocoa butter (see Table 4.3), can have very different triglyceride compositions. In lard, there is a definite tendency for unsaturated fatty acids to occupy the outer positions of the glycerol molecule, whereas in cocoa butter the converse is true. This is, in fact, a general difference between animal and plant fats, as is borne out by the further triglyceride compositions illustrated in Figure 4.16.

If the fatty acid residues at the two outer positions are different, then the four substituent groups attached to the central carbon atom of the glycerol moiety will be different. In this case the triglyceride molecule is asymmetric and optical activity is to be expected. It has been possible to show that a particular species of triglyceride in a natural fat occurs as a single enantiomer rather than as a racemic mixture. This indicates that the 1- and 3-positions are differentiated during triglyceride biosynthesis. However, in many fats there is, in fact, very little difference between the fatty acid compositions of these two positions, but in some, as implied by the data in Figure 4.16, there is a marked difference. Milk fats are exceptional in that their triglycerides fall into three broad classes. In the first, all three positions are occupied by long-chain fatty acids. The second class has long-chain acids at the 1- and 2-positions, but short-chain acids at position 3. The third class has medium-chain fatty acids at the 1- and 2-positions and medium- or short-chain fatty acids at the 3-position.

The determination of the triglyceride composition of a fat is not easy. The need to distinguish between isomeric pairs of glycerides, such as SUS and SSU, crucial to the difference between cocoa butter and lard, for example, makes special demands on chromatographic procedures. High-performance liquid chromatography, using silica gel impregnated with silver nitrate as the stationary phase, has been very successful because it can resolve these isomeric pairs. The silver ions are complexed by the double bonds of unsaturated triglycerides, whose mobility is thus reduced. The identity of a purified triglyceride can be established by means of pancreatic lipase. This enzyme

Table 4.6 Detailed triglyceride composition of lard, expressed as percentages. Simple three-letter codes identify the triglycerides; thus PMS has palmitic acid at position 1, myristic acid at position 2 and stearic at position 3. O = oleic acid; L = linoleic acid. As with the fatty acid composition of a particular fat, there is considerable variation in triglyceride composition from sample to sample, although the broad features remain consistent. (Based on data quoted in *The Lipid Handbook*, ed. F. D. Gunstone, J. L. Harwood and F. B. Padley, Chapman and Hall, London, 1986).

No double bond:										
PMS	SMS	PPP	PPS	SPS	PSS	SSS	Others			
0.4	0.4	0.5	2.0	2.0	0.4	0.4	0.5			
One double bond:										
POP	POS	SOS	PMO	SMO	MPO	PPO	SPO	PSO	SSO	Others
0.6	1.9	1.5	0.4	0.7	0.8	7.9	12.8	0.9	1.6	0.6
Three double bonds:										
OOO	SLO	OML	OPL	OSL	Others					
11.7	0.6	0.6	7.2	1.2	0.5					
Four or more double bonds:										
OLL	OLO	LPL	Others							
1.5	1.5	0.5	0.5							

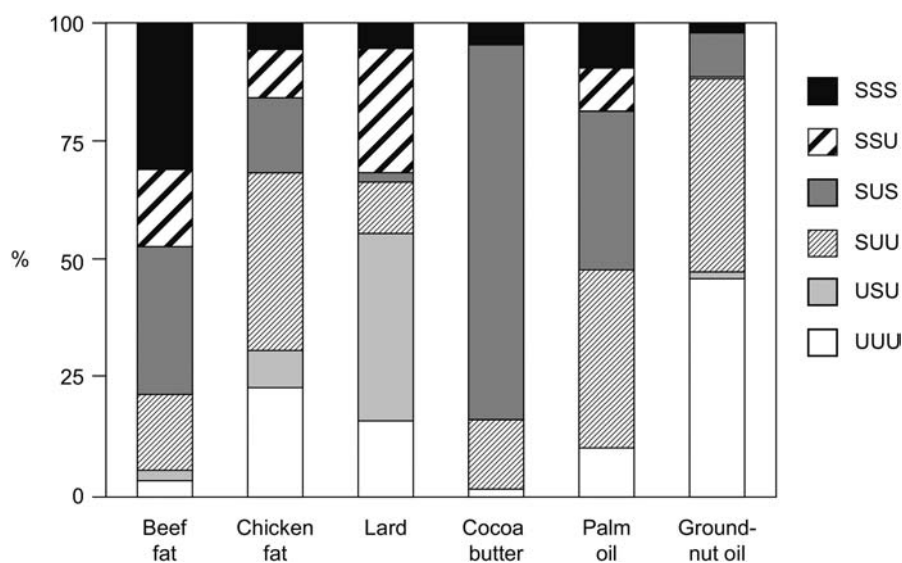


Figure 4.15 Simplified triglyceride compositions of some animal and plant fats. All saturated fatty acids are shown as S and unsaturated fatty acids as U.

specifically catalyses the hydrolysis of ester links in the 1- and 3-positions of a triglyceride and, in the laboratory, as in the small intestine, the products from a triglyceride molecule will be the two fatty acids from the outer positions and one 2-monoglyceride. The liberated fatty acids can be isolated from the reaction mixture and identified by gas chromatography.

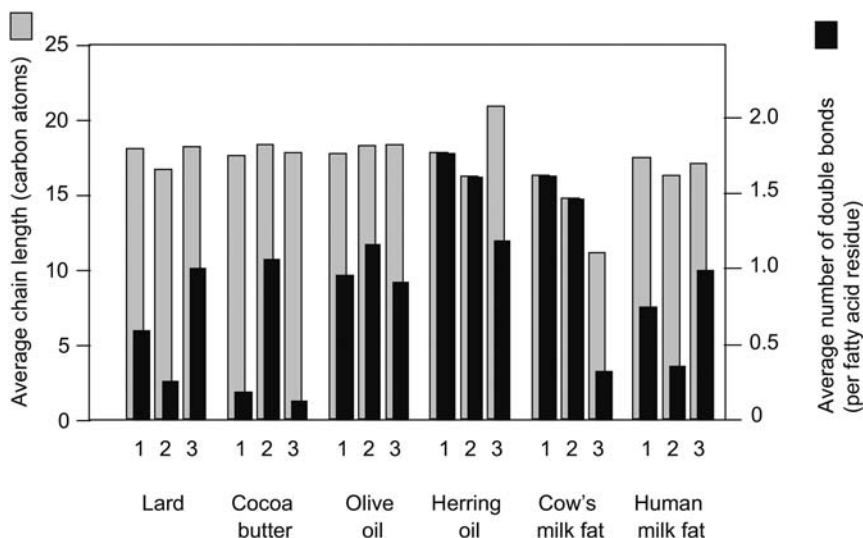


Figure 4.16 Distribution of fatty acids in a range of animal and plant fats. To simplify the presentation of a massive amount of data, the characteristics of the fatty acids esterified at each of the three positions of the glycerides are expressed in terms of their average chain length and average number of double bonds per fatty acid residue.

4.4.1 Melting and Crystallisation

The melting and crystallisation characteristics of a fat are physical properties of prime importance to food chemists. Although the melting points of pure triglycerides are a function of the chain lengths and the unsaturation of the component fatty acids, much as might be expected, the melting behaviour of fats is more complex. As natural fats are mixtures, each component having its own melting point, a fat does not have a discrete melting point, but a melting range. At temperatures below this range, all the component triglycerides will be below their individual melting points and the fat will be completely solid. At the bottom of the range, the lowest melting types, those with the lowest molecular weight or the most unsaturated, will liquefy. Some of the remaining solid triglycerides will probably dissolve in this liquid fraction. As the temperature is increased, the proportion of liquid to solid also increases and the fat becomes more plastic until, at the temperature arbitrarily defined as the “melting point”, there is little or no solid fat left.

This melting behaviour is illustrated in Figure 4.17 for cocoa butter and lard. The key difference between the shapes of the melting curves for these two fats is the steepness of the cocoa butter curve in the 32–36 °C region. This almost unique property of cocoa butter is due to the fact that about 80% of its triglycerides are of the SUS type, namely POP ~15%, POST ~40%, and StOSt ~25%.^{xi} All of these have very similar melting points. By contrast, we have seen that lard contains a large number of different types, with no similarity between the major ones.

^{xi}P = palmitic acid, O = oleic acid, St = stearic acid.

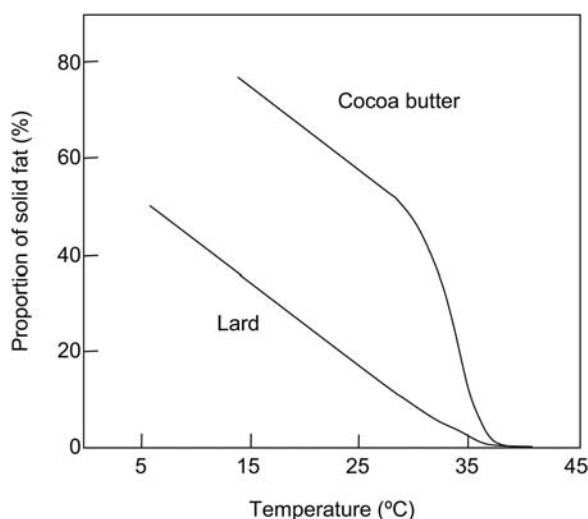


Figure 4.17 Typical melting curves for samples of cocoa butter and lard. The curves are based on data obtained by the use of the low-resolution nuclear magnetic resonance spectrometry, the theory of which lies well beyond the scope of this book.

Table 4.7 Melting points (°C) of the polymorphic forms of simple triglycerides.

	α	β'	β
Tricaprin	-15	—	32
Trilaurin	14	43	44
Trimyristin	32	44	56
Tripalmitin	44	56	66
Tristearin	54	64	73
Triolein	-32	-13	4

A second complication is that triglycerides are polymorphic, *i.e.* their molecules can pack into crystals in several different arrangements, each with its characteristic melting point and other properties. There are three basic types of crystal arrangement (polymorphic form), known as α , β' and β in order of increasing stability. A particular fat will sometimes show a small number of variants within the β' and β classes, and some fats (*e.g.* cocoa butter) may demonstrate as many as six different polymorphic types, each with its own distinct melting point. The melting points of the principal forms of a number of simple triglycerides are given in Table 4.7. When a melted triglyceride is cooled rapidly, it solidifies in the lowest melting point, unstable α form. If this is slowly heated, it will melt and then resolidify in the β' form. Repetition of this procedure will bring about a transition to the final stable β form. The β form is also obtained by recrystallisation from a solvent. Although each form and many of their variations have been fairly well characterised in terms of their X-ray diffraction patterns and infrared spectra, the actual arrangements of the triglyceride molecules in the crystals remain to be described, apart from a handful of purified triglycerides.

It is the occurrence of large numbers of different species of triglyceride in a natural fat that makes the situation so complex, but, as a rule, particular fats stabilise in either a β or β' state. Important examples of β stable fats and oils are cocoa butter, corn oil, groundnut oil, olive oil, safflower oil and lard. β' stable fats and oils include coconut oil, cottonseed oil, palm oil, palm kernel oil, rapeseed oil, beef fat (tallow), herring oil, whale oil and cow's milk fat. The third of the Special Topics at the end of this chapter provides further information about polymorphism.

The usefulness of an individual fat in a particular food application is crucially dependent on its melting and crystallisation characteristics. Fats to be spread on bread, or blended with flour for cakes or pastry, require the plasticity associated with a wide melting range. The term "shortening" is now applied to all manufactured fats used in baking (*i.e.* the production of bread, cakes and pastry). The literal use of the term refers to the tendency of shortenings to reduce the cohesion of the wheat gluten strands in baked goods (see Chapter 5), thereby "shortening" or softening them.

The different polymorphic types differ in the actual form of their crystals. The β' stable fats crystallise in small needle-like crystals. At the correct mixing temperatures, these fats consist of crystals embedded in a liquid fat matrix, giving a soft plastic consistency ideal for incorporating air bubbles and suspending the flour and sugar particles. The β stable fats form large crystals and these produce clumps that give a grainy texture. Although difficult to aerate, they are valuable in pastry-making. If shortenings are blended from mixtures of β and β' stable fats, it is the latter which dictate the crystallisation pattern.

The fatty acid composition, and the distribution between the different positions of the triglyceride molecules (*i.e.* 1, 2 and 3), all help to determine the stable crystal form of a fat. Although there are exceptions, it is generally true that when most of the triglycerides have a broadly similar arrangement of fatty acids, then the fat will readily form β crystals. However, fats with a more diverse mixture of triglyceride types, or those dominated by asymmetrical triglycerides (*i.e.* SSU or UUS), tend to form only β' crystals. Processes such as hydrogenation (Section 4.3.1) or interesterification (Section 4.4.4) provide the possibility of changing the physical characteristics of a fat by a number of different mechanisms. For example, lard, which typically contains about 60% palmitic acid in the 2-position, normally crystallises in the β form. Interesterification redistributes the palmitic acid around all three positions of the triglyceride, leading to a more diverse range of triglyceride types and a tendency to crystallise in the β' form.

4.4.2 Cocoa Butter and Chocolate

Cocoa butter is another good example of the importance of melting properties to a foodstuff, in this case chocolate. The reason for the most notable feature of chocolate, its sharp melting point ("it melts in your mouth but not in your hand"), has already been considered, but we also expect chocolate to have a very smooth texture and a glossy surface. However, before we examine the melting behaviour of cocoa butter in detail, a brief outline of the processes of chocolate manufacture may be valuable.

Chocolate's unique raw material is the seed pod of the cacao tree, *Theobroma cacao*.^{xii} This is grown in various parts of the tropics, notably West Africa and South America, but always within 20° of the Equator. When mature, the pods are 10–35 cm long and weigh 200 g to >1 kg each. After harvesting, the pods are split open and the beans, 30–45 per pod, are extracted, together with the surrounding mucilaginous pulp in which they are embedded. The pods and pulp are then prepared for the fermentation stage of processing.

In West Africa, the beans and pulp are placed in large heaps containing up to 2500 kg of beans and traditionally covered with banana leaves. Elsewhere, large, ventilated wooden boxes containing up to 2000 kg are preferred. Over the next six days or so, a variety of adventitious microorganisms, mostly bacteria and yeasts, break down the polysaccharides in the pulp to release sugars. Some of the sugar is fermented by naturally occurring yeasts to ethanol, which is oxidised to ethanoic acid (CH_3COOH) by bacteria towards the end of the fermentation period. The rest of the sugars are converted to lactic acid (6.8). All this microbial activity generates heat, which raises the temperature sufficiently to start the beans germinating. Part of the normal germination process is the breakdown of some of the bean's storage proteins, catalysed by enzymes secreted as part of the germination process, releasing free amino acids (see Chapter 5). However, the increasing temperature and the diffusion of acids from the fermenting pulp into the beans brings germination to a halt. After drying (ideally spread out in the sun for a week or so) the beans are shipped.

The industrial-scale processes that follow have evolved over the years and the following account should only be regarded as typical. For example, mechanical winnowing, the removal of the shells from the beans, is carried out before or after the roasting stage in which the beans are heated to about 140 °C for up to an hour. Winnowing also breaks the nibs (the chocolate industry's term for the cotyledons) down to fragments around 0.5 cm. In chemical terms, the Strecker degradation element (see Figure 2.16) of the Maillard reaction is the dominant process during roasting. A wide range of compounds emerge from the Strecker degradation: volatile, and usually pleasant smelling, aldehydes and numerous heterocyclic compounds, of which the pyrazines, with odour thresholds in the range 2 ng kg^{-1} to 10 mg kg^{-1} are probably most important.

The roasted nibs are then mixed with sucrose (600–1000 μm) and milled in a multi-stage process down to particles no larger than 30 μm . Particles any larger than this would be detected as graininess by the tongue. The heat generated during milling melts the fat content of the powder, the cocoa butter, so that the end-product is a liquid, known as cocoa liquor. When this has been allowed to cool and solidify, it is usually known as cocoa mass. If milk, as opposed to plain, chocolate is required, then dried milk powder is also added at this stage. The invention of roller-drying for milk powder was an essential element in the development of milk chocolate as even a trace of water will ruin the final product.

The next stage is conching, the particular addition to the process invented by Lindt in Switzerland. It was conceived as an additional mixing stage to enhance smoothness,

^{xii}The generic name *Theobroma* is derived from the Greek "food of the gods".

but was found to have several highly beneficial effects. The original conching machines consisted of a granite trough with a mechanical granite roller moving backwards and forwards. Over the years, massive conching machines have been developed that stir and mix, but retain the “smearing” action of the original machines. The liquor is pressed against the temperature-controlled walls, providing opportunities for the last traces of moisture and other unwanted volatiles, particularly ethanoic acid, to escape. Any aggregates of nib material and sugar crystals are broken up and the fragments become coated with fat, allowing easier flow. This is dependent on the presence of emulsifiers (see Section 4.5) to mask the hydrophilic surfaces of the nib particles and, more particularly, the sugar crystals.

The cocoa bean has a low level of its own membrane phospholipids, but it is usual to supplement these with soya lecithin, the crude residue from soya bean oil extraction containing mostly phosphatidyl choline. Other more modern emulsifiers are coming into use. A low viscosity is essential in the manufacture of items such as Easter eggs and chocolate rabbits, where a thin film of chocolate has to be spread around the inside surface of a mould.

The final stage of manufacture is tempering, ensuring that when the cocoa butter is allowed to cool and set in its final form it has crystallised in the correct polymorphic state, with a sharp melting point and glossy surface. Cocoa butter can occur in six different polymorphic states, with melting points reported to range from 17.3 to 36.4 °C (Table 4.8). In fact, the lowest melting states, γ and α , are only stable for no longer than a few hours at temperatures below about -5 and $+5$ °C, respectively. The γ state has not been described in crystallographic terms. Only the fifth of these (a β -3 type, melting point 33.8 °C) has the properties required for chocolate and the special skill of the chocolate-maker lies in ensuring that the fat is in this particular state in the finished product. The liquid chocolate is first cooled to initiate crystallisation and then reheated to just below the melting point of the β -3 type to melt out any of the undesirable types. The chocolate is then stirred at this temperature for some time to obtain a high proportion of the fat as very small crystals of the desired type by the time it finally solidifies in the mould, or when used to coat biscuits or confectionery. The third of the Special Topics at the end of this chapter is a detailed account of the structure of triglyceride crystals.

Chocolate that has been incorrectly tempered, or has been subjected to repeated fluctuations in temperature, for example in a shop window, develops a bloom. This is a grey film that resembles mould growth, but is actually caused by the transition of some of

Table 4.8 Melting points of the different polymorphic phases of cocoa butter. Details of the crystal structure of the γ state have not been established. The two β' phases, III and IV, are now regarded as the extreme members of a continuum of crystal forms. (From K. van Malssen, *et al.*, *J. Am. Oil Chem. Soc.*, 1999, **76**, 669.)

Phase	Crystal structure	MP (°C)
I	γ	~ -5
II	α	~ 5
III	β'	25.5
IV	β'	27.5
V	β	33.8
VI	β	36.3

the fat to a more stable polymorphic form, which crystallises out on the surface. The migration of triglycerides from the nut centres of chocolates or the crumb of chocolate-coated biscuits can cause similar problems. Milk fat is an effective bloom inhibitor and small amounts are often included in plain chocolate. Cocoa butter is obviously a very difficult fat to handle successfully in the domestic kitchen and chocolate substitutes are marketed for home cake-decorating. These contain fats such as hardened (*i.e.* partially hydrogenated) palm kernel oil. Although they have an inferior, somewhat greasy, texture, these artificially modified fats are stable in the β' polymorphic state and therefore do not present the cook with the problems of tempering.

A fat such as hardened palm kernel oil is referred to as a “cocoa butter replacer”. Such a fat cannot be used in combination with cocoa butter because their triglyceride composition and stable polymorphic states are different. A novel enzymic interesterification process (see later in this chapter) for producing cocoa butter equivalents (CBEs), which can be blended with cocoa butter but are rather cheaper, is now in commercial use. The inclusion of CBEs in chocolate can have other advantages, such as making it less prone to the formation of bloom. Since 2003, fats produced by enzymic interesterification have not been permitted for use in chocolate in the European Union. Some countries, such as Japan, still permit them, but many other countries, including the USA, do not allow any non-cocoa vegetable fats to be incorporated into chocolate.

4.4.3 Fractionation

Fats and oils have, over the years, found use in a wide range of food applications, in the kitchens of homes and restaurants and also in the industrial plants of food manufacturers. Although modern science has helped us to appreciate the reasons, the relationship between the physical properties of a fat or oil and its suitability for spreading on bread, frying chips or making cakes is probably just as well understood by cooks and chefs as it is by food technologists. Unfortunately, there has always been a poor match between what is available in world or local markets and what consumers require. The “margarine” of Mège Mouriès was an early attempt redress the balance, but whether the primary aim was the betterment of the working classes or providing food for the French military is not clear. Although hydrogenation has become the crucial process for converting abundant, but relatively useless, vegetable oils into plastic spreading fats, other processes are becoming increasingly important. Nutritional goals, such as low levels of *trans* fatty acids, have to be achieved, along with ever more subtle manipulations of the physical properties. One fundamental approach has been to separate a fat or oil into different fractions of different melting properties by virtue of differing proportions of more or less saturated triglycerides. A fraction thus obtained can then be used either as it is, or combined with other fats or oils by simple mixing or by interesterification, a process to be considered in the next section.

Cottonseed oil was one of the first oils to be subjected to a fractionation process. Cottonseed oil stored in large tanks out of doors over the winter months becomes cloudy as saturated triglycerides crystallise out. Based on this experience, all that is required to make the oil more acceptable for domestic use as a salad oil or mayonnaise is to chill the oil to about 0 °C for a few hours and then remove the crystalline material *via*

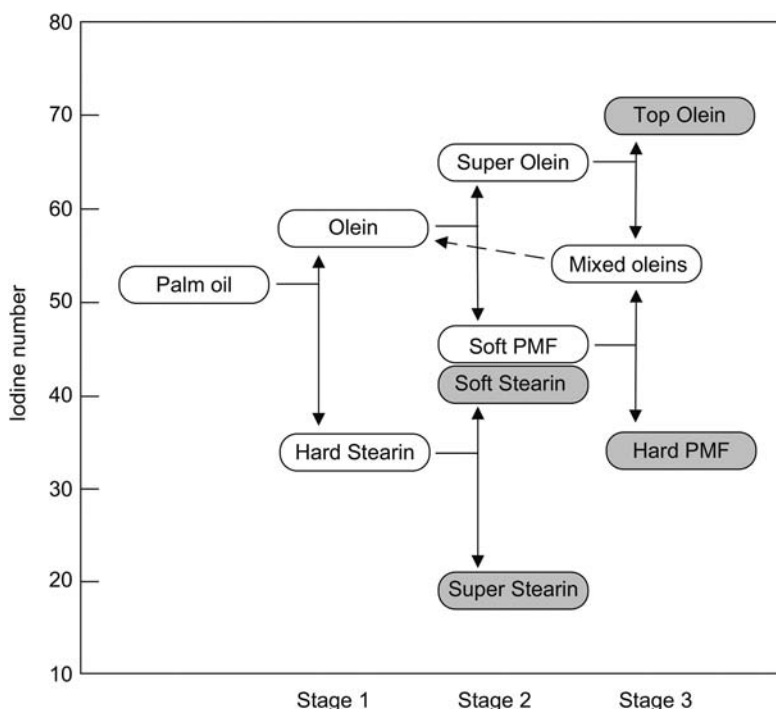


Figure 4.18 Fractionation of palm oil. The iodine number is an indication of the degree of unsaturation of the triglyceride fatty acids (see Section 4.2.1). (Based on data quoted by V. Gibon, in *Modifying Lipids for Use in Food*, see Further Reading.)

vacuum filtration. This technique, known as “winterisation”, is also commonly carried out with olive oil. It can also be applied to these and other oils after a hydrogenation stage to obtain fractions with particularly desirable characteristics. The two fractions obtained by winterisation are always referred to as “olein” and “stearin”. The sought-after, less saturated olein fraction is usually obtained in yields of 80–85%; the more saturated stearin fraction is not discarded, but is incorporated into the feedstock for the production of hydrogenated fats.

What are known as dry fractionation processes are a much more sophisticated version of winterisation, applied to higher melting oils such as palm oil (melting point 21–27 °C). The temperature of the oil is first increased to ensure that it is completely molten and then slowly cooled under continuous stirring (up to 24 h may be needed) until the requisite proportion has crystallised. The crystals are separated from the liquid oil by vacuum filtration. Depending on the objective, further rounds of fractionation may be carried out on the oil or crystal fractions. Figure 4.18 shows the fractionation stages that may be applied to palm oil. The oleins obtained are useful components of frying and salad oils. The first stage should completely separate the fully saturated triglyceride (mostly PPP^{xiii} content from the olein fraction and yields oleins free from cloud

^{xiii}The abbreviations used for triglyceride types are explained in Table 4.7.

formation. The stearin fractions are used in margarine and shortening formulations, in which they replace the need for hydrogenated triglycerides. The most valuable final fraction is the hard palm mid-fraction (hard PMF). This largely consists of the monounsaturated triglyceride POP. It has almost identical melting properties to those of cocoa butter and is therefore valuable as a cocoa butter substitute in chocolate manufacture.

Technologies such as fractionation and interesterification, which are discussed in the next section, have undoubtedly helped the oil and fat industry towards the nutritional goal of reduced *trans* fatty acid content. Since soya bean and palm oils account for about 60% of worldwide fat and oil consumption, the influence of oilseed manufacture cannot be underestimated. There are increasing concerns about the environmental impact of massive plantings of oil palms in South East Asia and soya in South America.

4.4.4 Interesterification

The previous sections have shown that although we do have some capability for changing the properties of a fat by manipulating its fatty acid composition, the properties we wish to alter often depend more on the distribution of the fatty acids among the triglycerides than the overall fatty acid composition. Interesterification is a procedure by which the fatty acids within the triglycerides of a fat or oil can be rearranged. The ester links between the fatty acids and glycerol are broken and then reformed, but the fatty acids do not necessarily return either to their original position or even to their parent glycerol molecule. When the reaction is carried out in the presence of a chemical catalyst, the fatty acids end up randomly distributed among all the glycerol hydroxyl groups in the fat (Figure 4.19).

After “clean-up” (as with hydrogenation) and the careful removal of all traces of water, the fat is exposed to the catalyst, usually sodium methoxide, at temperatures of 100–110

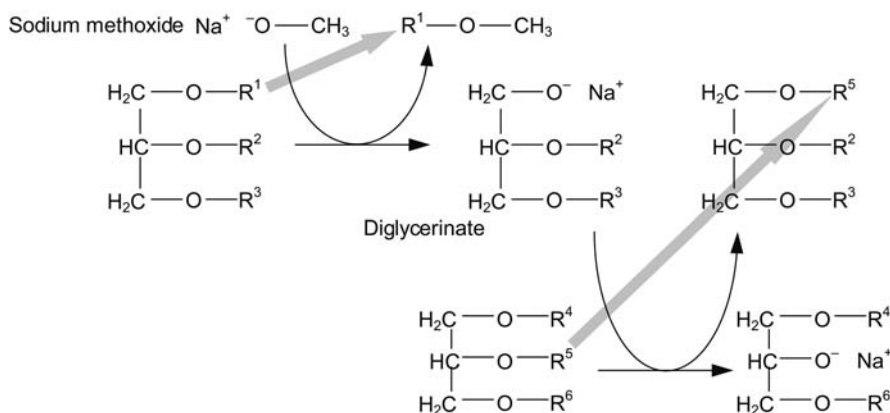


Figure 4.19 Triglyceride interesterification catalysed by sodium methoxide. The methoxide ion extracts a fatty acid from a triglyceride molecule to generate a diglycerinate. This then extracts a fatty acid from another triglyceride molecule, a process that can be repeated indefinitely. As fatty acids at all three positions on the triglyceride react, continuation of the sequence eventually leads to a completely random fatty acid distribution.

°C for about 30 minutes. At the end of the reaction, any residual traces of catalyst are readily washed out of the fat with water. In addition to the manipulation of lard crystallisation referred to earlier, this type of interesterification has been brought to bear on the *trans* acid problem in hydrogenated fats (see Section 4.3.1). Some of the vegetable oils converted into margarine or shortening are fully hydrogenated, so that they contain only saturated fatty acids, such as stearic and palmitic acids, and, of course, no residual unsaturated *trans* acids. They are then mixed in appropriate proportions with oil and subjected to interesterification. This is able to introduce just the right proportion of saturated acids into the oil to give an acceptable spreading fat without the elimination of desirable PUFAs or the introduction of *trans* acids. It is possible to avoid hydrogenation completely if fractions of fats or oils rich in saturated fatty acids, such as the stearin fractions from palm oil, are used in place of fully hydrogenated fats in interesterification.

A recent alternative to interesterification catalysed by sodium methoxide is enzymic interesterification. An appropriate blend of oils is passed through a series of reactors packed with silica particles coated with a thermostable lipase obtained from the thermophilic fungus *Thermomyces lanuginosus*. The entire system is maintained at 70 °C to ensure that the oil blend remains liquid. The specificity of the enzyme for the 1- and 3-positions of the triglyceride (like pancreatic lipase, see Section 4.4.1) results in the exchange reactions being largely restricted to the fatty acids at these positions. A particularly promising application of this concept uses the lipase from another fungus, *Mucor meihi*, to interesterify a mixture of a palm oil fraction rich in POP triglycerides and stearic acid. After diluting with hexane to ensure that both fat and fatty acid are in solution and that the resulting solution is not too viscous, the mixture is pumped slowly through a bed of polymer beads coated with enzyme in a reactor at about 60 °C. The enzyme attacks the palmitic acid rich 1- and 3-positions, but in the almost total absence of water the equilibrium of the reaction is such that the enzyme catalyses not only the removal of fatty acids from these positions, but also the reverse reaction. The end result is the formation of a low-cost fat with almost identical proportions of POP, POS and SOS to natural, and expensive, cocoa butter. As it also shares cocoa butter's polymorphic behaviour, it can be used as a cocoa butter extender (the commercial term) to enhance the profitability of chocolate manufacture (the consumer's term).

Another interesting application of this technique is the preparation of fats with special nutritive characteristics. Human digestion of fats depends on pancreatic lipase, with the fatty acids liberated from the 1- and 3-positions, together with 2-monoglycerides, being absorbed by the mucosa of the small intestine. It is observed that long-chain saturated fatty acids (*i.e.* 16 or more carbon atoms) are less well absorbed than their unsaturated counterparts, especially when they are in the outer positions of dietary triglycerides. This is because when the lipase liberates them, they readily form insoluble salts with any calcium ions in the vicinity. The calcium salts of unsaturated fatty acids are much more soluble. As we have seen, animal fats, including human milk fat, tend to have their saturated fatty acids in the 2-position, but plant fats are the reverse. This reduces the digestibility of plant fats when used in formula feeds for infants. The solution has been to use enzymic interesterification with tripalmitin and oleic acid as the feedstock, yielding a product with oleic acid in the 1- and

3-positions and palmitic acid in the 2-position. This is expected to find ready acceptance in infant formula feeds because it mimics human milk much more closely than cow's milk. Cow's milk has more or less the correct distribution of fatty acids in its triglycerides, but, compared with human milk, it is low in linoleic acid, an essential fatty acid.

4.5 Polar Lipids

The membranes of all living systems – not only the plasma membranes surrounding cells, but also those that enclose or comprise organelles such as mitochondria, vacuoles or the endoplasmic reticulum – are composed of an essentially common structure of specialised proteins and lipids. Polar lipids are also essential parts of the structure of the various lipoprotein particles (*e.g.* the chylomicrons) that are involved in the transport of lipids in the blood. A detailed account of these structures is outside the scope of this book, but the nature and properties of many of their lipid components are very important to food chemistry. Many polar lipids occur in small amounts in crude fats and oils and are also classically associated with egg yolk, in which they are major constituents.

The defining feature of polar lipids is that they are *amphiphilic*. This is to say that, although their molecules share with the triglycerides (and waxes) an affinity for non-polar environments (*i.e.* they are *lipophilic*),^{xiv} their molecules also include structural elements with an affinity for aqueous polar environments (*i.e.* they are *hydrophilic*). These two contrasting elements are apparent in the structure of phosphatidyl choline (Figure 4.20), also known as lecithin. Commercial lecithin actually contains a mixture of glycerophospholipids separated from crude soya bean oil. Typically, it contains about 15% phosphatidyl choline, 12% phosphatidyl ethanolamine and 10% phosphatidic acid, the remainder being mostly soya bean oil triglycerides.

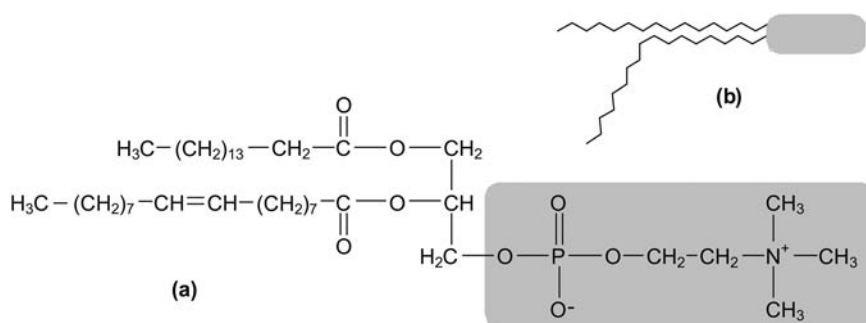


Figure 4.20 Phosphatidyl choline. (a) The molecule has two long-chain fatty acids, typically oleic and palmitic acids, as shown here, esterified to the central glycerol. This also carries choline attached through a phosphate group. The hydrophilic polar area of the molecule is shaded. (b) The small diagram shows that the molecule as a whole has a large hydrophilic head carrying a long hydrophobic tail.

^{xiv}Alternatively, *hydrophobic*.

Most of the important naturally occurring polar lipids are, like phosphatidyl choline, glycerophospholipids, with two long-chain fatty acid residues esterified with a glycerol molecule that also carries a phosphate group. The phosphate group is, in turn, esterified with one of a number of organic bases, amino acids or alcohols, which add to the hydrophilic character of the molecule. The structures of a range of glycerophospholipids are shown in Figure 4.21. Although a great many other phospholipid types have been described, from the point of view of the food chemist their properties are not significantly different from those shown here.

One other polar lipid that must be mentioned is cholesterol (4.12). Although not immediately obvious from the usual presentation of its chemical structure as shown here, the hydrocarbon part of the cholesterol molecule has similar dimensions to the paired fatty acid chains of the glycerophospholipids. The molecule is therefore able to occupy a similar position to the other polar lipids in membranes, *etc.* Esters of cholesterol with fatty acids occur as components of the lipoproteins in blood, but cholesterol is unesterified in membranes and in egg yolk.

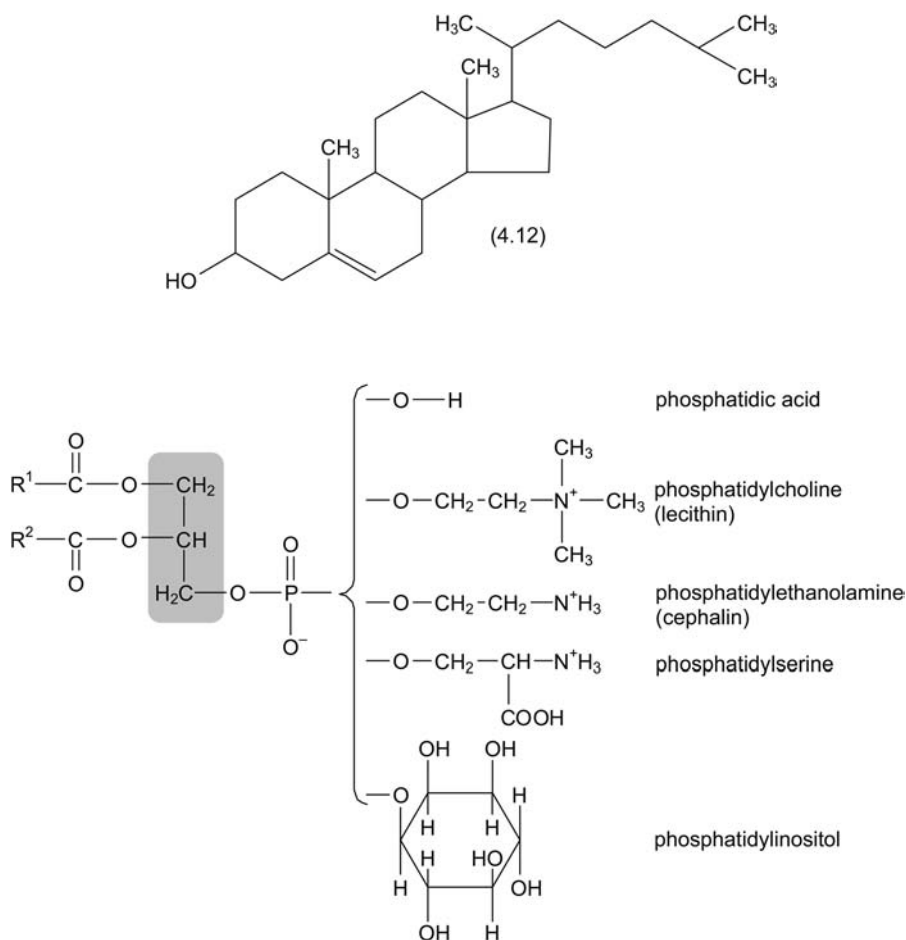


Figure 4.21 Glycerophospholipids. R¹-COO⁻ and R²-COO⁻ are fatty acids. The glycerol component is shaded.

4.5.1 Emulsifying Agents

The traditional importance of polar lipids in food systems is due to their ability to stabilise emulsions. Emulsions are colloidal systems of two immiscible liquids, one dispersed in the other, known as the continuous phase. Foods can provide examples of both oil-in-water emulsions (*e.g.* milk and mayonnaise) and water-in-oil emulsions (*e.g.* butter). In many cases the structure is complicated by the presence of suspended air, forming a foam, or solid particles.

Emulsions are prepared by vigorous mixing of the two immiscible liquids such that droplets of the dispersed phase are formed. However, a simple emulsion usually breaks down rapidly as the dispersed droplets coalesce to form a layer, which either floats on the surface or settles to the bottom of the vessel. The stability of an emulsion is enhanced by the presence of substances such as phospholipids, whose molecules contain both polar and non-polar regions. Figure 4.22 shows how such molecules are able to orientate themselves at the interface between the two phases and thus inhibit the coalescence of the droplets.

Emulsifiers, and their non-food relatives the soaps and detergents, are often described as *surfactants* (*surface active agents*). This is because one manifestation of their amphiphilic character is the ability to reduce the surface tension of water (see Chapter 13) as they dissolve. Not all surfactants have large lipophilic and hydrophilic groups. Polar organic solvents, which are miscible with both water and non-polar solvents, such as ethanol (C_2H_5OH) or acetone ($(CH_3)_2CO$), are regarded as surfactants because they lower the surface tension of water. The hydrophilic groups in many phospholipids include both phosphate groups (negatively charged at pH values usually found in food) and quaternary nitrogen atoms (positively charged) and are therefore classified as *zwitterionic* (see Chapter 5). Many other surfactants are acidic in character, *i.e.* they form

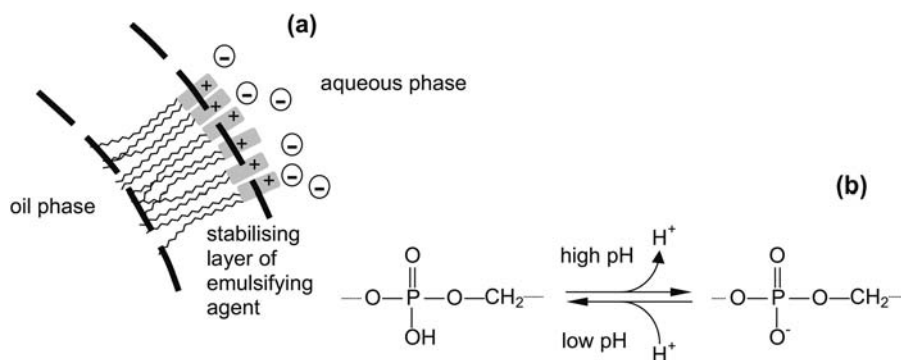


Figure 4.22 (a) Part of the surface of an oil droplet in an oil-in-water emulsion stabilised by a phospholipid such as lecithin. The phospholipid molecules orientate themselves at the oil–water interface to allow their polar groups to interact with the aqueous continuous phase. In most food situations the prevailing pH is low and the ionisable groups of the phospholipid will carry a net positive charge, as shown in (b). This, in turn, attracts the negatively charged ions present in the aqueous phase. The mutual repulsion of the layers of negative charges keeps the droplets apart and prevents them from coalescing.

Egg yolk actually consists of a suspension of lipid/protein particles in a protein/water matrix. Specific associations of lipids and proteins such as these are known as lipoproteins and are used in animal systems whenever lipid material has to be transported in an aqueous environment, such as blood. The best-known applications of egg yolk as an emulsifier are in mayonnaise and sauces such as hollandaise, but its role in cake-making is just the same. The lecithin recovered from soya bean oil during its preliminary purification is increasingly valued as an alternative natural emulsifying agent, particularly for use in chocolate and other confectionery, and also where a vegan product is required.

One important egg yolk protein, phosvitin (about 7% of the total yolk protein) should be mentioned here, although its emulsifying properties have not been much studied. Phosvitin is unusual in that serine constitutes around half of its total amino acid residues. A high proportion of the serine hydroxyl groups carry phosphate groups, so that about 60% of the egg's phosphate (obviously a key nutrient for the developing chick) is carried by this protein. Furthermore, almost all of the egg's iron is attached to this phosphate. The white of a newly laid egg has a pH of about 7.8, but over the next two to three weeks carbon dioxide diffuses out of the white through the shell and the pH of the white increases to 9 or higher (assuming that the egg does not contain a developing chick). Prolonged heating, such as when the egg is hard-boiled (*i.e.* 10 minutes or more in boiling water), leads to the release of hydrogen sulfide (H_2S) from the cysteine residues in various albumin proteins (see Figure 5.8), some of which diffuses into the yolk where it reacts with iron bound to the phosvitin to form iron sulfide. This gives a greenish-black colour to the surface of the yolk. Although in no way harmful to the consumers of hard-boiled eggs, it is very unsightly.

In 1965, Griffin introduced the hydrophile-lipophile balance (HLB) system, a scale of 1–20 for quantifying the emulsifying capability of surfactants. Although originally proposed for use with cosmetics, HLB values have become widely applied to food emulsifiers. The HLB value for an emulsifier was originally defined by the equation:

$$HLB = 20[1 - (S / A)]$$

where S is the *saponification number* of the ester and A is the *acid number* of the resulting acid. These are, to say the least, outdated chemical parameters and it is now common to regard S as the overall molecular weight of the emulsifier and A as the molecular weight of the hydrophilic part of the molecule. These definitions give no recognition to the differences in affinity for water between different hydrophilic groups – for example, the two hydroxyl groups of a monoglyceride compared with the phosphate and quaternary nitrogen of lecithin. Many quoted HLB values have been derived by empirical methods based on ascribing values to particular chemical groups, or from laboratory-based experimental comparisons. The HLB values of a selection of commercially important emulsifiers are given in Table 4.9. Surfactants with low HLB values, those between 4 and 6, are the most lipophilic and best suited to forming water-in-oil emulsions, whereas for oil-in-water emulsions HLB values in the range 8–18 are required. High HLB values indicate a high degree of hydrophilicity and solubility in water.

Table 4.9 HLB values of a range of permitted emulsifiers. The structures of the semi-synthetic emulsifiers asterisked are shown in Figure 4.23. The fact that the value for sodium stearyl-2-lactylate is >20 is a typical case of the more empirical approach to calculation of HLB values often adopted today. Only a selection of the synthetic emulsifiers used around the world is shown here. Details of the emulsifiers permitted in Europe, the USA, Australia and elsewhere are available on the websites listed in Appendix II.

Emulsifier	E number	Type	HLB value
Oleic acid	— ^a	Anionic	1.0
Glycerol dioleate	E471	Nonionic	1.8
Sorbitan tristearate (Span 65)*	E492	Nonionic	2.1
Glycerol monooleate	E471	Nonionic	3.4
Sorbitan monooleate (Span 80)	E494	Nonionic	4.3
Soya lecithin (crude)	E322	Zwitterionic	8.0
Diacetylated tartaric acid esters of monoglycerides*	E472	Anionic	8.0
Sucrose monolaurate*	E473	Nonionic	15.0
Polyoxyethylene sorbitan monopalmitate (Tween 40)*	E434	Nonionic	15.6
Sodium stearyl-2-lactylate*	E481	Anionic	21.0

^aOleic acid itself is not classified as a food additive in the EU but its sodium, potassium, calcium *etc.* salts (and those of other fatty acids) are permitted in the EC with the number E470.

Unfortunately, most food systems are far too complex for the simplistic application of HLB values. Almost all foods containing emulsified fats or oils also contain protein, and protein molecules are extremely effective emulsifying agents whose complex structures rule out the possibility of calculating HLB values. The normal folding of the polypeptide chains of proteins (see Chapter 5) ensures that the outer surface of the protein molecule is dominated by hydrophilic amino acid side-chains. By contrast, the interior of the molecule is dominated by hydrophobic amino acids. A major contributor to the maintenance of the correct folding of the protein is the drive to separate these hydrophobic side-chains from the aqueous environment surrounding the protein. When a protein molecule encounters an oil droplet, it naturally unfolds to expose its hydrophobic elements to the oil surface and, at the same time, presents a hydrophilic surface to the surrounding water – Figure 4.22 on a larger scale. Loops consisting of hydrophilic sections of the polypeptide chains will protrude some distance above the surface of the oil droplet. The adsorption of the protein on to the oil surface is effectively an irreversible denaturation.

The major role of food emulsifiers is usually assumed to be in foods that are most obviously emulsions, such as mayonnaise, in which an oil-in-water emulsion (actually olive oil and vinegar) is stabilised by egg yolk. However, food emulsions are much more widespread. For example, the fat in cake batters is dispersed by the egg yolk phospholipids. Soya lecithin is often incorporated into commercial cake recipes to reduce the amount of the (more expensive) egg required. The proteins from meat are responsible for emulsifying the fat in sausages, especially the featureless pink objects revered in the UK. One reason why so much fat pours out of meat sausages and burgers when they are grilled is that the thermal denaturation of the meat proteins (in particular the so-called sarcoplasmic proteins, see Chapter 5) destroys their fat-binding capability. The term “emulsifying salts” refers to substances often included alongside added emulsifiers in meat products and processed cheese, such as the sodium or potassium

liquid very viscous. The non-fatty particulate material from the bean, mostly polysaccharides and protein, accentuates this. The emulsifier molecules bind to the surface of the particles and give them a lipophilic surface, which keeps them apart and keeps the viscosity of the liquid chocolate low. Melted chocolate needs to be free-flowing if it is to be used to coat biscuits or to flow into elaborate moulded shapes for Easter eggs.

4.5.2 Milk Fat, Cream and Butter

Dairy products provide examples of both oil-in-water and water-in-oil emulsions. The fat in milk occurs in very stable globules, mostly between 4 and 10 μm in diameter. There are $1.5\text{--}3.0 \times 10^{12}$ globules in 1 L of milk. Globules of such a small size should, according to Stokes' law, take about 50 h to float to the top of a pint bottle of milk and establish the familiar layer of cream, but we know that half an hour will in fact suffice. This implies that much larger particles are involved, with effective diameters up to 800 μm . This clustering of globules is clearly no ordinary coalescence because evaporated milk, which is sterilised after canning by holding it at temperatures $>100^\circ\text{C}$ for several minutes, shows no tendency to "cream". The explanation for this curious behaviour lies in the nature of the milk fat globule membrane that surrounds each globule. This consists of specific lipoproteins and polar lipids arranged around the globule in a similar arrangement to that shown in Figure 4.22. The lipids in this membrane, including those associated with proteins to form lipoproteins, are typical of those found in the plasma membrane of animal cells, including cholesterol and its esters formed with long-chain fatty acids, phosphatidyl ethanolamine, choline, serine and inositol, and sphingomyelin. The membrane is most probably acquired as the milk fat globule is secreted through the plasma membrane of the mammary gland cells into the milk ducts. Clustering is caused by cross-linking through one specific type of protein, macroglobulin, which occurs in the aqueous phase of the milk in very small amounts. Heating above 100°C for a few minutes (but not pasteurisation) denatures this protein and creaming is prevented.

Creaming is also prevented in homogenised milk, in which the fat globules are reduced to about 1 μm in diameter by passage through very small holes at high pressures and velocities of about 250 m s^{-1} . The vast increase in total surface area of the fat globules, from about $75\text{ m}^2\text{ dm}^{-3}$ of milk to about $400\text{ m}^2\text{ dm}^{-3}$, results in extra proteins being adsorbed from the aqueous phase of the milk. These proteins inhibit the coalescence of the globules, but do not interact strongly with the macroglobulin, so creaming no longer occurs.

Butter-making results in the formation of a water-in-oil emulsion. Cream with a fat content of 30–35% is inoculated with a culture of bacteria and incubated for a few hours. The bacteria produce characteristic flavour compounds, such as diacetyl (butanedione). The cream is then mechanically agitated (churned) sufficiently to disrupt the membranes of the fat globules and allow them to coalesce. During mixing, a proportion of the aqueous phase, known as buttermilk, is trapped as small droplets. These are prevented from coalescing by the rigidity of the fat and by the layer of proteins and polar lipids that forms at the fat–water interface. Butter usually contains about 20% water, to which salt is added, nowadays as a flavouring, but originally to deter the growth of unwanted microorganisms. Margarine has buttermilk and salt added to it during the final blending stages, but because it lacks its own emulsifying agents, soya lecithin and other emulsifiers also have to be added.

4.5.3 Synthetic Emulsifiers

Figure 4.23 shows a range of semi-synthetic emulsifiers and their structures. These are semi-synthetic in the sense that they are based on naturally occurring fats or oils, but are modified in one of several chemical processes that each give a mixture of closely related chemical species – for example, polyglyceryl polyricinoleate (PGPR), an emulsifier often used in place of lecithin in chocolate. As shown in outline in Figure 4.24, PGPR is synthesised from glycerol and ricinoleic acid (from castor bean oil), but the final product contains individual molecules differing in both the number of glycerol residues and the number of ricinoleic acid residues.

In terms of bulk usage, monoglycerides are by far the most important, as well as being the starting point for the industrial synthesis of many other types of emulsifiers. They are manufactured by the interesterification of triglycerides with excess glycerol in the presence of sodium hydroxide. The fatty acids become randomly distributed throughout the glycerol molecules present, resulting in a mixture of “monoglycerides”, which also contains some glycerol and di- and triglycerides. If required, reasonably pure monoglycerides can be obtained from this mixture by fractional distillation under vacuum.

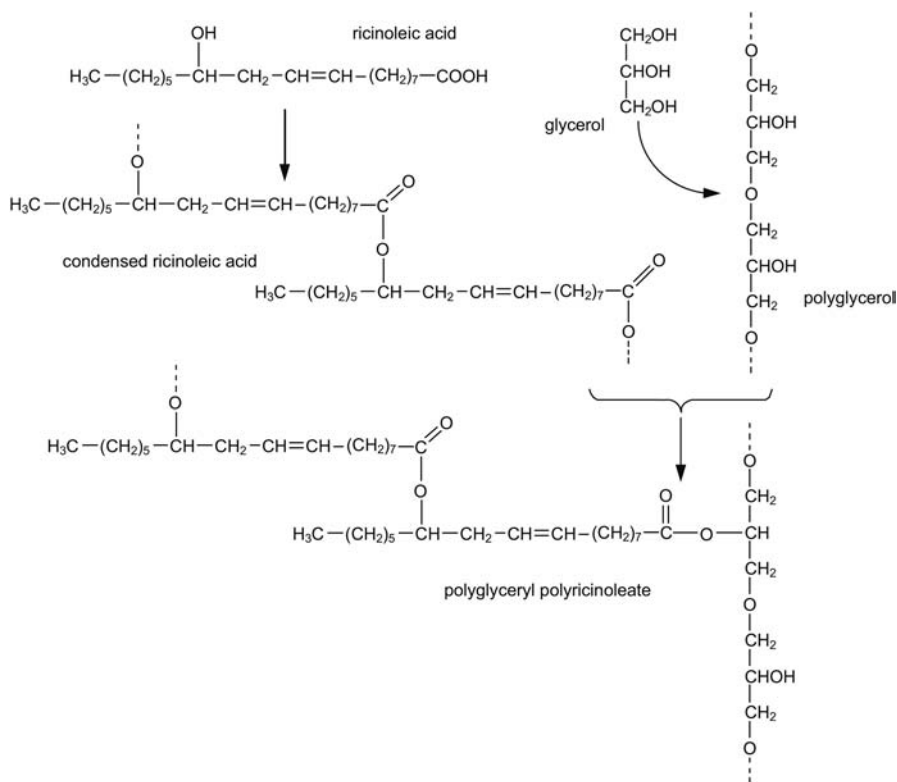


Figure 4.24 An outline of the synthesis of polyglyceryl polyricinoleate (PGPR). The polyglycerol moiety usually contains two to four glycerol units and the polyricinoleate moiety about five fatty acid units. There may be several polyricinoleate chains attached to the polyglycerol moiety.

Baked goods (including bread, burger buns, cakes and biscuits) account for a high proportion of the usage of semi-synthetic emulsifiers. Monoglycerides of saturated fatty acids are useful for slowing down the rate of staling. The straight hydrocarbon chains are able to occupy the central core of the amylose and amylopectin helices, slowing down retrogradation (see Chapter 3, Section 3.1). Diacetyl tartaric acid esters of monoglycerides (DATEMS) are included at levels of about 0.5% of the weight of flour in many commercial bread recipes, where they not only delay staling, but also increase the loaf volume and improve the crumb texture, apparently by binding to gluten proteins.

4.5.4 Phytosteroids

Cholesterol and bile salts were mentioned earlier in this chapter and provide the justification for including a brief consideration of a further group of steroids here, although the phytosteroids can hardly be classified as polar. Although their presence in plant tissues has long been recognised, their recent inclusion in some margarines and low-fat spreads has brought them into prominence because their consumption can have a beneficial effect on serum cholesterol levels.

The three sterols shown in Figure 4.25 are abundant in plants, in which, together with smaller amounts of the phytostanols, they have the same role in cell membranes as cholesterol does in animal cells.

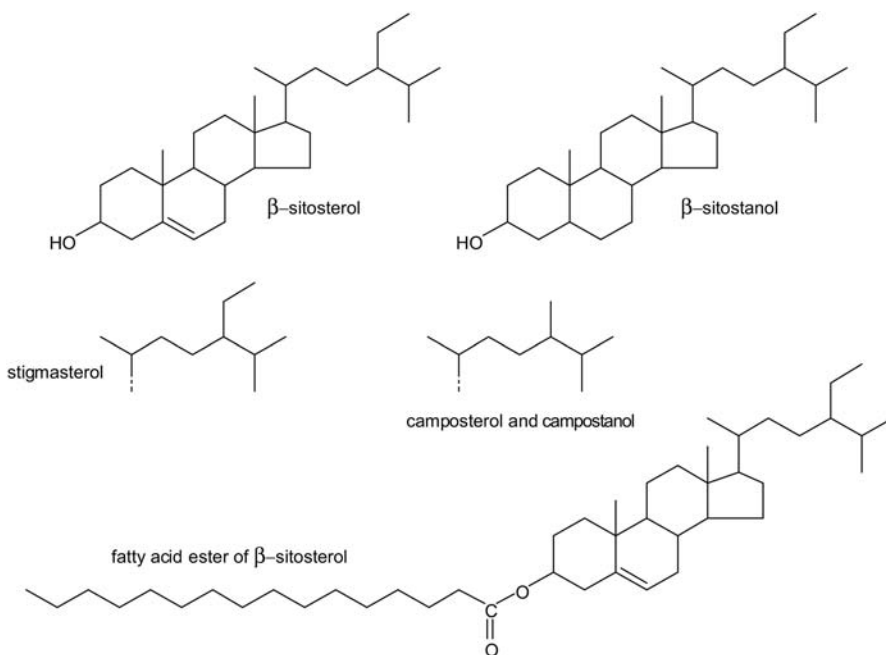


Figure 4.25 Structures of the common phytosterols and phytostanols. The phytostanols differ from the phytosterols in being fully saturated; otherwise, the differences between them lie entirely within the side-chains. The fatty acid (normally stearic) esters are the form added to margarines.

Western diets usually deliver about 200–400 mg daily of these plant sterols (with vegetarians and vegans consuming amounts towards the top of this range), about the same as the amount of cholesterol, plus about one-tenth of this amount of phytosteranols. One of them, β -sistosterol, has been prescribed for patients with hypercholesterolaemia for many years, but over the last decade or so the phytosterols have been used more widely as “nutraceuticals”^{xv} available to the general public.

Cholesterol is a major component of bile (alongside the bile salts, see Section 4.5), which is secreted into the small intestine from the gall bladder. (Cholesterol appears to have a role in protecting the membranes of the bile duct from the damaging effects of bile acids.) However, the cholesterol and bile acids (once their roles in emulsifying dietary fats are complete) are promptly reabsorbed into the bloodstream for return to the liver. Phytosteroids inhibit this reabsorption and, in doing so, place a strain on the cholesterol economy of the body that can lead to significant reductions in the levels of cholesterol circulating in the bloodstream. Some non-starch polysaccharides also bind cholesterol and bile acids (see Chapter 3, Section 3.4), leading to their excretion in faeces, which has a similar effect on blood cholesterol levels. Attaching a fatty acid chain to the phytosterols greatly increases their solubility in oils and fats and makes them effective at much lower doses.

The usual sources of phytosterols are vegetable oils, such as soya and corn, from which they are extracted along with lecithin and tocopherols during the refinement of the oil prior to hydrogenation. One important, if unlikely, source is tall oil. Tall oil is extracted from pine trees during the manufacture of wood pulp for paper-making.

In studies of patients with elevated serum cholesterol levels, the daily consumption of 2 g or more of phytosterols has been shown to reduce levels of serum cholesterol by 8–13%, particularly the LDL form. It is elevated levels of LDL cholesterol rather than the overall cholesterol figure that is a major risk factor for heart disease. Bearing in mind that the typical daily consumption of spreading fats, butter, margarine, *etc.* in Western Europe is about 20 g, levels of up to 20% of phytosterols (as esters) are being added to supplement margarines and low-fat spreads. It has been calculated that the consumption of 3 g per day of phytosterols could lower the risk of heart disease by between 15 and 40%, depending on other risk factors.

Only a small proportion of the phytosterol is absorbed, and even this is rapidly eliminated *via* the bile. There appears to be no significant safety issue associated with consumption at even the highest levels suggested here. However, the widespread consumption of margarines and low-fat spreads supplemented by phytosterols faces a number of obstacles, in particular their cost. At the present time, supplemented products (in the UK) cost about twice as much as their non-supplemented counterparts and manufacturers have little to say on the amounts of their spreads that would need to be consumed to have a measurable effect on health. The question of their suitability for healthy young children does not seem to have been addressed as extensively as it has

^{xv}The term “nutraceutical” (combining “nutrition” and “pharmaceutical”) first appeared in 1989. It is used to describe substances deliberately included in manufactured food products (either as food additives or as constituents of other ingredients) that have a recognised health benefit without qualifying – as do, for example, vitamins – as essential nutrients.

for adults with hypercholesterolaemia. In real families, offering two distinct piles of sandwiches may not be as straightforward as one would wish.

4.6 Special Topics

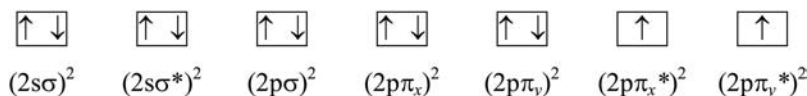
4.6.1 Hydrogenation in Detail

The manufacture of margarines and shortenings is highly dependent on the process of hydrogenation for the conversion of liquid oils into plastic fats. The conditions used for the reaction (temperature, catalysis, *etc.*) were discussed earlier in this chapter, but the following is a brief account of the detailed chemistry. The process involves a number of reactions, illustrated in Figure 4.26.

The tendency of linoleic acid residues to be converted into oleic acid (desirable), compared with the conversion of oleic acid to stearic acid (undesirable), is expressed as the selectivity ratio (SR), the ratio of the rate constants for the two processes. The SR is affected by several operating parameters, including temperature, pressure (*i.e.* H_2 concentration), the catalyst concentration and the degree of agitation (stirring rate) in the reactor. An increase in any of these parameters increases the rate of hydrogenation, but higher temperatures and catalyst concentrations each lead to high SR values and high proportions of *trans* acids, whereas higher pressures and vigorous stirring have the opposite effect. If the catalyst surface is densely packed with hydrogen atoms, then hydrogenation is more likely and selectivity is low. By contrast, if hydrogen atoms are sparse, then the SR will be high, but there will be a much greater likelihood of isomerisation because there will not be a second conveniently placed hydrogen atom to complete the hydrogenation process.

4.6.2 Singlet and Triplet Oxygen

These terms relate to the bonding between the two atoms in an oxygen molecule. The oxygen molecule, O_2 , has a total of 12 valency electrons originating in the outer shells of the two oxygen atoms. In its usual low-energy ground state, eight of these are located in seven different molecular orbitals. In five of the orbitals, the paired electrons spin in opposite directions, as indicated by the arrows in the first five of the following boxes:



The asterisks indicate antibonding orbitals. The number of bonds between the atoms is obtained by subtracting the number of electrons in the antibonding orbitals from the number of electrons in bonding orbitals and dividing the result by two.

The two highest molecular orbitals contain single electrons with parallel spin. This gives the molecule a *multiplicity* (S) of 3, from the equation:

$$S = 2s + 1$$

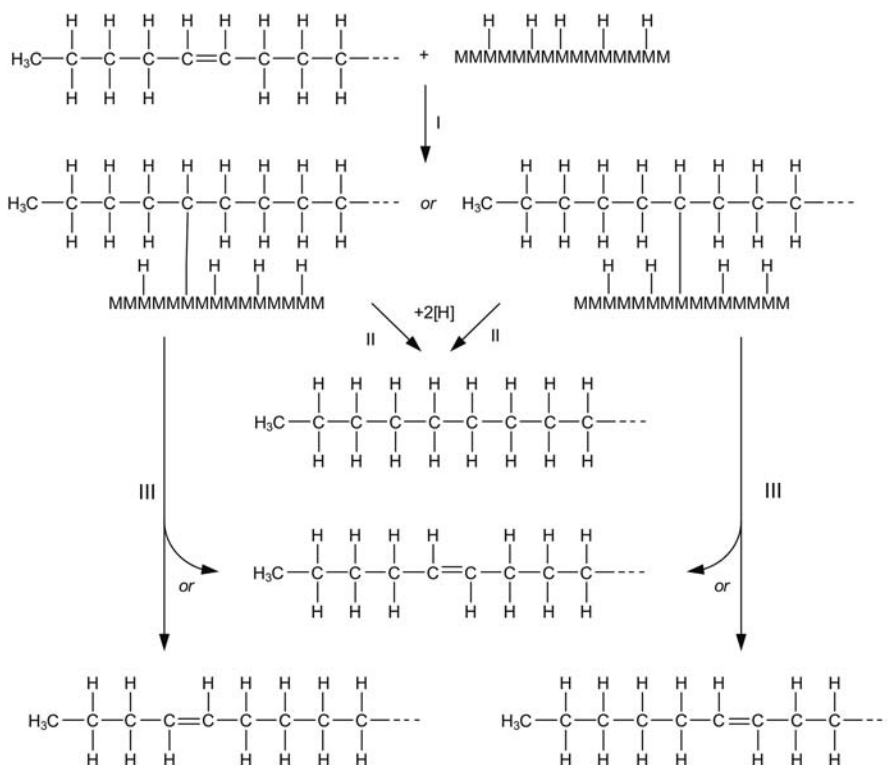


Figure 4.26 Hydrogenation reactions. (Reaction IV, the re-entry of isomerised by-products into the sequence, has been omitted.)

- I Hydrogen is absorbed onto the metal surface of the catalyst and is effectively converted from H_2 into individual hydrogen atoms bound to metal atoms. At the site of a double bond, the fatty acid chain is bound to both the metal surface and to one hydrogen atom from the metal surface. This link may be formed at either end of the double bond. From this point on, there are two possible outcomes.
- II A second hydrogen atom reacts to fully saturate the double bond and the hydrogenated fatty acid chain dissociates from the catalyst.
- III The fatty acid chain dissociates from the catalyst without further reaction and the double bond is restored. However, there is no special reason why the hydrogen returned to the catalyst should be the original one donated by the catalyst, so the resulting double bond can be in the original position or between a neighbouring pair of carbon atoms in the chain. The result is an apparent migration of the double bond, *e.g.* from 9,10 to 10,11 or 8,9. The newly formed bonds are most likely to be in the *trans* rather than the original *cis* configuration because this is the thermodynamically favoured form.
- IV A proportion of the products of the migration will re-enter the sequence, some ending up with *trans* double bonds where they originated, but others ending up even further down the chain.

where s is the sum of the spins of all the electrons, counting $\uparrow$ as $+1/2$ and $\downarrow$ as $-1/2$. A multiplicity of three is known as the *triplet* state. The triplet state of oxygen molecules in their lowest energy condition, the ground state, is unusual. Most molecules have *singlet* ground states, in which all the electron spins are paired. Triplet oxygen is denoted as $^3O_2$. In addition to its triplet ground state, oxygen molecules can also occur

in two *excited*, high-energy singlet states with the two highest orbitals filled in the following manner $-\frac{1}{2}$: $\uparrow\downarrow$ $\square$ or $\square$ $\uparrow\downarrow$, written as $^1\text{O}_2$ and $^1\text{O}_2^*$, respectively. The other orbitals are as shown previously. These excited states are extremely short-lived.

Only the first of these excited states, $^1\text{O}_2$, is of relevance to us. This has the two electrons that were unpaired in the singlet oxygen in a $2p\pi$ orbital, leaving the corresponding antibonding $2p\pi^*$ orbital empty. The resulting molecule is an extremely reactive electrophile because it seeks to fill the empty orbital – for example, by reaction with unsaturated fatty acids. The energy difference between this and triplet oxygen is $+24 \text{ kcal mol}^{-1}$.

4.6.3 Triglyceride Crystals

The fatty acid chains in an individual triglyceride molecule are organised into either a “chair” or a “tuning fork” arrangement (Figure 4.27). Which of these is preferred depends on the symmetry of the triglyceride. The tuning fork arrangement is favoured in symmetrical triglycerides because it brings the two identical fatty acids in the 1- and 3-positions alongside each other. By contrast, the chair arrangement is favoured in asymmetrical triglycerides. Neighbouring triglyceride molecules in a fat crystal are stacked in layers, with the parallel hydrocarbon chains packed closely together and running more or less perpendicular to the parallel planes of the glycerol groups and the planes of terminal methyl groups. These planes are sometimes referred to as the “methyl terraces”. Neighbouring triglyceride molecules overlap in different patterns to place the methyl terraces either two or three chain lengths apart. As with the choice between the tuning fork and chair arrangement, the overlap pattern is dictated by the

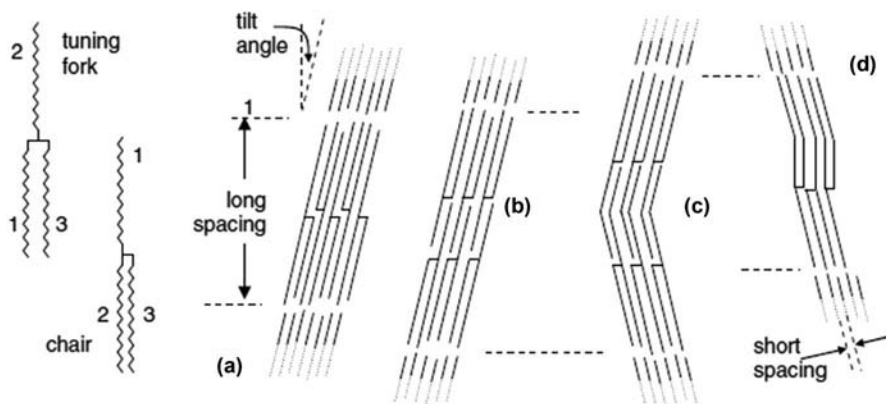


Figure 4.27 Organisation of triglyceride molecules in fat crystals. In the more stable crystal types shown here (β' and β , as discussed in the text), the triglyceride molecules stack alongside each other so that the terminal methyl groups form horizontal planes, the methyl terraces (shown as heavy dashed lines). The dimensions of the long and short spacings and the tilt angle, established by X-ray crystallography, characterise the different forms. The long spacing reflects the different overlap patterns, double chain length in (a) and (d) and triple chain length in (b) and (c).

need to accommodate fatty acids of different chain lengths and the distortions introduced by double bonds (Figure 4.27b–d).

The most important differences between the three crystal types are in their melting points and crystal forms. In the crystalline state, the hydrocarbon chains are arranged in extended zigzags with all the carbon atoms in the same vertical, or near-vertical, plane. Figure 4.28a–c show the chains viewed end-on, along their long axes.

In the unstable α forms, Figure 4.28a and b, the chains are arranged in a hexagonal pattern, but the planes of the hydrocarbon zigzag are oriented randomly. The crystal structures of the β' and β forms are formally described as orthorhombic and triclinic, respectively. The tilting of the chains in the triclinic form, shown in Figure 4.28f, allows the most compact packing of the chains. Although not allowed for in the formal definition of an orthorhombic crystal, these fatty acid chains are also assumed to tilt.

Recent X-ray crystallographic studies of cocoa butter and its constituent triglycerides provide an excellent illustration of how an understanding of crystal structure can explain aspects of fat behaviour. Figure 4.29 shows, in simplified form, the crystal packing of 1-palmityl-2-oleyl-3-stearyl glycerol (POS), the dominant triglyceride of cocoa butter in its two most stable polymorphic forms, β -V and β -VI. Essentially similar patterns are also seen in the corresponding polymorphic forms of pure cocoa butter, in which POP and SOS, along with POS, make up the bulk of the triglycerides. The first of these, β -V, is the form which gives the physical characteristics desired by the chocolate-maker. The second, the β -VI form, with a slightly higher melting point, is the form found in the unwanted crystalline bloom on the surface of chocolate that has been mishandled. The

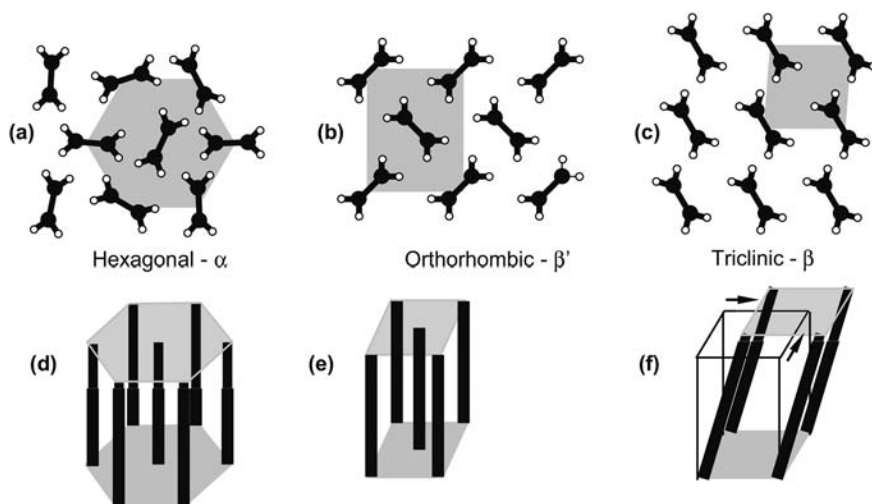


Figure 4.28 Crystal packing in triglycerides. In (a), (b) and (c), the zigzag hydrocarbon chains of the fatty acid residues are viewed end-on. The grey areas emphasise the geometrical relationships of the chains. In the hexagonal and orthorhombic arrangements, (d) and (e), the chains are shown perpendicular to the planes formed by their CH_3 ends, the so-called methyl terraces, whereas in the triclinic arrangement (f) the chains are tilted in both vertical planes. It is generally assumed that the chains in triglycerides are tilted in an orthorhombic arrangement.

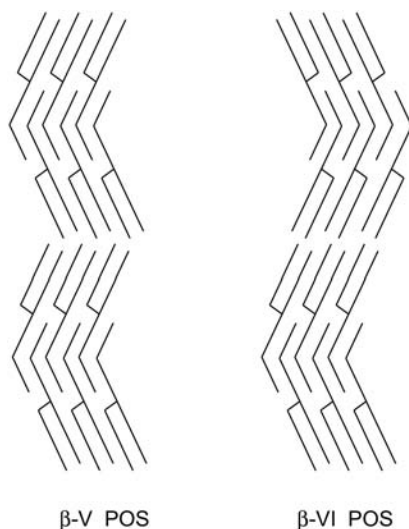


Figure 4.29 Crystal packing of 1-palmityl-2-oleyl-3-stearyl glycerol (POS), redrawn from the data of J. B. van Mechelen, *et al.*, *Acta Crystallogr. B*, 2006, **62**, 1121-1130 and 1131-1138, with the kind permission of the International Union of Crystallography, Chester, UK. The kinked oleic acid chains are packed between layers of straight saturated fatty acid chains, here illustrated as palmitic acid, but in cocoa butter also stearic acid. The difference between the two arrangements lies solely in the relationship between the successive layers.

packing of the β -VI form is just sufficiently favoured thermodynamically to provide the explanation for the transition between the two forms.

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5 Proteins

The proteins are the third class of macrocomponents in living systems, and therefore in foodstuffs, that we will consider.ⁱ Proteins are polymers with molecular weights ranging from about 10 000 to several million and are often described as having a highly complex structure. In fact, there is a great deal about the structure of proteins that is fairly straightforward. The monomeric units of which they are composed, the amino acids, are linked together by a single type of bond, the peptide bond, and the range of different amino acids is both limited in number and essentially common to all proteins. Furthermore, the polypeptide chains of proteins are never branched.

The special character of proteins lies in the subtlety and diversity of the variations, both in structure and function, which nature works on this simple theme. The properties and functions of a particular type of protein depend entirely on the precise sequence of its amino acids, unique to that protein. Unlike the polysaccharides, there cannot be anything vague about the exact length of the chain. If even one amino acid in the sequence is out of place, then it is likely that the protein will lose all or some of its biological activity. It is the sequences of the amino acids in proteins that are defined by the sequences of bases in the DNA that make up our genes. The amino acid sequence of a protein, the β -lactoglobulin of cow's milk, is shown in Figure 5.1. Although in some respects typical, this protein has been selected for illustration purposes solely because its unusually small size makes it easier to fit on the page. Most proteins have polypeptide chains many times this length. Sequences such as these may appear random, but are, in fact, very tightly controlled and reproduced exactly in every molecule of the protein synthesised by a particular organism. With possibly only a few changes, the sequence will normally be reproduced in closely related species and often throughout much larger taxonomic groups.

ⁱIn spite of their fundamental importance to living systems, the nucleic acids RNA and DNA are of almost no significance as dietary components in their own right. However, their presence has from time to time caused concern to opponents of genetically modified food crops. One class of their breakdown products, the purines, are implicated as a cause of the painful joint condition, gout. Some of the same purines also contribute to the desirable taste of many foods, as considered in Chapter 7.

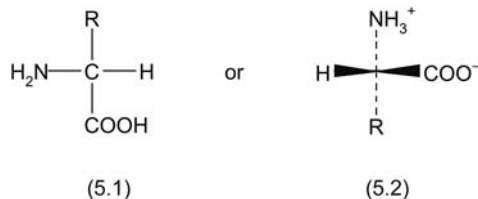
NH_2 - Glu-GIN-Leu-Thr-Lys-Cys-Glu-Val-Phe-Arg-Glu-Leu-Lys-Asp-Leu-Lys-Gly-
 Tyr-Gly-Gly-Val-Ser-Leu-Pro-Glu-Trp-Val-Cys-Thr-Thr-Phe-His-Thr-Ser-Gly-
 Tyr-Asp-Thr-Glu-Ala-Ile-Val-Glu-Asn-AsN-GIN-Ser-Thr-Asp-Tyr-Gly-Leu-Phe-
 GIN-Ile-AsN-AsN-Lys-Ile-Trp-Cys-Lys-AsN-Asp-GIN-Asp-Pro-His-Ser-Ser-
 Asn-Ile-Cys-Asn-Ile-Ser-Cys-Asp-Lys-Phe-Leu-AsN-AsN-Asp-Leu-Thr-AsN-
 AsN-Ile-Met-Cys-Val-Lys-Lys-Ile-Leu-Asp-Lys-Val-Gly-Ile-AsN-Tyr-Trp-Leu-
 Ala-His-Lys-Ala-Leu-Cys-Ser-Glu-Lys-Leu-Asp-GIN-Trp-Leu-Cys-Glu-Lys-
 Leu- COOH

Figure 5.1 Amino acid sequence of bovine β -lactoglobulin, one of the principal whey proteins. The standard three-letter codes shown in Figure 5.2 are used. The start and finish of the sequence are known as the amino (NH_2) and carboxyl (COOH) ends, respectively. The use of these terms is explained in Figure 5.3.

As we will see later in this chapter, the proportions of the various amino acids in the proteins we consume are important, but the total quantity is equally important. Table 5.1 lists the protein content of a wide variety of different foodstuffs consumed in the UK.

5.1 Amino Acids

All but two of the amino acids that occur in proteins have the same general formula (5.1). The structural formulae in Figure 5.2 show how the exceptions, proline and hydroxyproline, do not fit this pattern. The central carbon atom carrying the side-chain (R), which characterises the particular amino acid in addition to carrying the carboxyl and amino groups, is known as the α -carbon.ⁱⁱ Except in the case of glycine, where $\text{R} = \text{H}$, each of the four groups attached to the α -carbon atom is different, *i.e.* the α -carbon is asymmetrically substituted. This means that amino acids, like sugars (see Chapter 2, Section 2.1), are optically active. All the amino acids found in proteins are members of the L-series, *i.e.* they have the optical configuration shown in formula (5.2).



Formula (5.2) shows both the amino and carboxyl groups in their ionised condition, giving what is known as a zwitterion, the state that prevails at neutral pH. D-Amino acids do, in fact, occur in nature, but not in proteins; they are found in bacteria as components of the cell wall and in certain antibiotics. Free amino acids are not very important to food chemists, although they do contribute to the flavour of some foods, as indicated in Chapters 2 and 7.

ⁱⁱThis use of the Greek alphabet was first introduced in the context of fatty acids, see Figure 4.2.

Table 5.1 Total protein contents of a range of foods consumed in the UK. These figures, which should be regarded as typical rather than absolute values for the particular food concerned, are derived from McCance and Widdowson's 'Composition of Foods Integrated Dataset' on the nutrient content of the UK food supply, <https://www.gov.uk/government/publications/composition-of-foods-integrated-dataset-cofid> (last accessed in 2020). This site provides access to the database of nutrient composition of nearly 3000 different foods, including both raw materials and food products. It can also be expected to provide indirect access to updated data published in the future.

Food	Total protein (%)	Food	Total protein (%)
White bread	8.7	Hen's egg white	10.8
Wholemeal bread	9.4	Hen's egg yolk	16.4
Rice, white, uncooked	6.4–8.1	Beef (fillet steak, grilled)	21.9
Pasta, dried, white	6.6	Lamb's liver (raw)	20.3
Cornflakes	7.1	Cod fillet (raw)	17.5
Cow's milk (whole)	3.4	Salmon (canned)	23.6
Human milk	1.3	New potatoes (boiled)	1.8
Soya milk	2.4	Potato chips (fried)	3.2
Cheese, hard (<i>e.g.</i> Cheddar)	23–25	Frozen peas (boiled)	5.5
Cheese, soft (<i>e.g.</i> Camembert)	21.5	Soya beans (raw, dried)	35.9
Tofu (steamed)	8.1	Almonds	21.2
Yoghurt (plain)	5.7	Peanuts (dry roasted)	25.7

The structures of the side-chains of the 20 amino acids found in most proteins are shown in Figure 5.2. Two other amino acids, hydroxyproline and hydroxylysine, are also included. These two only occur in some of the structural proteins of animals, notably collagen (see Section 5.5.4). They are not included in the genetic code, but are the product of hydroxylation reactions that occur after proline and lysine have been incorporated into protein molecules (see Section 5.5.4). Various approaches are used to classify amino acids, but the most useful is to consider them in terms of the properties of their side-chains rather than their chemical structures. The ionisation of some side-chains is dependent on the pH of the protein's immediate environment or the special conditions prevailing at the active site of an enzyme.

5.2 Protein Structure

The amino acids of a protein are linked together by peptide bonds formed by the loss of a molecule of water when the amino group of one amino acid reacts with the carboxyl of another. The peptide bond has the amide structure shown in Figure 5.3.

The actual reactions involved in protein synthesis in living systems are, of course, much more complex than the simple arrow in Figure 5.3 might imply. This is not least because the enzymes concerned, part of the cell structures known as ribosomes, not only have to forge the links, but also to ensure that the amino acids are combined in the correct sequence.

The electrons of the carbonyl group are delocalised and give the C–N bond considerable double-bond character. As a result, free rotation about the C–N bond is inhibited and, as shown in Figure 5.4, all six atoms lie fixed in a single “amide plane”. Figure 5.4 shows how a length of polypeptide chain can be visualised as a series of amide planes

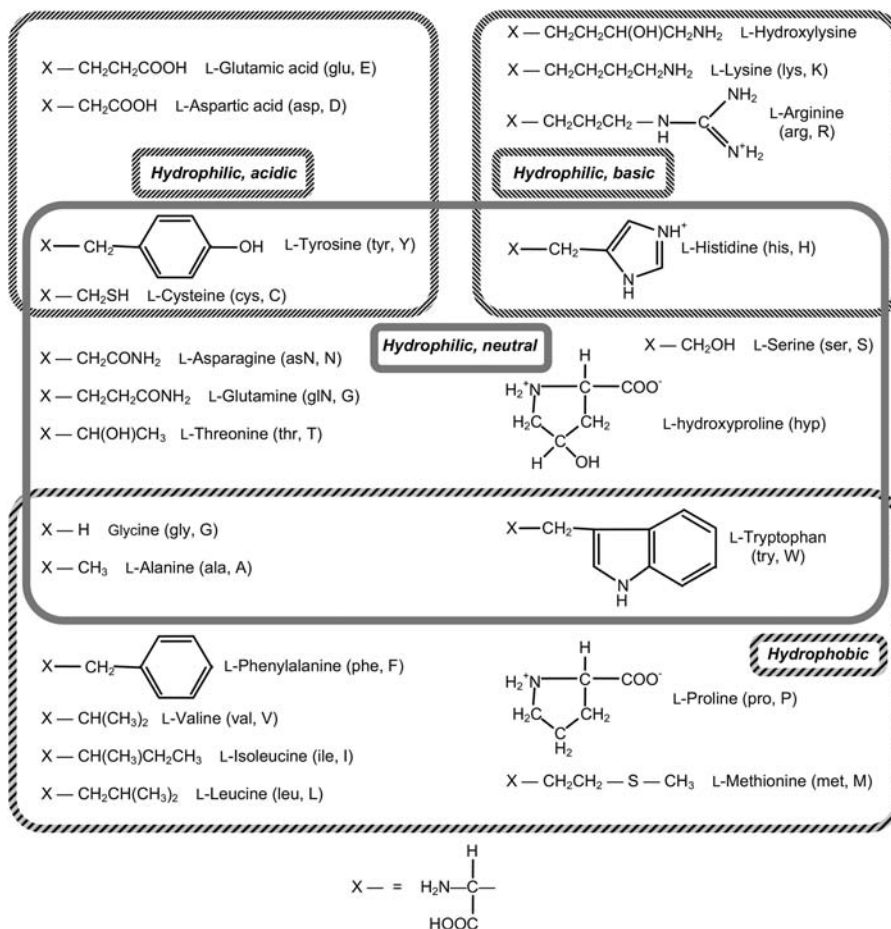


Figure 5.2 Structures of the amino acids commonly found in proteins. All these amino acids, except L-hydroxylysine and L-hydroxyproline, are primary amines, *i.e.* the nitrogen atom is attached to one carbon and two hydrogen atoms. The two exceptions are secondary amines, in which the nitrogen atom is attached to two carbon atoms, the second of these being in effect the far end of the chain curled round and attached to form a ring. Apart from L-hydroxylysine and L-hydroxyproline, the names of all these amino acids have two abbreviated forms, shown here in parentheses. The single letter codes are more convenient when writing long amino acid sequences, such as those of entire proteins.

linked at the α -carbon atoms of successive amino acid residues. The polypeptide chain in Figure 5.4 has been drawn with scant regard for the tetrahedral distribution of the bonds about the α -carbon atoms. In reality, the chain takes up more compact folding arrangements that can be defined by the angles of rotation about the $C_\alpha-N$ and $C_\alpha-C$ bonds. When a particular pair of such angles is repeated at successive α -carbon atoms, then the polypeptide chain takes on a visibly regular shape, usually a helix, but at the extreme will form a zigzag arrangement not unlike that in Figure 5.4.

The folding of the polypeptide chain in a protein is determined by its amino acid sequence in ways which molecular biologists are beginning to understand. Sophisticated

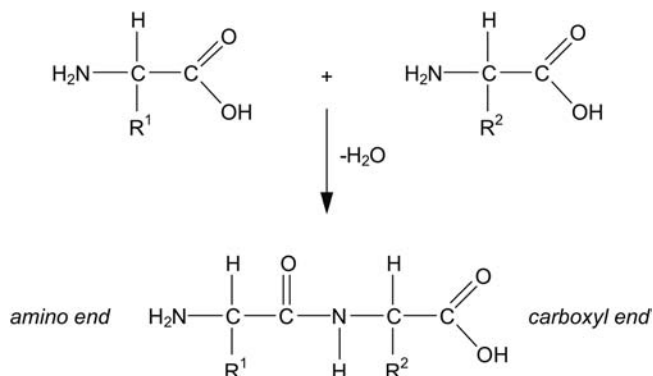


Figure 5.3 Formation of a peptide bond.

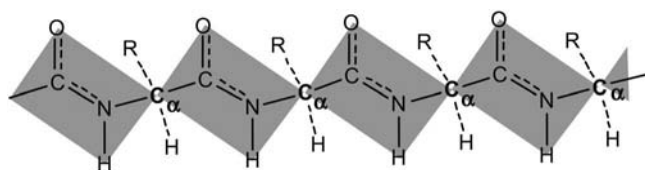
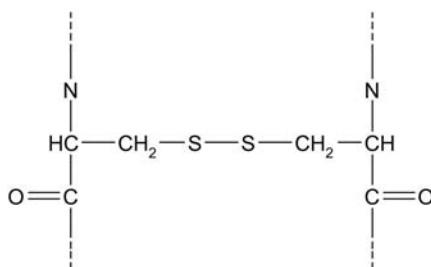


Figure 5.4 The amide planes of the polypeptide chain.

computer programs are able to predict with considerable accuracy the most likely configuration that a given sequence of amino acids will take up. Within the molecule, the unique patterns of folding of the peptide chain are maintained by bonds of various types. Covalent linkages, so-called sulfur bridges, are found linking cysteine residues (5.3).ⁱⁱⁱ



(5.3)

Hydrogen bonding is especially important in maintaining ordered spatial relationships along the polypeptide chain. When hydrogen bonds repeatedly join the carboxyl oxygen of one amino acid to the amino hydrogen of the amino acid next but three along the chain, then the well-known α -helix is formed (Figure 5.5).

ⁱⁱⁱThe dimer of two cysteine molecules linked through their sulphydryl groups is known as cystine.

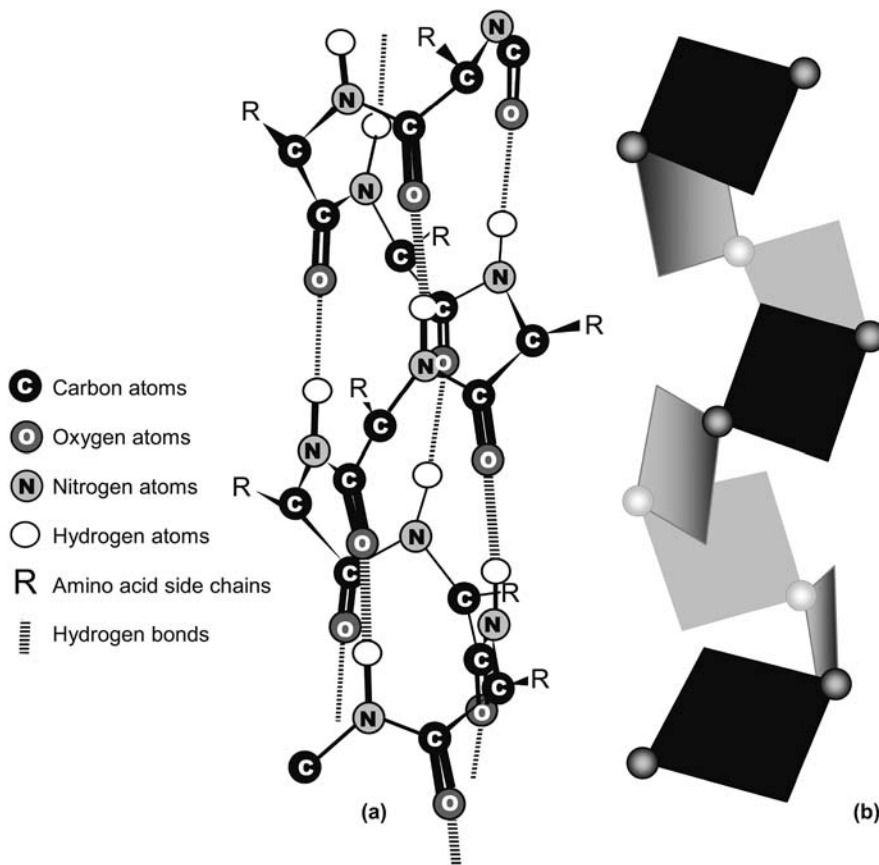


Figure 5.5 The α -helix. Only the atoms of the backbone and those involved in hydrogen bonding are shown. There are 3.6 amino acid residues per turn of the helix. Other helical patterns occur with other patterns of hydrogen bonding, but these do not give such a compact structure without straining the hydrogen bonds away from the preferred linear arrangement of the four atoms N-H...C=O.

The side-chains of the amino acids in helical structures such as these point outwards (*i.e.* away from the axis of the helix) and some are able to form bonds between different regions of the polypeptide chain. These can stabilise the overall folding of the polypeptide chain in globular proteins. One example of such bonds is the cysteine–cysteine bridges already mentioned. Inspection of the amino acid structures in Figure 5.2 shows ample scope for hydrogen bonding between side-chains, particularly those involving the amide groups of glutamine and asparagine. Hydrophobic side-chains tend to be orientated in a manner that minimises their interaction with the aqueous environment of the protein. Such residues are therefore usually directed towards the centre of the folded molecule, where they will be among other non-polar residues. The tendency of the amino acids to act in this way is a major factor in maintaining the correct folding of the polypeptide chain and is often referred to as hydrophobic bonding, unfortunately a far from ideal term (see Chapter 13).

Whatever biological role a particular protein may have, it is always dependent on the correct folding of the backbone to maintain the correct spatial relationships of its amino acid side-chains. Not surprisingly, extremes of pH and temperature disrupt the forces maintaining the correct folding and lead to “denaturation” of the protein. In a few cases, highly purified proteins can be persuaded to return to their correct arrangement after denaturation, but this is highly unlikely to occur in food situations. It is much more probable that the unfolded proteins will form new interactions with their neighbours, leading to precipitation, solidification or gel formation. For example, egg white is almost entirely composed of water (about 88%) and proteins (about 12%). When egg white is heated, denaturation leads to the formation of a solid gel network in which the water is trapped. Similarly, when liver is cooked, the proteins contained in the liver cells are denatured; if cooking is continued for too long, then an unpalatable hard texture results from the rigid network of polypeptide chains.

The denaturation of proteins in foodstuffs is not always undesirable, however. The steam or boiling water used when vegetables are blanched before freezing will inactivate certain enzymes, particularly lipoxygenase (see Chapter 7, Section 7.2.3), whose activity can lead to the generation of off-flavours. The link between protein denaturation and enzyme inactivation is considered in more detail in Chapter 12 (see Section 12.1.3).

Proteins perform numerous roles in living systems. The chemical processes on which all life processes depend require enzymes, which are proteins, as catalysts. Carrier molecules – for example, haemoglobin, which carries oxygen around the body in blood – are proteins. So are the permeases, which control the transport of substances across cell membranes, often against concentration gradients. Another group of proteins are the immunoglobulins, which form the antibodies that provide defence against invading microorganisms and certain toxins. These classes of proteins, of which enzymes are by far the most numerous, are all characterised by their physiological functions. In structural terms they are all globular, *i.e.* their polypeptide chains are folded into a compact arrangement. Just as the exact amino acid sequence is characteristic of a particular protein, so also is their pattern of folding into a globular structure. Figure 5.6 is a diagrammatic representation of the protein myoglobin, which illustrates many of the structural features of more complex proteins with much larger molecules.

Like many enzymes and other carrier proteins, myoglobin has a prosthetic group, *i.e.* a non-protein component, which is an essential participant in the catalytic or carrier function. In this case, the prosthetic group is a porphyrin, known as haem. The iron atom at the centre of the porphyrin ring can form a temporary coordinate link with an oxygen molecule, so that the myoglobin created can function as an oxygen carrier in muscle cells. It should be pointed out that the proportion of the polypeptide chain of myoglobin in the α -helix configuration is particularly high, about 75%; most proteins have no more than 10% as an α -helix, plus a significant proportion of other ordered structures. The sequence of amino acids in the polypeptide chain is referred to as the *primary structure* of the protein. The term *secondary structure* refers to regular locally ordered arrangements of the chain, including the α -helix (Figure 5.5). The overall folding pattern, *e.g.* that shown in Figure 5.6, is referred to as the *tertiary structure*.

If a protein has the angles of rotation about the $N-C_\alpha$ and $C_\alpha-C$ bonds repeated along the majority of its polypeptide chain, this will obviously rule out any sort of compact, globular folding arrangement. Instead, we can expect extended, highly ordered molecules. Proteins with such a configuration have structural roles in animal tissues and one in particular, collagen, makes a crucial contribution to the texture of meat. We will return to meat and muscle proteins later in this chapter.

Another group of proteins are those with a nutritional function, either in the transmission of nutrients from mother to offspring (the caseins and whey proteins in milk) or in the storage of nutrients to be utilised by an embryo (the seed proteins of plants and egg proteins of birds). In these cases the physical characteristics of the protein are of secondary importance to its overall chemical composition. For example, gluten, the principal protein of wheat, is rich in the amino acid glutamine, which has two nitrogen atoms, giving gluten a higher nitrogen content than most other proteins. Among their other distinctive characteristics, seed proteins and caseins share a tendency to form fairly ill-defined, but compact, aggregates. Biologically, such behaviour ensures that large amounts of nutrient can be concentrated without the problems of osmotic pressure associated with high solute concentrations. This behaviour has hindered efforts to elucidate the structure of these proteins. For example, it is only in recent decades that the structure of gluten has begun to be properly understood.

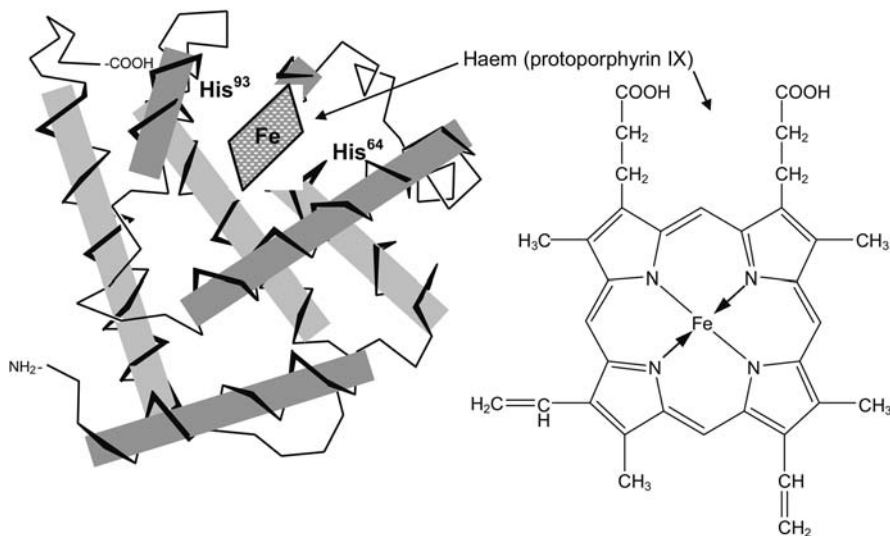


Figure 5.6 Myoglobin. Only the backbone ($-C_\alpha-N-C-C_\alpha-N-C-C_\alpha-$) of the polypeptide chain is shown, with the angles between successive bonds showing the positions of the α -carbon atoms. The shaded rods mark the axes of regions of the polypeptide chain arranged in the α -helix configuration. The central shaded area shows the position of the haem prosthetic group, which is surrounded by amino acid side-chains to form a hydrophobic pocket. The two histidine residues linked to the haem iron atom are marked as His.

5.3 Indispensable Amino Acids and Protein Quality

The protein in our diet provides the amino acids from which the body synthesises its own proteins, the major constituents of our tissues. The proportions of the different amino acids supplied by a selection of foodstuffs are presented in Table 5.2. The action of hydrolytic enzymes, initially in the stomach and then in the small intestine, breaks down food proteins to their component amino acids. On absorption into the bloodstream, they then become part of the body's amino acid pool. Breakdown of the body's own tissue proteins, an essential part of the process of the renewal of ageing or redundant cells, also contributes to this pool. The amino acid pool is drawn upon not only for protein synthesis, but also to provide the raw materials for the synthesis of purines, pyrimidines, porphyrins and other substances.

Many amino acids can be synthesised by mammals when adequate supplies of amino nitrogen and carbohydrate are available. However, some amino acids cannot be synthesised by mammals and must be supplied in the diet. These two groups are known, respectively, as dispensable and indispensable amino acids^{iv} and are listed in

Table 5.2 Amino acid composition of some dietary proteins. The data (calculated from those in the First Supplement to McCance and Widdowson's *The Composition of Foods*, ed. A. A. Paul, D. A. T. Southgate and J. Russell, HMSO, London, 1980) show the proportions of each amino acid in the total protein content of the foodstuff.

	Fresh peas	Wheat flour	Chicken breast	Beef steak	Whole egg	Cow's milk	Human milk	Cod fillet
	g per 100 g total protein							
Isoleucine	4.7	3.9	4.8	4.9	5.6	4.9	5.3	5.2
Leucine	7.5	7.0	7.8	7.6	8.3	9.1	9.9	8.3
Lysine	8.0	1.9	9.3	8.7	6.3	7.4	7.1	9.6
Methionine	1.0	1.6	2.5	2.6	3.2	2.6	1.5	2.8
Cysteine	1.2	2.6	1.3	1.2	1.8	0.8	2.0	1.1
Phenylalanine	5.0	4.8	4.7	4.3	5.1	4.9	3.8	4.0
Tyrosine	3.0	2.6	3.6	3.7	4.0	4.1	3.0	3.4
Threonine	4.3	2.7	4.3	4.5	5.1	4.4	4.5	4.7
Tryptophan	1.0	1.1	1.1	1.2	1.8	1.3	2.3	1.1
Valine	5.0	4.4	5.0	5.1	7.6	6.6	6.8	5.6
Arginine	10.0	3.6	6.5	6.4	6.1	3.6	3.8	6.2
Histidine	2.4	2.1	3.1	3.5	2.4	2.7	2.5	2.8
Alanine	4.5	3.1	6.0	6.1	5.4	3.6	4.2	6.7
Aspartic acid ^a	11.9	4.4	9.4	9.1	10.7	7.7	9.1	10.2
Glutamic acid ^b	17.3	32.9	17.1	16.5	12.0	20.6	17.4	14.8
Glycine	4.3	3.2	5.1	5.6	3.0	2.0	2.5	4.6
Proline ^c	4.1	1.3	4.3	4.9	3.8	8.5	9.9	4.0
Serine	4.7	5.6	4.1	4.3	7.9	5.2	4.3	4.8

^aIncludes asparagine.

^bIncludes glutamine.

^cIncludes hydroxyproline. The older techniques used to obtain this data were not normally able to separate these pairs of amino acids.

^{iv}The terms dispensable and indispensable amino acids are now preferred by professional nutritionists to the long-established terms "non-essential" and "essential" amino acids.

Table 5.3. It is important to recognise that the distinction between these terms must not be regarded as absolute. Thus an amino acid may be regarded as dispensable because an animal can synthesise it, but not at a sufficiently rapidly rate to meet the needs of a rapidly growing young animal. Two amino acids among those normally listed as dispensable, histidine and arginine, were originally regarded as indispensable following experiments carried out in the 1930s on laboratory rats. However, subsequent work showed that humans could synthesise these for themselves. The rate at which humans are able to synthesise histidine, although adequate for adults, is insufficient to meet the needs of children. Two other amino acids, cysteine and tyrosine, also have an intermediate status and are sometimes referred to as semi-indispensable. Humans require supplies of the indispensable amino acid methionine to synthesise cysteine, plus similar supplies of phenylalanine for the synthesis of tyrosine. As implied in Figure 5.7, when the nutritional quality of food proteins is considered, histidine should be regarded as indispensable.

Ideally, the protein in the diet should provide amino acids in the same relative proportions as the body's requirements, but, in practice, such an ideal is never attained. Balancing the pattern of amino acid supply against the needs for protein synthesis is a major function of the liver. Excess supplies of particular amino acids are broken down and the carbon skeletons either oxidised to provide energy or converted to fat for energy storage. The nitrogen is either converted to urea for excretion *via* the kidneys or utilised in the synthesis of any dispensable amino acids that may be in short supply. Difficulties arise when the supply of one or more of the indispensable amino acids is restricted. The absence of even one of these will effectively result in the cessation of all protein synthesis because the molecular structure of virtually all proteins includes at least one molecule of each of the amino acids shown in Figure 5.2 (the exceptions are hydroxyproline and hydroxylysine).

The diets of typical well-fed Europeans or North Americans usually contain more than ample supplies of both total protein and the individual indispensable amino acids, but for many people living in Africa and Asia the supply of protein in their diet is inadequate in both respects. Nutritionists have established that the proportions of the different amino acids required by human infants correspond closely to the amino acid

Table 5.3 Status of amino acids in human nutrition.

Indispensable	Dispensable
Isoleucine	Alanine
Leucine	Arginine
Lysine	Asparagine
Methionine	Aspartic acid
Phenylalanine	Cysteine
Threonine	Glutamic acid
Tryptophan	Glutamine
Valine	Glycine
	Histidine
	Proline
	Serine
	Tyrosine

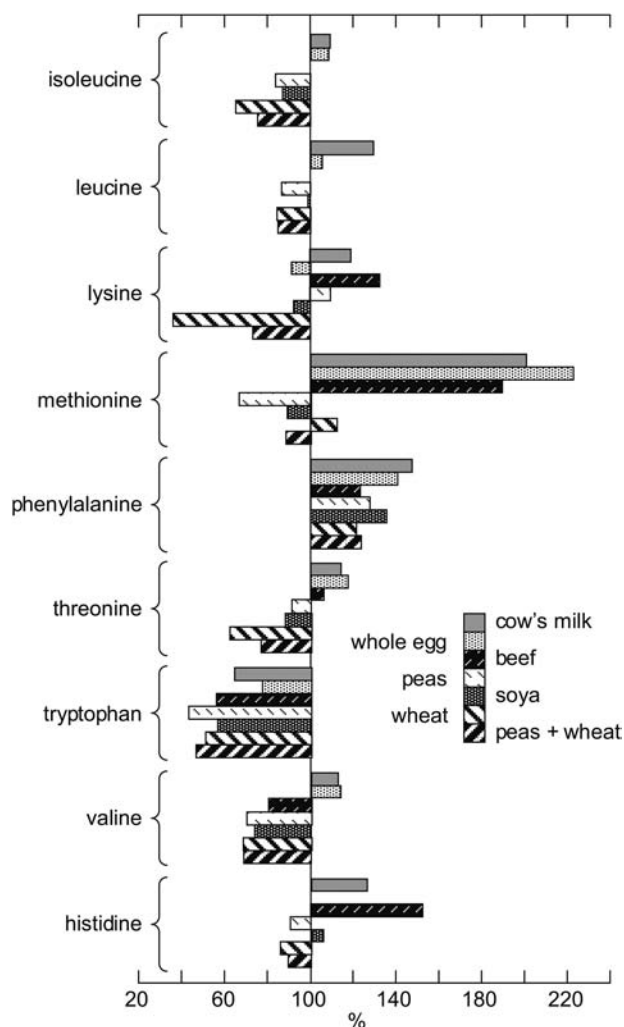


Figure 5.7 Indispensable amino acids in some important food proteins. The values shown for each amino acid indicate the proportion present (as a percentage) relative to that present in human milk. Thus the provision of methionine by animal proteins is seen to compare very favourably with this standard, whereas the provision of lysine by wheat protein is inadequate. The total amino acid composition of human milk and some other important foodstuffs is given in Table 5.2.

composition of human milk and this is now accepted as the standard against which the nutritional value of the protein content of other foodstuffs is judged. Figure 5.7 shows the relative proportions (molar rather than by weight) of the essential amino acids of a number of key foodstuffs compared with human milk.

It is immediately apparent from these data that the universality of the roles of the different amino acid side-chains in the maintenance of protein structure leads to broadly similar amino acid compositions in foodstuffs of quite diverse origins. Animal protein foods (eggs, milk and meat) do not differ significantly from human milk in regard to any particular amino acid, but the plant protein foods do present some

problems. The low proportion of lysine in wheat protein means that wheat is only about 50% as efficient as human milk as a source of protein. This means, in turn, that the amount of wheat protein that provides just adequate levels of lysine supplies wasteful levels of the other amino acids. Legumes, such as soya and peas, provide ample proportions of lysine, but are deficient in methionine, so they also are inefficient as sources of protein. A diet containing a mixture of both cereal and legume proteins will clearly be much more efficient than either alone. The efficiency of an individual protein, foodstuff or whole diet can be described numerically by calculation from the relative proportions of the amino acids present. The proportions of each indispensable amino acid are compared with those in human milk protein and the lowest of these (as a percentage) is the “chemical score”.

Chemical scores (a selection is shown in Table 5.4) provide a reasonable guide to the actual performance of proteins in human nutrition. Experiments measuring efficiency in terms of the proportion of the nitrogen that the body utilises from dietary protein indicate that we never actually attain the theoretical efficiency. The inevitable inefficiencies of our digestive system and the effects of cooking are usually blamed for this, and also for the wide variations in published data, most of which have been determined in the context of the nutrition of laboratory or farm animals rather than humans. The protein content of a typical well-balanced European diet gives a chemical score of about 70%. Adults in the UK consume between 60 and 90 g of protein per day, so that any reasonably balanced diet will provide at least 3 g of even the least abundant amino acids. Although plant proteins are used less efficiently than animal proteins, one cannot ignore the much greater efficiency, in terms of energy, land and water, of agriculture when providing sources of plant protein.

The problems posed by the low levels of lysine and methionine in what would otherwise be ideal proteins are exacerbated by the tendency of these two amino acids to undergo reactions that destroy their nutritional activity during the storage and processing of foods. The Maillard reaction between the lysine amino groups and reducing sugars is the most important of these and has been considered in Chapter 2, but this is not the only route by which the lysine in a protein may be lost. During processing at high temperatures, especially under alkaline conditions, several amino acids undergo changes in their side-chains, resulting in nutritional loss.

Table 5.4 Nutritional efficiencies of proteins calculated from the amino acid composition (the chemical score) and determined experimentally. The experimental values are variously known as “biological values” and “net protein utilisation, NPU”, values. The wide range due to the potential influence of cooking means that a single experimental value for meat is not practicable.

Protein source	Chemical score (%)	Approximate experimental value (%)
Human milk	100	94
Whole egg	100	87
Cow's milk	95	81
Peanuts	65	47
Beef	57	—
Wheat	53	49

Cysteine and serine are both liable to be converted into dehydroalanine, which can form links with lysine side-chains that result in irreversible links being formed between different sections of the polypeptide chain. The pH of the albumin of newly laid hen's eggs is about 7.8, but, over time, carbon dioxide diffuses out through the shell and the pH increases to about 9. At this pH, cysteine in some albumin proteins releases hydrogen sulfide if the egg is subjected to temperatures approaching 100 °C for 5–10 minutes (*i.e.* hard-boiled). The hydrogen sulfide diffuses from the albumin to the outer layers of the yolk, where it can react with traces of iron to give a harmless, if unsightly, dirty brown layer of ferrous sulfide. (Affluent readers may have observed that their silver egg spoons are similarly tarnished by the hydrogen sulfide released in their breakfast egg.)

In addition to reducing the number of lysine residues available, the formation of cross-links of this type between neighbouring polypeptide chains will tend to prevent the assimilation of much of the remainder of the protein molecule because unfolding and access for the proteolytic enzymes of the intestine will be inhibited. The reactions involved in these processes are shown in Figure 5.8, which also indicates how arginine and glutamine may be lost under these conditions.

5.4 Analysis

Measuring the amount of protein in a food material is far from straightforward and the method chosen tends to be strongly influenced by the subsequent application of the data obtained. For this reason, the analysis of protein is given more prominence in this book than the analysis of other food components.

The nutritional quality of a food protein can really only be determined by feeding trials, but sufficient is now known about protein digestion and the effects of processing techniques to enable fairly accurate predictions to be made. These predictions require

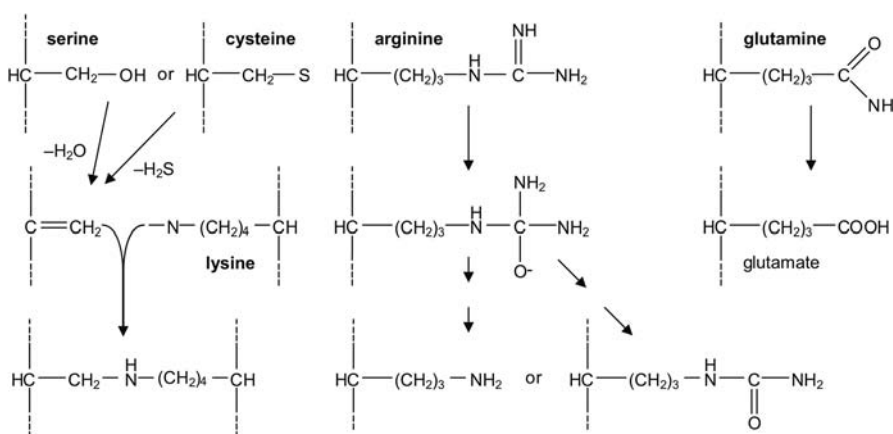
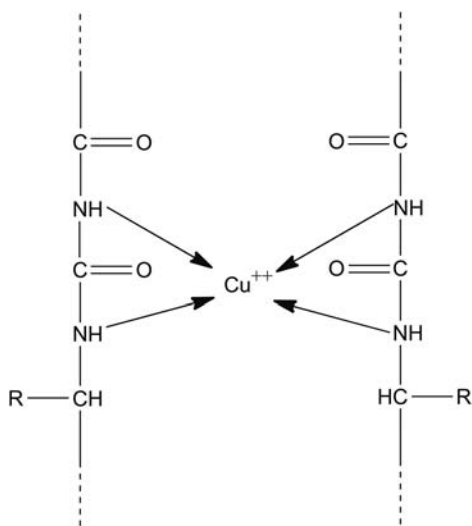


Figure 5.8 Pathways of amino acid breakdown occurring when proteins are subjected to heat at alkaline pH. Asparagine is converted to aspartic acid in a reaction similar to that shown for glutamine. The loss of water from serine is to some extent a reversible reaction, but the product of the reverse reaction is just as likely to be in the D -configuration, in which case its nutritional value is still lost.

knowledge of both the total protein content of the food material and its amino acid composition.

The determination of the total protein content of a foodstuff or raw material is by no means as simple as might be imagined. To be specific to individual proteins, almost any chemical test will depend on the presence of a particular amino acid side-chain. The analytical results will therefore be subject to variations in the proportions of the target amino acid in the proteins under test and will generally need to be relative to some arbitrarily selected standard protein. An example of this approach is the Lowry method, which is more popular with biochemists than food chemists because it is only applicable to fully dissolved proteins in solution at concentrations up to $300 \mu\text{g cm}^{-3}$. In alkaline solution and in the presence of copper ions, tyrosine residues reduce phosphomolybdotungstate to a blue compound that can be estimated by spectrophotometry.

Another colorimetric-spectrophotometric procedure which is fairly uniform in its response to different proteins is the Biuret method. Like the Lowry method, this too is limited to proteins in solution. Under alkaline conditions, which ensure that the polypeptide chain is unfolded and fully accessible, Cu^{2+} ions are complexed by the nitrogen atoms of the polypeptide chain to give a characteristic violet colour (5.4). Potassium tartrate is included in the reaction mixture to ensure that excess Cu^{2+} ions are not precipitated as $\text{Cu}(\text{OH})_2$. Although much less sensitive than the Lowry method, it gives a linear response up to about 10 mg cm^{-3} and has the merit of being easy to carry out.



(5.4)

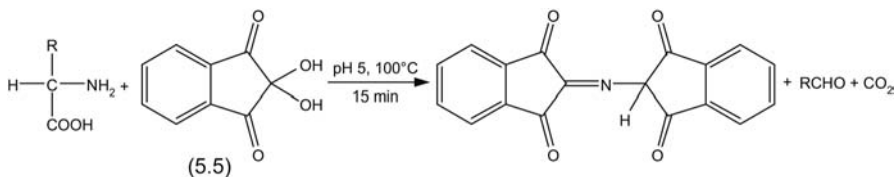
By far the most common method for protein determination in food materials, especially in official laboratories, is the Kjeldahl procedure. This method gives a figure for total nitrogen and its use depends on the reasonable assumption that this can provide an accurate measure of the total protein. In typical food materials the proportion of non-protein organic nitrogen is too small to be significant, as are the concentrations of nitrate or nitrite ions, even in cured meats. The proportion of nitrogen in most proteins is roughly 16% by weight and a factor of 6.25 is used to convert the nitrogen percentage

into the protein content. Variations in the amino acid composition of different foodstuffs inevitably require slightly different factors to be used in accurate work on certain foodstuffs. For example, the unusually high proportion of the amino acid glutamine in cereal proteins gives these proteins a nitrogen content that is higher than average and, as a consequence, a lower conversion factor of 5.70 is used. About one-third of the amino acid in gelatine is glycine and this similarly increases the nitrogen content, with a factor of 5.55 required. Meat requires the standard 6.25, but the highest factors, 6.38 and 6.68, are reserved for milk and eggs, respectively. Almost without exception, the protein content of food products recorded on their labels has been derived from the total nitrogen content multiplied by the most appropriate factor.

In the Kjeldahl procedure, the entire food sample is digested in concentrated sulfuric acid at temperatures approaching 400 °C, with a heavy metal salt, such as copper sulfate, as a catalyst. Sodium sulfate is added to increase the boiling point of the sulfuric acid. Under these conditions, organic material is oxidised, with any organic nitrogen being retained in solution as ammonium ions. On completion of the digestion, an aliquot is withdrawn and transferred to an apparatus known as a Markham still. Here it is first made alkaline and then the liberated ammonia is steam distilled off into an aliquot of boric acid solution. The ammonia is determined by titration. Modern laboratories use a semi-automated system for this procedure.

Near-infrared reflectance spectrophotometry is a valuable instrumental procedure, useful when large numbers of similar solid samples – for example, of flour – are being routinely tested. Measurements are made at 2100 nm (characteristic of the hydroxyl groups in starch), 2180 nm (characteristic of the amide groups in protein) and 1680 nm (a background reference value). Calculation of the protein content is based on subtraction of the reference value plus the starch shoulder from the amide peak. The test can be extended to give a measure of the moisture content. Although the technique demands the accurate control of particle size, it is rapid and lends itself to automation. Samples taken from a lorry-load of wheat can be checked while waiting to be unloaded at the flour mill.

Many colour reactions specific to particular amino acids have been developed. Unfortunately, few are quantitative and most are unable to provide the comprehensive data required. For this reason, chromatographic techniques are the obvious choice for determining the amino acid composition of a protein hydrolysate. Although well-established systems are available for paper and thin-layer chromatography, these methods have been displaced in modern laboratories by high-performance liquid chromatography, which lends itself to automation. In some methods, the amino acids are first converted to a derivative that is more amenable to chromatographic separation and detection. Alternatively, the amino acids can be detected in the eluate by reaction with ninhydrin (triketohydrindene hydrate, (5.5)), which gives a blue colour with most amino acids, but a yellow colour with the secondary amines proline and hydroxyproline.



5.5 Food Protein Systems

5.5.1 Milk

The milk from the domestic cow, *Bos taurus*, is an important protein source for humans, particularly for children. The milk of other ruminant species, notably sheep (*Ovis aries*), goats (*Capra hircus*) and camels (*Camelus dromedaries* or *bactrianus*), also make also significant contributions to human nutrition, but have not received the extensive scientific attention that has been devoted to the cow's milk. Milk is an aqueous solution of proteins, lactose, vitamins and minerals, carrying emulsified globules of fat. It also contains particles known as casein micelles, consisting of a number of related protein species, collectively known simply as the casein proteins, together with phosphate, calcium and trace amounts of citrate. If the fat is removed from milk, the product is known as skimmed, or skim, milk.^v If the casein micelles are also removed (they can be precipitated out at about pH 4.6), the residual solution is known as serum (by analogy with blood) or, more commonly, whey. The proportions of the various casein and whey proteins vary considerably, as does the total protein content, but the values shown in Table 5.5 are typical. The whey obtained during cheese-making has a slightly different composition because some of the casein is solubilised and some of the lactose will have been converted into lactic acid by the action of bacteria. In the case of cheddar and similar cheeses a small percentage of salt (sodium chloride) will also have been added.

The Greek letters used in the names of the different proteins were originally assigned on the basis of the mobility of the protein during electrophoresis. The “s” in α_{s1} -casein refers to its sensitivity to precipitation by calcium ions. Although the prefixes α_{s1} , β , *etc.* define particular protein species, it is now recognised that there are a number of different versions of each protein that differ slightly in their amino acid sequence. For example, α_{s1} C-casein differs from α_{s1} B-casein (the most common variant) in having a glycine residue in place of a glutamic acid residue at position 192 in the polypeptide chain. The breed of cow from which a sample of milk has originated can be identified by examining the proportions of these variants.

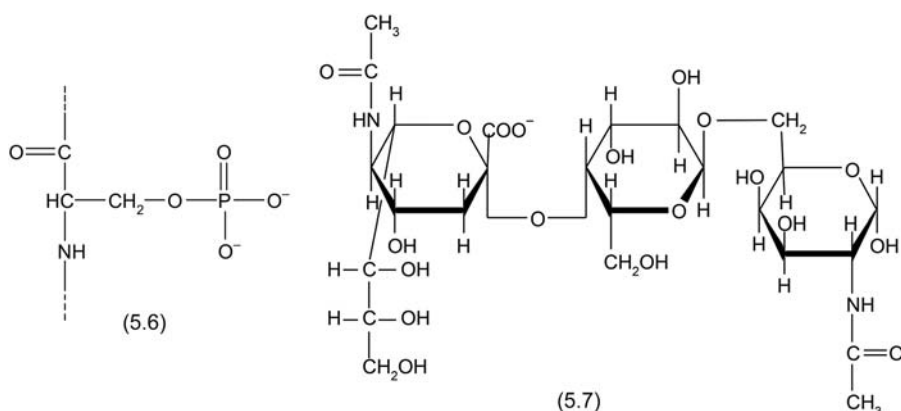
Table 5.5 Typical proportions of major proteins in skimmed cow's milk. The α_s -casein fraction consists of two proteins, α_{s1} and α_{s2} , present in the approximate ratio 3:1.

	Quantity (g l ⁻¹)	Total protein (%)
Casein proteins (total)	~27	~80
α_s -Casein	10–16	31–45
β -Casein	8–12	24–34
κ -Casein	10–15	3–5
γ -Casein	4–6	1.0–1.5
Whey proteins (total)	~6	~20
α -Lactalbumin	1.0–1.5	3.3–5.0
β -Lactoglobulin	2–4	6.6–13.3
Immunoglobulins	0.7–0.8	2.3–2.7
Others	1–2	3–7

^vSemi-skimmed milk, with about 2% fat, is manufactured by mixing equal proportions of skimmed milk and whole milk; a much simpler procedure than attempting to remove half the fat from whole milk.

The casein micelles of milk are roughly spherical, with diameters up to 600 nm. However, about half of the total casein is found in micelles with diameters between 130 and 250 nm, with the remainder fairly equally divided between micelles above and below this range. The micelles are sufficiently large and abundant to be the source of the opalescence of skimmed milk. Milk contains about 10^{15} micelles per L. A typical micelle contains 10^4 – 10^5 casein molecules (the molecular weights of α_{S1} -, β - and κ -casein are 2.35×10^4 , 2.40×10^4 and 1.90×10^4 , respectively). γ -Casein is believed to be an artefact of the laboratory procedures used to separate and purify casein molecules and is a fragment of β -casein resulting from limited breakdown caused by the proteolytic enzymes in milk.

Chemists have long sought to describe the structure and organisation of the casein micelle in relation to both its natural nutritional role and the behaviour of milk proteins during processing operations, such as cheese-making. Milk evolved as a vehicle for the nutrients required by a new born mammal and its value to humans for the manufacture of cheese and butter, and for inclusion in tea and coffee, is decidedly secondary. The organisation of much of milk's total protein, and all of its calcium and phosphate, into micelles overcomes two major problems. First, in the absence of aggregation, the quantity of protein present in milk would form highly viscous solutions that would pose problems for secretion and delivery. Second, the calcium and phosphate, each essential for the growth of the new born skeleton, would be able to form large, relatively insoluble crystals of calcium phosphate, again posing problems for secretion and also digestion. It is now recognised that the role of the casein proteins as a vehicle for calcium and phosphate has been a major determinant in the evolution of their structure.



The most popular theory for the structure of the casein micelle was for many years based on the proposals of Slattery and Evard in 1973, and subsequent work by Schmidt. In this theory, the micelle structure is composed of spherical aggregates of 25–30 casein molecules, the submicelles. Within these submicelles, individual protein molecules are held together by hydrophobic interaction.^{vi} The phosphoserine side-chains (5.6) of the casein molecules provide the attachments for the calcium phosphate bridges which

^{vi}Hydrophobic interactions, sometimes referred to as hydrophobic bonds, were described in Section 5.2 in the context of the forces that maintain the folded of peptide chains, and are given further attention in Chapter 13.

hold the submicelles together. The submicelle theory has since been challenged by the results of various studies, both relating to the micelles themselves and also to the interactions between the casein, phosphate and calcium. The attention devoted to the submicelle theory in previous editions of this book is now transferred to what have now become known as the internal structure models. These were initially proposed by Carl Holt and subsequently developed by David Horne.

Studies which have told us a great deal about the three-dimensional structure of large numbers of different proteins have been much less successful when applied to the caseins. Although their amino acid sequences are well established, illustrated in simplified form in Figure 5.9, there has been no agreement about the secondary and tertiary structures (see Section 5.2) of the caseins. Most authorities now agree that it is likely that they do not take up the compact tertiary structures associated with most proteins, instead tending to exist in irregular flexible conformations that link up to form extended three-dimensional networks. As shown in Figure 5.9, the polypeptide chains of all four caseins include extensive regions dominated by hydrophobic amino acids, which tend to associate and form links between protein molecules. Hydrogen bonding between amino acid side-chains is also expected to strengthen these links.

The phosphoserine residues (5.6) found in clusters in the hydrophilic regions of the polypeptide chains of α - and β -caseins provide the second type of bonding, which maintains micelle structure. Casein micelles contain a significant amount (about 6% on a dry basis) of colloidal calcium phosphate. The calcium phosphate occurs in nanoclusters a few nanometres in diameter, with a chemical composition approximating to $\text{Ca}_9(\text{PO}_4)_6$. The α - and β -casein molecules link to the nanoclusters through their own clusters of phosphoserine groups. The combination of these links and the association between the hydrophobic regions of the molecules allows the formation of a stable three-dimensional network. In contrast with the α - and β -caseins, κ -casein has no more than a single phosphoserine. Instead, the extremely hydrophilic character of its C-terminal region is maintained by the presence of at least one trisaccharide unit, α -N-acetylneuramyl-(2 $\rightarrow$ 6)- α -galactosyl-(1 $\rightarrow$ 6)-N-acetyl-galactosamine (5.7).

It is important to remember that, in the formation of any branched or net-like polymer, the monomeric units must each contain at least three functional, *i.e.* linking, groups, as illustrated in Figure 5.10. In the case of the casein micelle, the α - and β -caseins both contain a number of regions capable of forming links with each other, either directly by hydrophobic bonding or indirectly *via* the calcium phosphate nanoclusters. As the network grows, it will be joined by κ -caseins. However, these are only capable of integrating into the network *via* their N-terminal ends^{vii} and their C-terminal ends will form a barrier to expansion in that region. It is not difficult to see that the growing micelle will eventually spontaneously acquire a surface dominated by κ -casein and growth will cease. Although the mathematics of this situation is beyond the scope of this book, even a superficial consideration of the process will show that smaller micelles will contain a higher proportion of κ -casein.

^{vii}When separated from other caseins in the laboratory, κ -casein forms dimers through its cysteine sulfhydryl groups and it is likely that κ -casein also exists in this form in the casein micelle. However, the dimers would still retain their chain-terminating function.

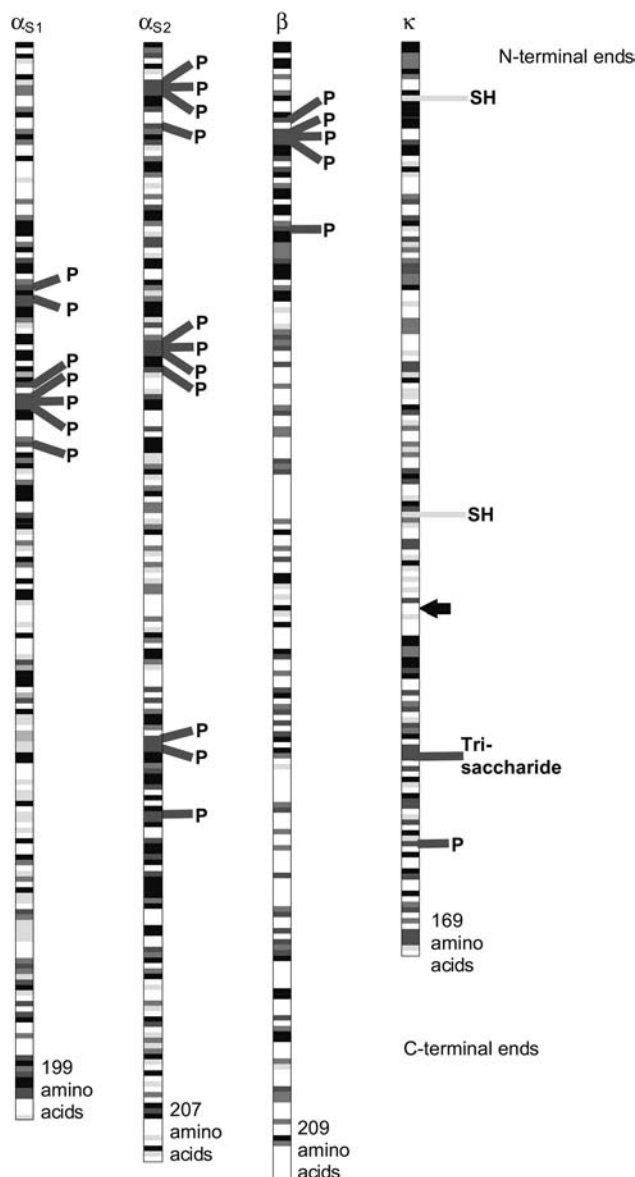


Figure 5.9 Polypeptide chains of bovine caseins. The characteristics of the amino acids are indicated by shading: black for the most hydrophilic and white for the most hydrophobic. Serine residues esterified with phosphate are identified by **P** and the threonine (134) that carries κ -casein's trisaccharide unit by **Trisaccharide**. The locations of the cysteine residues of κ -casein are indicated by **SH**. The arrow marks the site between phenylalanine-105 and methionine-106, where chymosin hydrolyses the peptide bond to liberate the glycopeptide during cheese-making.

The model for the casein micelle that emerges from this is shown in Figure 5.11. The core of the micelle is a three-dimensional network of α - and β -casein chains, linked both by attraction between the extensive hydrophobic regions of the polypeptide chains and by the binding of their clusters of phosphoserine residues and the colloidal calcium phosphate nanoclusters. It has been calculated that a typical micelle might contain

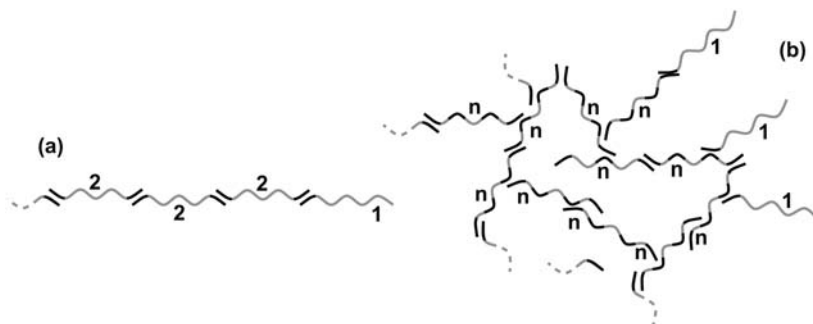


Figure 5.10 Polymer formation. In (a), monomers (grey) with two linking groups (shown in black) form an unbranched chain that is terminated at one end when a monomer with only one linking group joins the chain. In (b), monomers with three or more linking groups (n) readily form a branched network. However, when single linking monomers join the network, as on the right-hand side, further growth of the network is prevented on this side.

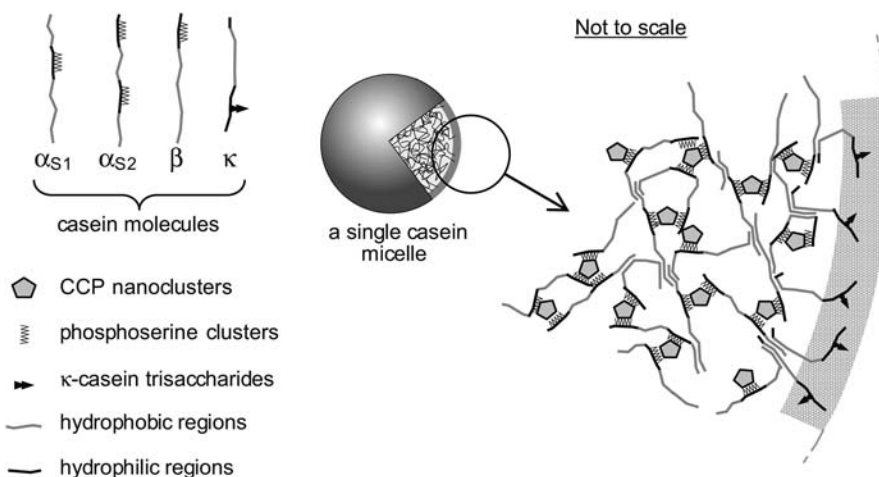


Figure 5.11 A diagrammatic impression of the structure of the casein micelle based on the proposals of Horne and Holt. The surface of the micelle, shown by the shaded area in the enlarged cross-section, is dominated by the polar C-terminal regions of the κ -casein polypeptide chains carrying trisaccharide units.

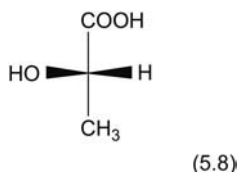
800 nanoclusters. The surface of the micelle is dominated by the hydrophilic C-terminal ends of the κ -casein.

The most abundant serum proteins, α -lactalbumin and β -lactoglobulin, have attracted considerable attention from protein chemists. They have been interested in their physical properties and structure, but these studies have revealed little about the reason for their presence in milk beyond their contribution to the total protein content. By contrast, the immunoglobulins have proved to be of great interest to food scientists. The role of one class of immunoglobulins, the macroglobulins, in the creaming phenomenon has been discussed in Chapter 4, but the biological role of immunoglobulins is much more important. Immunoglobulins are popularly known as antibodies, which

are proteins synthesised in various parts of the body in response to the invasion of tissues by foreign matter, particularly bacteria, viruses and toxins. Their reaction with these antigens is remarkably specific and facilitates their neutralisation or destruction by other elements of the body's defence mechanisms. It is now well established that the exposure of a mother to many common pathogenic bacteria, especially those causing intestinal diseases such as diarrhoea that are very dangerous to new born infants, leads to the appearance of appropriate antibodies in the mother's milk. These undoubtedly play a part in protecting new born infants from many dangerous diseases. However closely the manufacturers of infant milk products based on cow's milk are able to match the nutrient content of human milk, it is increasingly clear that they will not be able to match these unique advantages of human milk for new born infants.

5.5.2 Cheese

The fundamental process involved in cheese-making is the precipitation of casein to form a curd. In the case of yoghurt and some cottage cheeses, the precipitation is caused entirely by a low pH. The growth of lactobacilli in milk is accompanied by their fermentation of the milk's lactose to L-lactic acid (5.8). The lactobacilli involved are sometimes those present naturally in the milk, but usually a starter culture of a strain with particularly desirable characteristics is added. Complete precipitation of the casein does not occur at the pH of these types of fermented milk product. Weak associations between the casein micelles give yoghurt its characteristic gel-like texture. Crème fraîche is manufactured from fresh cream (30–45% fat) using a bacterial culture to generate a limited amount of lactic acid from the lactose in the aqueous phase of the cream. In this case, the decrease in pH is sufficient to cause some thickening, but not gel formation.



In the production of hard cheeses such as Cheddar, Stilton and Gruyère, microbial action is allowed to bring the pH down to around 5.5. At this point, rennet is added to cause extensive precipitation and curd formation. Rennet is a preparation of the enzyme chymosin (known in the past as rennin) obtained from the lining of the abomasum (the fourth stomach) of calves. Chymosin specifically catalyses the hydrolysis of one particular peptide bond in κ -casein, as shown in Figure 5.12. The κ -casein is split into two fragments. One, para- κ -casein, remains as part of the micelle and includes the hydrophobic section of the molecules. The other, κ -casein glycopeptide, is lost into the whey. This fragment carries with it all the carbohydrate units. The loss of their carbohydrate coating means that the formation of strong cross-links between the micelles is no longer blocked and a curd rapidly develops.

The curd is held for several hours, during which time the acidity increases and the curd acquires the correct degree of firmness. Once this is achieved, the curd is chopped into small pieces to allow the whey to run out as the curd is stirred and finally pressed.

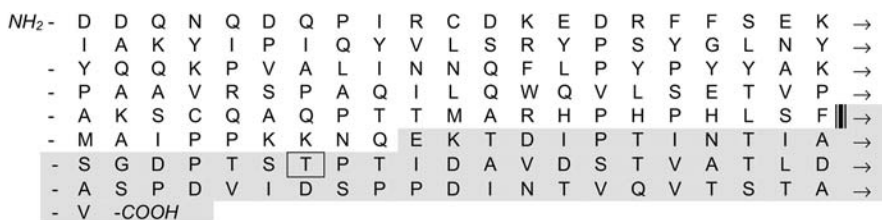


Figure 5.12 Amino acid sequence of κ -casein using the single letter code for amino acids. The bar marks the peptide bond between phenylalanine-105 and methionine-106. This is hydrolysed by the enzyme chymosin during cheese manufacture, liberating the fragment (shaded) known as the κ -casein glycopeptide into the whey. The boxed threonine residue (133) is the normal site of attachment for the trisaccharide. Trisaccharide units are also occasionally found attached at a number of nearby threonine and serine residues.

In the production of many types of hard cheese, notably Cheddar, salt (around 20 g per kilo of curd) is added before pressing to hasten the removal of the last of the whey and to depress the growth of unwanted microorganisms. The final stage of cheese manufacture, ripening, requires low temperature storage for several months. Other enzymes present in rennet (notably pepsin), together with proteases from the microorganisms, cause a limited degree of protein breakdown, which is important for both the texture and flavour of the finished cheese (many small peptides have distinctive bitter or meaty tastes). Lipases from the milk liberate short-chain fatty acids from the milk fat triglycerides, which also contribute to the flavour.

In recent years, the increasing yields of milk per cow have meant that fewer calves are born relative to the amount of milk converted into cheese. As a result, rennin supplies have not kept pace with demand. This has forced cheese-makers to examine other enzymes as substitutes. Proteases from fungi (notably *Rhizomucor meihei*) and porcine pepsin have been tried, but these have the disadvantage of causing excessive breakdown of other proteins.^{viii} Chemically modified *R. meihei* proteases are becoming popular with the manufacturers of cheeses suitable for lacto vegetarians. They are modified to make them much more sensitive to heat and therefore less likely to remain active in the finished product. The modern techniques of genetic engineering have also been brought to bear on the problem. The DNA coding for calf rennin has been successfully inserted into the chromosomes of the yeast *Kluveromyces lactis* and the bacterium *Escherichia coli*. Both of these microorganisms can then be persuaded to produce large quantities of the enzyme, identical in every way to that produced by calves.

5.5.3 Eggs

The yolk fraction of eggs from the domestic hen, *Gallus domesticus*, was discussed briefly in Chapter 4, Section 4.5. This section will be devoted to the egg white, or albumen, fraction. Egg white consists almost entirely of protein, 11–13%, and water. Many

^{viii}Cheese manufactured using fungal rennet is sometimes labelled as “vegetarian”. Bearing in mind that cows have to give birth to a calf before they can produce milk and that only a small proportion of calves live to become milk producers themselves, it may be thought eccentric to focus on the source of chymosin as the qualification for vegetarian status.

of the applications of eggs in cooking can be explained in terms of the properties of this protein.

In raw eggs, the white is not homogeneous, but occurs in four layers, two thick and two thin. The inner thick layer nearest the yolk is continuous with the chalazas, the stringy structures that suspend the yolk in the centre of the egg. The protein composition of the different layers is identical, except that the more viscous layers have a higher proportion of the protein ovomucin. For all culinary purposes, the different layers of the egg white are thoroughly mixed together and the white can therefore be regarded as a simple aqueous solution of all but one of the globular proteins listed in Table 5.6. The exception is the ovomucin, which forms fibres that make a major contribution to the viscosity of the egg white.

The predominant egg white protein, ovalbumin, is a glycoprophosphoprotein, *i.e.* a protein with both phosphate and carbohydrate groups attached. Up to two phosphates are attached to serine residues (as in α - and β -caseins) and an asparagine residue carries the carbohydrate unit. This consists of two or more *N*-acetylglucosamine (3.1) units carrying several mannose units. Ovalbumin is readily denatured, the process we observe when egg white sets on heating. The polypeptide chain of ovalbumin includes six cysteine residues. Two of these residues form a sulfhydryl bridge (5.3), which helps to maintain the three-dimensional structure of the protein. As the temperature is increased, the polypeptide chain unfolds and new sulfhydryl bridges become possible, not only within the molecule, but also linking neighbouring molecules together, forming the rigid gel of cooked egg white.

Ovalbumin is also particularly susceptible to denaturation at air–water and oil–water interfaces. It will be recalled that the polypeptide chains of globular proteins tend to fold up so that amino acids with hydrophobic side-chains are located in the centre of the molecule away from the surrounding water. If the water on one side of a protein molecule is displaced by air or oil, then the advantage of keeping the hydrophobic amino acids tucked away in the centre of the molecule disappears and the molecule unfolds, *i.e.* it becomes denatured. As before, the denatured molecules readily interact together, but instead of forming a solid gel they form a film around air bubbles or fat droplets, stabilising, respectively, foams (as in meringue) or emulsions (as in cake batters). Subsequent heating causes further denaturation, which reinforces these films.

Beating egg white to a stable foam is a difficult process to control. If the beating is excessive, then too much of the ovalbumin becomes denatured, leaving insufficient undenatured protein to bind the water. Beating must stop as soon as peak stiffness is achieved because further beating to incorporate more air will only weaken the foam.

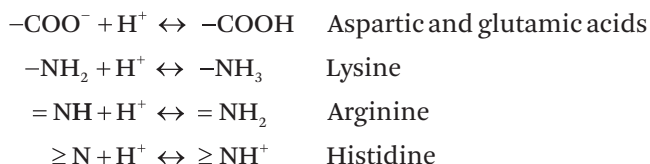
Table 5.6 Major proteins in the white of hen's eggs. Conalbumin is also known as ovotransferrin.

	Approximate total protein (%)
Ovalbumin	54
Conalbumin	12
Ovomucoid	11
Ovomucin	1.5–1.3
Lysozyme	3.4
Ovoglobulins	8

Another glycoprotein, conalbumin, is a metal-binding protein analogous to lactoferrin in milk (see Chapter 11, Section 11.2.1). It behaves similarly to ovalbumin, but has been implicated in one rather odd aspect of cookery technique. Glass bowls are preferred to plastic bowls for beating egg white because the surface of even well-cleaned plastic bowls is likely to be contaminated with traces of fat, which interferes with foam formation. However, copper bowls are claimed to be the very best. It has been suggested that the conalbumin binds with Cu^{2+} ions from the bowl and that the resulting copper-protein complex is more resistant to surface denaturation, thereby providing some protection against over-beating.

Ovomucoid, also a glycoprotein, gives the albumen its high viscosity. Although a certain level of viscosity is important for foam stability, whipping becomes difficult if it is too high. This is why it is important that eggs for meringue- or cake-making should not be used straight from the refrigerator because their low temperature would cause too high a viscosity.

Foam stability is enhanced when the pH is close to the isoelectric points of the proteins. Many protein amino acid side-chains include ionisable groups, notably aspartic and glutamic acids, lysine, arginine and histidine. The proportion of a particular amino acid side-chain that is ionised is clearly a function of the hydrogen ion (H^+) concentration, *i.e.* the pH:



The isoelectric point of a protein is the pH at which the number of positively charged groups exactly equals the number of negatively charged groups, *i.e.* the protein molecule carries no net charge. Protein molecules do not repel each other as much when they are uncharged and therefore interact more strongly. Cooks use cream of tartar (potassium hydrogen tartrate) or a trace of vinegar to bring the pH of the egg white down to about 4.5–5. The isoelectric point of ovalbumin is reported to be about 4.7.

5.5.4 Meat

When we consume the proteins in milk and eggs, we are taking in proteins that have evolved purely as foods. When we consume meat, however, or if we are studying the processes involved in the conversion of muscle tissue into the slice of roast beef on our plate, we must remember that the biologist's view of its function will be quite different from that of the food chemist, nutritionist or diner. The structure of muscle tissue^{ix} is exceedingly complex and readers with biological or biochemical interests are strongly

^{ix}Although the term "meat" can encompass fish and poultry as well as other mammalian tissues such as liver, kidney and intestine (offal), this account is largely directed towards the properties of the skeletal muscles of mammals such as pigs, sheep and cattle. Some particular features of fish muscle are dealt with in Section 5.5.4.

advised to supplement the present account with the details to be found in text books of mammalian physiology or biochemistry.

A typical joint of meat from the butcher's shop (or supermarket) is cut from a number of muscles, each originally having had its own independent attachment to the skeleton, its own blood supply and its own nerves. Each muscle is surrounded by a layer of connective tissue consisting almost entirely of the protein collagen. This layer contains and supports the contractile tissues of the muscle and, at its extremities, provides the connections to the skeleton. The contractile units of the muscle are the muscle fibres. These are exceptionally elongated cells, 10–100 μm in diameter, and can be as long as 30 cm. Examined in cross-section, the muscle fibres can be seen to be organised into bundles separated by connective tissue, which is continuous with that surrounding the muscle and provides the connections to the skeleton. Blood vessels, adipose (*i.e.* fatty) tissue and nerves are also embedded in the connective tissue of the muscle.

Muscle fibres share most of the features found in more typical animal cells. Surrounding the fibre is the cell membrane, known as the sarcolemma (the prefix *sarco* is derived from the Greek for *flesh*). Along the length of the cell, just inside the sarcolemma, are numerous nuclei. Muscle fibres have two elaborate intracellular membrane systems, the transverse tubules, or T-tubules, which are invaginations of the sarcolemma, and the sarcoplasmic reticulum, which is the counterpart of the endoplasmic reticulum of other cells. Both participate in the transmission of the signal from the motor nerve endings on the surface of the fibre to the contractile elements of the fibre, the myofibrils. The T-tubules and sarcoplasmic reticulum wind around each myofibril, so that the contraction events in each myofibril are accurately synchronised. Innumerable mitochondria are also found lying between the myofibrils to ensure that the supply of adenosine triphosphate (ATP), the chemical fuel for the contraction process, is maintained.

The myofibrils themselves are composed of bundles of protein filaments arranged as shown in Figure 5.13a. The thin filaments are composed mostly of the protein *actin*, together with smaller amounts of *tropomyosin* and *troponin* (Figure 5.13b). The thick filaments are aggregates of the very large elongated protein *myosin*, molecular weight 5×10^5 , arranged as shown in Figure 5.13c. The sequence of events in muscle contraction is as follows:

- (i) The nerve impulse is received at the membrane surface of the fibre, the sarcolemma, transmitted throughout the cell by the T-tubules and provokes the release of Ca^{2+} ions from the reservoir of vesicles formed by the membranes of the sarcoplasmic reticulum.
- (ii) The Ca^{2+} ions bind to the troponin of the thin filaments. This causes a change in the shape of the troponin molecules that move the adjoining tropomyosin protein complex. The change in the position of the tropomyosin exposes the active site of the actin molecules.
- (iii) The activated actin molecules are now able to react repeatedly with the myosin of the thick filament and ATP, the energy source for contraction, as shown in Figure 5.14.
- (iv) The heads of the myosin molecules are at different angles to the thick filaments at different stages in the cycle, so that, with each turn of the cycle and with the

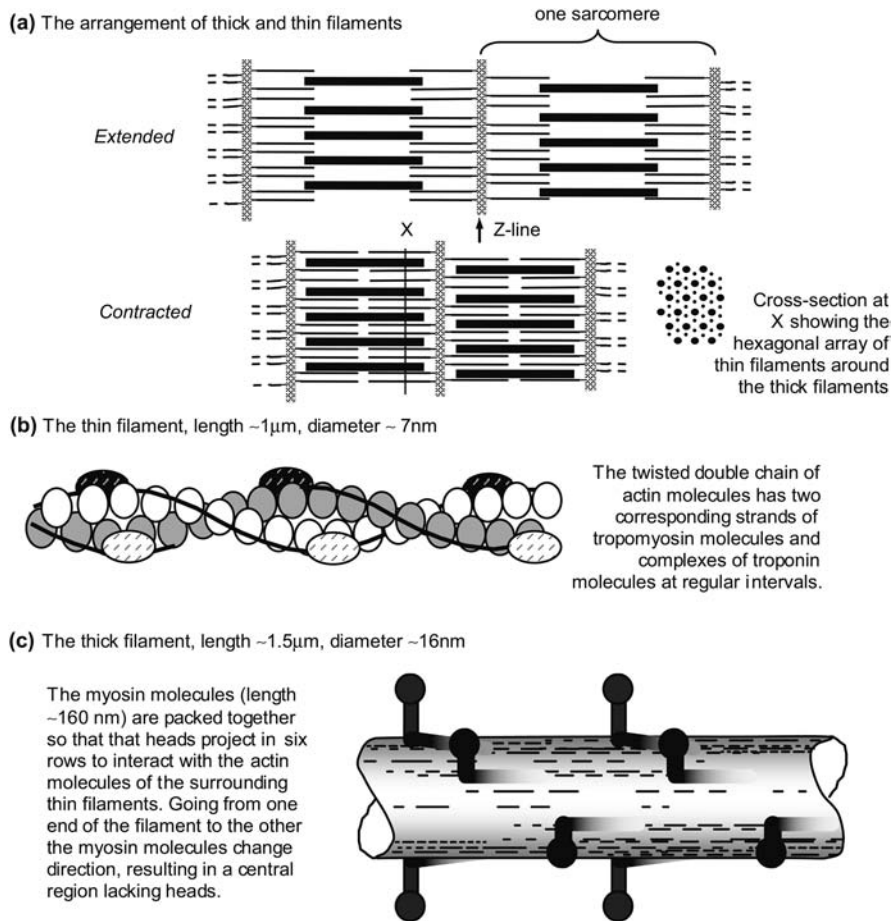


Figure 5.13 The myofilaments of skeletal muscle.

- hydrolysis of one ATP molecule, the myosin head engages with a different actin molecule, the two filaments move relative to each other, and contraction occurs.
- (v) When the nerve impulse ceases, calcium is pumped back into the sarcoplasmic reticulum and the cycle comes to rest at stage (ii). The tropomyosin returns to its original position and the actin can no longer interact with a myosin head. The muscle is now relaxed and will be returned to its original extended state by the action of other muscles.

The energy in the form of ATP, for contraction and for the active uptake of Ca^{2+} ions by the sarcoplasmic reticulum, is derived from two sources. When moderate levels of muscle activity are demanded, pyruvic acid (2-oxopropanoic acid, (5.9)), derived from carbohydrate or fatty acid breakdown, is oxidised in the mitochondria of the muscle fibres. The energy made available by the oxidation is utilised in the mitochondria for the phosphorylation of adenosine diphosphate to the triphosphate. Approximately $2\frac{1}{2}$ molecules of ATP are formed for every $\frac{1}{2}\text{O}_2$ reduced to H_2O . The oxygen is carried from

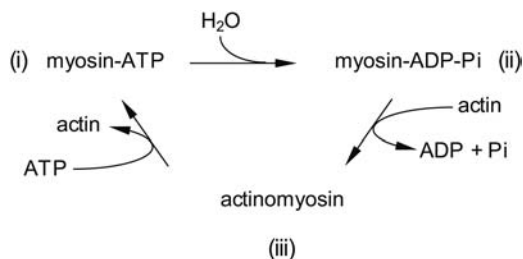
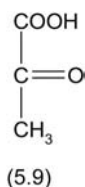


Figure 5.14 The myosin-actinomyosin cycle. When the muscle is in the process of contracting, the cycle functions continuously, as shown here. When the concentration of Ca^{2+} ions decreases, the cycle comes to rest at stage (ii) because linkages between actin and myosin can no longer be formed. If the supply of ATP fails, then the cycle comes to rest at stage (iii).

the blood to the mitochondria as an oxygen adduct of myoglobin, the source of the red colour of fresh meat.



When short bursts of extreme muscle activity are demanded – for example, when sprinting or when a bird like a pheasant suddenly takes to the air – the limited rate at which the lungs can supply oxygen to the muscles *via* the bloodstream is insufficient to ensure an adequate supply of ATP. At these times the muscle obtains its energy by the much less efficient, but much more rapid, anaerobic conversion of glycogen or glucose into lactic acid by the metabolic process of glycolysis. Only two molecules of ATP per molecule of glucose are obtained in glycolysis, compared with 32 when the glucose is oxidised completely to CO_2 . In the muscles of living animals, anaerobic glycolysis cannot be maintained for many seconds before the accumulation of lactic acid becomes excessive and the resulting pain brings the exertion to a halt. As soon as demands on the muscles ease, the accumulated lactic acid is readily oxidised back to pyruvate; then either the pyruvate can be oxidised to CO_2 or some of it is converted back to glucose in the liver.

On the death of the animal, there is still a demand for ATP in the muscle from the pumping systems of the sarcoplasmic reticulum and other reactions, even though the muscles are no longer called upon to contract by signals from the nervous system. As there is obviously no further availability of oxygen from the bloodstream, a supply of ATP is maintained for a time by glycolysis until one of two possible circumstances brings it to a halt. If the animal was starved and/or otherwise stressed by bad treatment before slaughter, the reserves of glycogen in the muscles will have been depleted and will rapidly be exhausted after slaughter. In this situation, glycolysis will quickly come to a halt. However, if the animal's muscles had adequate glycogen reserves, then glycolysis will still cease, but only after a few hours when the accumulation of lactic acid

has reduced the pH to about 5.0–5.5, sufficient to inhibit the activity of the glycolytic enzymes. When the ATP level in the muscle has consequently fallen, Ca^{2+} ions will no longer be pumped out of the sarcoplasm into the vesicles of the sarcoplasmic reticulum. These Ca^{2+} ions allow the myosin to interact with the actin, but the lack of ATP will prevent the operation of the full cycle that would normally lead to contraction. The thick and thin filaments will therefore form a permanent link at stage (iii) and the muscle will become rigid—the state of *rigor mortis*. It is normally impossible to cook meat before it goes into rigor; meat cooked during rigor is said to be exceedingly tough.

The answer to the problem of *rigor mortis* and, as we shall see, other aspects of meat quality, lies in butchers ensuring that the critical pH range of 5.0–5.5 is achieved. (Suffice to say, it is the application of sound pre- and post-slaughter practices rather than the possession of a pH meter that makes a good butcher.) The most obvious effect of low pH is to deter the growth of the putrefactive and pathogenic microorganisms that spread from the hide, intestines, *etc.*, during the preparation of the carcass. The toughness due to *rigor mortis* slowly disappears during conditioning (hanging) of the carcass. The phrase “resolution of *rigor*” is often applied to the disappearance of toughness.^x

Certain specific changes in the myofibrillar proteins are now associated with the resolution of *rigor*. These involve the activity of a group of proteolytic enzymes (*i.e.* enzymes that hydrolyse peptide bonds) known as calcium-activated neutral proteinases or calpains. These enzymes occur in the sarcoplasm of the muscle cell, but require levels of Ca^{2+} ions that only become available once the muscle’s ATP supply is exhausted and the sarcoplasmic reticulum releases them. Several calpains are known to attack a group of minor proteins, sometimes referred to as “gap filaments”, which are responsible for maintaining the organisation of the major myofibrillar proteins, actin and myosin. One of these, titin, appears to provide an elastic connection between the thick filament and the Z-line (see Figure 5.13). The breakdown of these proteins during conditioning creates planes of weakness across the muscle fibres, which allow the muscle fibres to break when they are stretched – in effect, become tender.

Muscle tissue contains a great deal of water, between 55 and 80%. Some of this water is directly bound to the proteins of the muscle, both the myofilaments and the enzyme proteins of the sarcoplasm. The bulk of the water is, however, in the spaces between the filaments. Protein denaturation on cooking releases some of this water and gives meat its desirable moistness; in the raw state, a moist and shiny cut surface of a joint of meat is favoured by purchasers. Although the sarcoplasmic proteins will bind less water at pH values near to their isoelectric points, mostly around pH 5, the principal cause of moisture release (drip) from well-conditioned meat is that, as the pH falls, the gap between neighbouring filaments is reduced and some of the water is squeezed out. Although the butcher would obviously not wish to lose too much moisture from a joint before it is weighed and sold, the increased mobility of the muscle water has another beneficial effect besides giving the meat an attractive moist appearance.

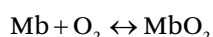
Another effect is, as we shall see, on the colour of the meat. It has already been mentioned that the red colour of myoglobin varies with the age of the animal, from species

^xAssessments of the degree of rigor in the muscles of fictional, and real life, murder victims are routinely used by pathologists to estimate the time at which the crime was committed.

to species, and from muscle to muscle. Some typical data for cattle and pigs are given in Table 5.7, based on results quoted by R. A. Lawrie and D. A. Ledward (2006), see Further Reading. As a general rule, muscles used for intermittent bursts of activity, which are fuelled predominantly by anaerobic glycolysis, contain only low levels of myoglobin and few mitochondria and will thus be pale in colour. The breast, *i.e.* the flight muscle, of poultry is an extreme example. By contrast, muscles used more or less continuously, such as those in the legs of poultry, will be fuelled by oxidative reactions that require high levels of myoglobin and are consequently much darker.

The state of the iron in myoglobin also affects its colour. The structure of myoglobin was shown in Figure 5.6, but we need to concentrate on the iron atom. As shown in Figure 5.15, the state of the iron atom differs in the three important forms of myoglobin. In its normal state, referred to simply as “myoglobin” and abbreviated to Mb, the iron is in its reduced (ferrous) state (Fe^{2+} or Fe^{II}). When it binds an oxygen molecule in the living animal as part of the system for transferring oxygen from the haemoglobin of the bloodstream to the muscle cell mitochondria, the resulting compound is known as oxymyoglobin (MbO_2). Despite the presence of the oxygen, the iron is not oxidised and an oxygen molecule can be released again if the local oxygen concentration is low. Oxymyoglobin is bright red in colour (its spectrum has a double peak, λ_{max} at 544 and 581 nm),^{xi} whereas myoglobin is a dark purplish red (λ_{max} 558 nm).^{xii}

In the living animal, the simple interchange:



is all that matters because the myoglobin only encounters oxygen in high concentrations when it collects oxygen from the blood, or in very low concentrations in the muscle cells to which the oxygen is delivered. However, in meat, the essentially irreversible formation of metmyoglobin takes place at intermediate oxygen concentrations, a process discussed in detail in the first of the Special Topics at the end of this chapter.

Table 5.7 Myoglobin content of different muscles.

Animal	Age	Muscle	Myoglobin content (g per 100 g fresh weight)
Calf ^a	12 days	<i>Longissimus dorsi</i> ^b	0.07
Steer ^c	3 years	<i>Longissimus dorsi</i>	0.46
Pig ^d	5 months	<i>Longissimus dorsi</i>	0.030
Pig ^e	7 months	<i>Longissimus dorsi</i>	0.044
Pig ^f	7 months	<i>Rectus femoris</i> ^g	0.086

^aVeal.

^bThe principal muscle of rib of beef or short back bacon.

^cBeef.

^dPork.

^eUsed for bacon.

^fUsed for pork sausage.

^gThe principal muscle of leg of pork.

^{xi}The term λ_{max} is explained in footnote ii of Chapter 3.

^{xii}The difference between the colours of arterial and venous blood is the result of a similar relationship between the corresponding forms of the haemoglobin in red blood cells (erythrocytes).

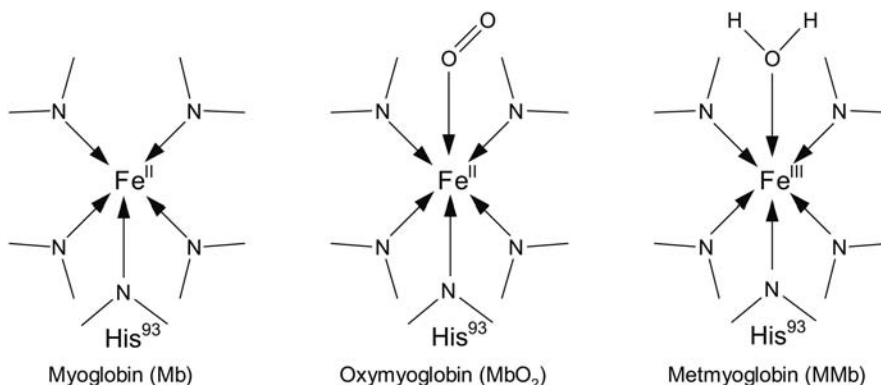


Figure 5.15 The three states of iron in myoglobin. The four nitrogen atoms in a square around the iron atom are those of the porphyrin ring and the fifth nitrogen, marked *His93* is part of the histidine imidazole ring, the 93rd amino acid counting from the NH_2 terminal. The sixth coordination position is occupied by oxygen or water. All six atoms attached to the iron are linked by coordinate bonds, in which the lone pair electrons of the nitrogen or the oxygen atoms (in H_2O or O_2) occupy the empty d-orbitals of the iron.

The interior of the muscle tissue of a carcass will obviously be devoid of oxygen and the freshly cut surface of a piece of raw meat is, as one would expect, the corresponding shade of dark reddish-purple. The diffusion of oxygen into the surface soon begins to convert myoglobin (Mb) to oxymyoglobin (MbO_2) and the meat acquires an attractive bright red appearance as a layer of MbO_2 develops. The importance of an adequate *post mortem* decrease in pH is that oxygen diffuses through the free moisture in the meat and, if the pH remains high, then there is much less free moisture available. The respiratory systems of the muscle continue to consume any available oxygen until the meat is actually cooked, so the MbO_2 layer never becomes more than a few millimetres deep. The relationship between the oxygen concentration and the proportions of the different forms of myoglobin is illustrated in Figure 5.16.

At the intermediate oxygen concentrations existing at the interface between the MbO_2 and Mb layers, the iron of the myoglobin reacts with the oxygen and is converted to the ferric state (Fe^{3+} or Fe^{III}). The resulting metmyoglobin (MMb) is an unattractive shade of brown (λ_{max} 507 nm). Meat kept at room temperature for too long therefore develops a brown layer just below the surface and, as time goes on, this widens until the surface of the meat looks brown rather than red, a sure sign that the meat is no longer fresh. Another consequence of the oxidation of Fe^{II} to Fe^{III} is that a superoxide free radical is generated during the reaction. Although very short-lived, this free radical may well have time to initiate the chain reactions of fatty acid autoxidation, described in Chapter 4, leading to rancidity of the fatty parts of the meat. There is therefore a clear association between the appearance of the raw meat and its flavour when cooked.

Compared with mammals and birds, fish have a relatively simple skeleton and associated musculature. There is a clear distinction between the white muscle, which constitutes the bulk of the edible part of the fish, and the red or dark muscle. In so-called white fish, such as haddock or cod, the dark muscle is in a thin layer under the skin and

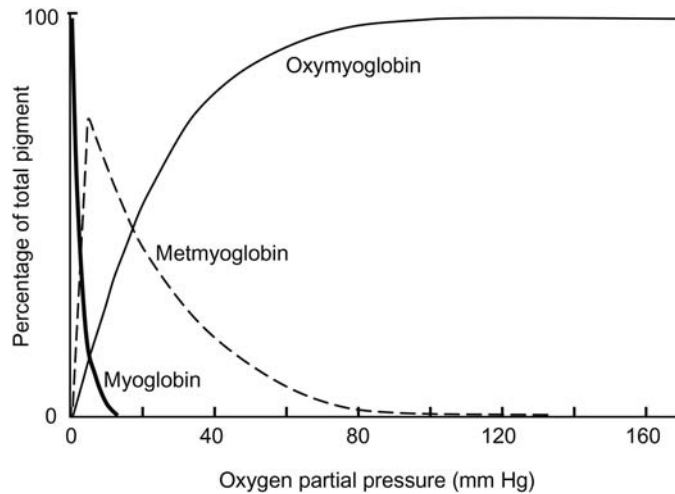


Figure 5.16 Effect of the oxygen concentration on the proportions of different forms of myoglobin. In air at atmospheric pressure, the concentration of oxygen is at a partial pressure of 170 mmHg. (From J. C. Forrest, *et al.*, *Principles of Meat Science*, W. H. Freeman, San Francisco, 1975.)

concentrated in a region under the lateral line on each side. Fatty fish such as herring (*Clupea* sp.) have a larger proportion of dark muscle, which is also rich in fat. The functional distinction between the two types of muscle is essentially similar to that between the light and dark muscle of mammals and birds described earlier in this chapter. The dark muscle provides the motive power for ordinary progress through the water, whereas the white muscle provides the relatively short-lived bursts of activity when, for example, a fish tries to escape from a predator or the fisherman's line. Tuna are unusual in that their entire musculature operates on a more aerobic basis, so tuna flesh is rich in myoglobin and, consequently, red or pink in colour. In some species of marine fish, notably salmon and trout, the "white" muscle is actually bright orange in colour due to the carotenoid astaxanthin (see Chapter 6, Section 6.2) derived from the fish's diet.

Although *post mortem* changes in the myofibrillar structures are an important factor in the texture of meat, the consumer is much more aware of differences in texture that stem from the maturity of the animal and the part of the carcass the meat came from. These variations arise in the connective tissue rather than the contractile tissues of the muscle. The connective tissue of muscles is composed almost entirely of fibres of the protein collagen. Collagen fibres consist of cross-linked, longitudinally arranged tropocollagen molecules, each of which is 280–300 nm long. The tropocollagen molecule itself consists of three very similar polypeptide chains. The conformation of each chain is an extended left-handed helix (remember that the classic α -helix shown in Figure 5.5 is a compact right-handed helix) and the three chains are then wound together in a right-handed helix (Figure 5.17a). The alternation of left- and right-handed helices at different levels of the organisation of the molecule reflects that applied in rope and textile threads, in which it makes spontaneous unravelling much less likely.

The amino acid composition and sequence of the tropocollagen chains are striking. Roughly one-third of all the amino acid residues are glycine; proline plus hydroxyproline

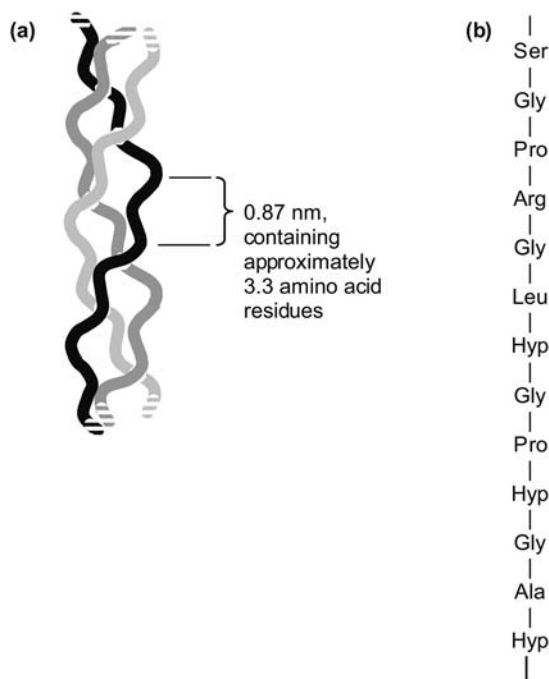


Figure 5.17 Molecular structure of collagen. (a) A section of a tropocollagen molecule showing only the $-C-N-C-C-N-C-$ backbones of the three polypeptide chains. Each chain forms an extended left-handed helix. The three polypeptide chains twist around each other in a right-handed helix. (b) A section of the amino acid sequence of a tropocollagen polypeptide chain.

account for 20–25%. The brief sequence given in Figure 5.17b shows that glycine occurs at every third residue along the chain. Structural studies indicate that the glycine residues always occur at points in the helical structure where the three chains approach closely. Only the minimal side-chain of glycine, a solitary hydrogen atom, can be accommodated in the small space available. The two secondary amino acids (proline and hydroxyproline) are important because their particular form of the peptide bond gives the ideal bond angles for the tropocollagen helix. The structure is maintained by hydrogen bonding between the three chains and also by hydrogen bonds involving the hydroxyproline hydroxyl group. The hydroxylation of proline to hydroxyproline occurs after the protein has been synthesised. The observation that ascorbic acid, vitamin C, is the reducing agent in the hydroxylation reaction can be readily correlated with the clinical signs of the deficiency of this vitamin discussed in Chapter 8. However strong an individual tropocollagen molecule is, the strength of the fibre as a whole will depend on the lateral cross-links to ensure that neighbouring molecules do not slide over each other under tension. Several types of cross-links have been identified, of which two are shown in Figure 5.18. Cross-links are most abundant in connective tissues in which the greatest strength is required, such as the Achilles tendon.

It is a common observation that meat from young animals is more tender than that from older ones. It has now been realised that this is due to an increase in the number

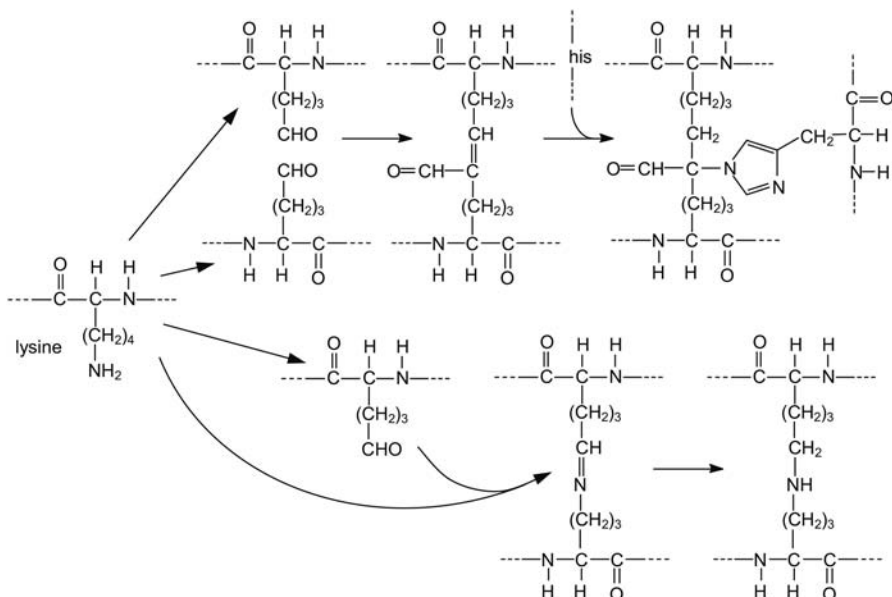


Figure 5.18 Formation of tropocollagen cross-links involving lysine side-chains. The initial conversion of lysine into an aldehyde derivative is catalysed by the enzyme lysine oxidase, but all the subsequent reactions are spontaneous. The two linkages shown here are usually found between neighbouring tropocollagen molecules. The attachment of the histidine side-chains provides a three-way linkage. Other types of link are present, supplementing the hydrogen bonds between chains within the same tropocollagen molecule.

of cross-links as the animal ages, rather than an increase in the proportion of total connective tissue. There are, of course, variations in the amount of collagen between different muscles in the same animal and these are very important to the texture of meat. The most tender cuts, and the most expensive, are those with the lowest proportion of connective tissue. For example, in beef, the connective tissue content of the *psoas major* muscle (in fillet steak) is one-third of that in the *triceps brachii* muscle (stewing beef).

When a solution of tropocollagen is heated, a temperature is reached at which the regular helical structure starts to be lost. The temperature at which this process is half complete is called the melting temperature, T_m . In intact collagen fibres, as in muscle tissue (*i.e.* meat), this loss of ordered structure during cooking results in fibre shortening and is associated with the desirable softening of the meat that is the cook's objective. If heating is prolonged, as in a casserole, not only the hydrogen bonds, but also some of the weaker covalent cross-links will be broken. The result will be solubilisation of the collagen, some of which will be leached out and will cause gelatinisation of the gravy when it cools and the hydrogen bonds are re-established. The thermal denaturation of the proteins of the muscle cells has a rather different outcome. The enzymes and other freely soluble proteins mostly denature, and coagulate, at around 60–70 °C, but the process may not be complete until reaching temperatures of about 90 °C. However, the coagulation of these proteins is not believed to have a major effect on meat

texture. By contrast, the myofibrillar proteins, the proteins that make up the contractile thick and thin filaments, denature over a wide temperature range, some at about 55 °C, but most at about 80 °C.

The different amounts of connective tissue in different muscles, and cuts of meat, are thus a major determinant of the cooking style to be adopted. When we cook a steak, ideally for only a few minutes under a very hot grill, the outer surface will reach temperatures of about 150 °C, giving a tough, but easily cut, shell of denatured protein and also a vast array of flavour compounds generated by the Maillard reaction. Inside this darkened shell, the lower temperature will result in much less protein denaturation, how much depending on the diner's taste for "well done", "medium rare" *etc.* However, the temperature will have been high enough to soften the small proportion of connective tissue collagen without causing extensive coagulation and toughening of the other proteins.

In a casserole, there is sufficient time at high temperatures to soften all the connective tissues and loosen the structure. The toughening of the myofibrillar proteins that occurs is not a problem because our teeth slide easily between the bundles of muscle cells.

Muscle tissue accounts for only a modest proportion of an animal's total collagen; most is located in the skin (in large mammals known as the hide) and the bones. Although these tissues are of little importance as food, they are the usual source of collagen for conversion into an important food ingredient, gelatin. Gelatin is widely used as a gelling or thickening agent, particularly in confectionery, desserts (the "jelly" of children's parties in the UK and "jello" in the USA), some sauces and in meat products. When used to embed pieces of cooked meat or fish, which can then be served cold in slices, it is often referred to as "aspic".

When gelatin is produced from bones, they are first crushed and then steeped in dilute hydrochloric acid for up to two weeks to solubilise and remove the calcium phosphate. The residue can then be processed along similar lines to cattle hides. This involves an initial treatment with lime (calcium hydroxide, Ca(OH)_2) for several weeks. The alkaline conditions break down the cross-links between the collagen chains. When the lime is eventually washed out, other proteins and fatty residues are also washed away. After neutralisation, the residue is then extracted with hot water at progressively higher temperatures. The gelatin, as it now is, dissolves and, after filtration, the solution can be concentrated and finally dehydrated.

Rather like the polysaccharide gel-formers described in Chapter 3, gelatin is insoluble in cold water, but dissolves readily in hot water. When the solution is cooled, the polymer molecules revert from their disorganised "random coil" state to a helical arrangement similar to that in the original connective tissue. In this state, segments of the polymer chains associate together, forming junction zones based on hydrogen bonding, as occurs in polysaccharide gels. The temperature at which solutions of gelatin from different sources form gel as they cool is closely related to the normal body temperature of the source animal. This relationship can be traced to a chemical characteristic of the original collagen, the proportions of the two secondary amine amino acids, proline and hydroxyproline, illustrated by the data in Table 5.8.

Table 5.8 Relationship of collagen's amino acid composition with its melting properties^a.

Collagen source	Hydroxyproline plus proline (per 1000 amino acid residues)	Melting temperature, T_m (°C)	Body temperature (°C)
Calf skin	232	39	37
Shark skin	191	29	24–28
Cod skin	155	16	10–14

^aBased on data in L. Stryer, *Biochemistry*, Freeman, New York, 3rd edn, 1988.

Despite its many good features as a gelling agent, gelatin use is restricted by vegetarian and religious issues. For example, it is difficult for Jewish or Muslim consumers to be confident that the gelatin in an otherwise acceptable product did not come from pig skins or bones. The use of gelatin obtained from fish could allay some consumers' fears, but most of the fish caught commercially are species native to colder seas, such as the North Atlantic, and their collagen melts at too low a temperature to be useful (see Table 5.8). Gelatin from tropical freshwater fish, such as the giant catfish (*Pangasianodon gigas*) and tilapia (*Oreochromis* spp.), is now becoming commercially available.

5.5.5 Bread

Cereals have been an important source of human food for thousands of years. Archaeologists have been able to relate the development of human civilisations, particularly those in the Middle East, to the evolution of what is now regarded as the modern species of bread wheat, *Triticum aestivum*, from its primitive ancestors. The other major cereal crops grown around the world today are as follows:

Rye	<i>Secale cereale</i>
Barley	<i>Hordeum vulgare</i>
Oats	<i>Avena sativa</i>
Sorghum	<i>Sorghum bicolor</i>
Maize (corn)	<i>Zea mays</i>
Rice	<i>Oryza sativa</i>

Comparing these, it is only the flour made from wheat that can be made into loaves with a leavened, open crumb structure. Wheat bread has been eaten in the temperate zones of the world for thousands of years. Some aspects of the ancestry of *T. aestivum* are given more consideration in the second of the Special Topics at the end of the chapter.

The grains (*i.e.* the seeds) of the cereals have a great deal in common. As shown in Figure 5.19, they consist of three major structures: (1) the embryo or germ of the new plant; (2) the endosperm, which is the store of nutrients for the germinating plant; and (3) the protective layers of the seed coat, which are regarded by the miller as bran. The endosperm is about 80% of the bulk of grain. White flour is almost pure endosperm

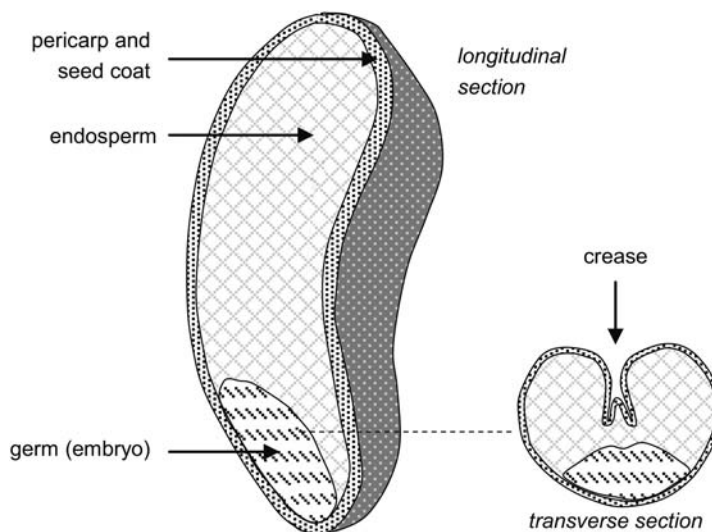


Figure 5.19 Sections through a wheat grain.

and, because we are primarily interested in bread, what follows refers to the endosperm and the endosperm constituents of wheat.

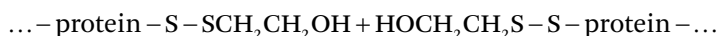
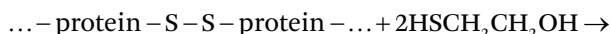
The endosperm consists of tightly packed, thin-walled cells of variable size and shape. The cell walls are the origin of the small proportions of cellulose and hemicelluloses in white flour. The cells are packed with starch granules lying in a matrix of protein. The protein, about 7–15% of the flour, is of two types. One of these (about 15% of the total) consists of the residues of the typical structural and metabolic proteins, mostly enzymes, which are soluble in water or dilute salt solutions. The remaining 85% are the storage proteins of the seed, insoluble in ordinary aqueous media and responsible for dough formation. These dough-forming proteins are collectively referred to as gluten. The gluten can be readily extracted from flour by adding water and mixing it to form a dough, leaving the dough to stand for half an hour or so, then finally kneading the dough under a stream of cold water, which washes out all the soluble material and the starch granules. The resulting tough viscoelastic and sticky material is about one-third protein and two-thirds water.

Flours from different wheat varieties vary in protein content. In general, flours that are good for bread-making (*i.e.* which give a good loaf volume for a given weight of flour) have a high protein content (12–14%), such as the North American spring wheats. Good bread-making wheats are usually the type described by millers as “hard”. Hard wheats have a brittle endosperm and require more energy for milling due to the stronger adhesion of starch and protein. The starch granules of hard wheats undergo more physical damage during milling, which aids their water absorption during bread-making. The baker’s description of a good bread-making flour as “strong” refers to the characteristics of the dough: it is more elastic and more resistant to stretching than dough made from a “weak” flour.

Weak flours are essential for making biscuits and shortcrust pastry. These flours are usually obtained from the “soft” winter wheats (sown in the autumn) grown in Europe.^{xiii} Their protein content is usually <10%. Despite the general correlation between protein content and milling/baking properties, there are enough exceptions to demonstrate that it is the properties of different wheat proteins rather than their abundance that is the major factor. The so-called “patent” or “household” flour that is sold for domestic use is blended from different types to give a product with intermediate properties to provide a reasonable compromise for home baking.

The gluten proteins can be fractionated on the basis of their solubilities. The most soluble, the gliadins, can be extracted using 70% ethanol. These constitute between one-third and one-half of the total gluten. The remainder are the glutenins, which are extremely difficult to dissolve fully. A solution consisting of 0.1 mol dm⁻³ ethanoic acid, 3 mol dm⁻³ urea and 0.01 mol dm⁻³ cetyltrimethylammonium bromide (cetrimide, a surfactant) will dissolve all but 5% of the glutenins. What is known of the molecular structure of the gluten proteins goes a long way towards explaining their remarkable dough-forming and solubility characteristics. Using elaborate electrophoretic techniques, it has been shown that a single variety of wheat may contain >40 different gliadin proteins.

The glutenins are much more difficult to characterise. They form very large aggregates (molecular weights >1 million, mostly about 2 million) of individual proteins known as glutenin subunits. Two different classes of these are recognised, high molecular weight (HMW) and low molecular weight (LMW) subunits. The distinctive elastic properties of dough are largely attributable to the molecular features of the HMW subunits. The glutenin “macropolymers” can be broken down into these subunits by treatment with reagents such as mercaptoethanol, which break the disulfide bridges between the cysteine sulphydryl groups:



Some features of the gliadins and glutenin subunits are shown in Table 5.9.

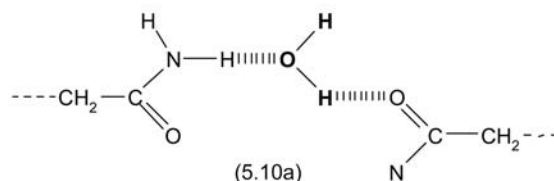
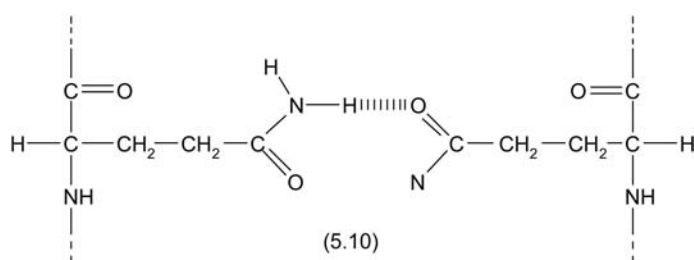
The amino acid composition of these proteins explains their lack of solubility in water. Compared with more typical proteins, the gluten proteins contain a low proportion of charged amino acids (*e.g.* in gliadins, glutamic acid is about 2% and the

^{xiii}British bread-making grists, *i.e.* the blends of grain being milled for a particular batch of flour, now contain significant quantities of European-grown wheat varieties that do give a strong flour. These varieties have been developed as a result of the increasing tendency for the UK and European Union countries to import less of their wheat from outside Europe. Another technique for utilising European wheat is the manufacture of so-called “vital gluten”. This is manufactured by first mixing the flour with water and forming a dough. The starch (and soluble proteins, *etc.*) is then washed out of the dough by prolonged kneading in a stream of cold water and the remaining gluten is dried and milled. As long as high temperatures are avoided at the drying stage, the gluten retains its dough-forming properties, hence the adjective “vital”. The manufacture of QuornTM, the mycoprotein food ingredient used as a meat substitute, was originally devised as an outlet for the considerable quantities of starch that were an inevitable by-product of gluten manufacture. The starch is hydrolysed to glucose, the carbon source in the growth medium for the fungus (*Fusarium venenatum*) from which the mycoprotein is obtained.

Table 5.9 Essential features of the gluten proteins.

		Total gluten protein (%)	Molecular weight ($\times 1000$)	Distinctive features of amino acid composition
Gliadins	Sulfur-rich (α , β and γ)	51–64	32–42	Glutamine 32–42%, proline 15–24%, cysteine 0–2%, phenylalanine 7–9%
	Sulfur-poor (ω)	7–13	55–79	Glutamine 42–53%, proline 20–31%, cysteine 0%
Glutenin subunits	Low MW	19–25	36 – 44 } 90 – 24 }	{ Glutamine 33 – 39 % , glycine 13 – 18 % , cysteine 0.5 – 1 %
	High MW	7–13		

three basic amino acids, histidine, lysine and arginine, total about 3%). The unusually high proportions of glutamine and proline indicated in Table 5.9 are readily explained. These two amino acids contain an above-average proportion of nitrogen and are also particularly easily utilised by the germinating seed as sources of both nitrogen and carbon. By comparison, the metabolic pathways for the breakdown of the other nitrogen-rich amino acids (arginine, lysine and histidine) are much more complex. The high proportion of glutamine might be expected to impart considerable water solubility as glutamine's side-chain may be regarded as hydrophilic. However, the glutamine side-chains instead form large numbers of hydrogen bond cross-links between polypeptide chains (5.10), many with an intermediate water molecule (5.10a).



Complete amino acid sequences have now been obtained for large numbers of gluten proteins and reveal a number of fascinating features. The first is the extensive homology between the amino acid sequences of different proteins within the same class. For example, the first 22 amino acids (counting as usual from the N-terminal end) of α -gliadins A, 2, 10, 11 and 12 and also γ -gliadin 1 are all identical. Similar homologies

have been found within the ω -gliadin class and between many HMW glutenin subunits. These homologies indicate that, in spite of the extensive changes in the wheat brought about by natural evolution and plant-breeders over the centuries, their common genetic ancestry is still evident.

Another feature of all classes of gluten proteins is that their amino acid sequences are highly repetitive, as illustrated by the example of α -gliadin A. The sequence of its first 95 amino acids is based on a 12-amino acid sequence (–PQPQPFPPQQPY–) repeated eight times.^{xiv} The repetition may not be immediately obvious because there is often a small number of changes from the “consensus” sequence in each of the repeats. These changes may arise from the insertion of an extra amino acid, a deletion from the sequence, or a substitution of one amino acid for another. Continuous sequences of 10–20 glutamine residues also occur in many gluten proteins. The third of the Special Topics at the end of this chapter provides further insights into the amino acid sequences of gluten proteins.

Comparison of these sequences with those in proteins whose secondary and tertiary structures are well established has suggested that the gluten proteins are unusually rich in an arrangement known to protein chemists as a “ β -bend” or “ β -turn”. β -Bends are normally found in proteins in which the polypeptide chain undergoes a sharp change in direction (such as at the corners of the α -helical sections of myoglobin, Figure 5.6). The hydrogen bonds linking successive turns in an α -helix (see Figure 5.5) instead cut across the corner. Scanning tunnelling electron microscopy of an HMW gluten subunit, and physical measurements using the sophisticated techniques of Fourier transform infrared spectrometry and far-UV circular dichroism,^{xv} have confirmed this suggestion. The only other protein known to possess an abundance of β -bends is the mammalian connective tissue protein elastin. Ligaments and artery walls are very rich in elastin and it is now believed that they owe their elasticity to this protein as a result of its large numbers of β -bends arranged in sequence to form a β -spiral. There is therefore plenty of support for the idea that the viscoelasticity of a dough can be accounted for by the amino acid sequences of its proteins. The β -spiral structure that has been proposed for one class of gliadin proteins is shown in Figure 5.20.

The current view of the structure of glutenin that emerges from these studies is of a backbone of HMW subunits linked nose-to-tail by disulfide bridges and carrying short, possibly branched, chains of LMW subunits.

Extensive comparison of the bread-making performance of a wheat cultivar with its complement of gliadins and glutenin subunits has shown that HMW glutenin subunits are a major factor. Close genetic linkage (*i.e.* genes within the same chromosome lying very close to each other) means that subunits occur in pairs. For example, subunits 5 + 10 impart a much better baking performance than 2 + 12. It is possible that the best subunit types are those with multiple cysteine sulfhydryl groups for the formation of branched gluten macropolymer chains, producing strong doughs and high loaf volumes.

^{xiv}The single letter codes used for the amino acids are as follows: P or pro = proline, Q or glN = glutamine, F or phe = phenylalanine, Y or tyr = tyrosine.

^{xv}The theory of these techniques is outside the scope of this book.

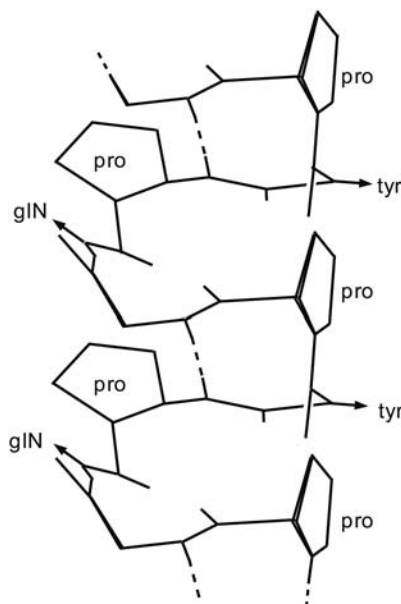
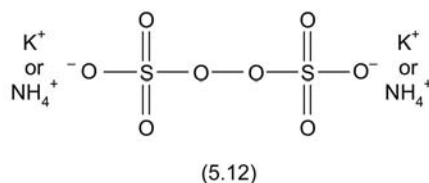
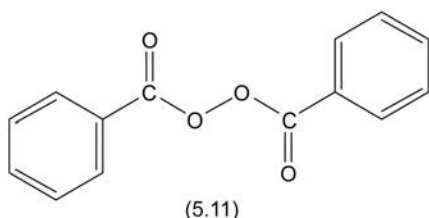


Figure 5.20 The gluten β -spiral. The conformation of the polypeptide chain illustrated is proposed for the repeating pentapeptide units (-pro-tyr-pro-gln-gln-) of ω -gliadin, but the β -spiral regions of other gluten proteins are unlikely to be very different. The -N-C-C-N-C- backbone of the polypeptide chain is shown with proline rings, including the N-H...O=C hydrogen bonds and (arrowed) the positions of the tyrosine and glutamine side-chains. The spiral is stabilised by hydrogen bonding across the β -spiral rather than along it, as in the α -helix. This allows some degree of stretch by rotation of the bonds around the glutamine residues. Interaction between the π -electron clouds of the stacked tyrosine side-chains gives further stabilisation.

When water is added to flour, dough can be formed as the gluten proteins become hydrated. The viscoelastic properties of the dough depend on the glutenin fraction, which is able to form an extended three-dimensional network. Links between the glutenin chains depend on various types of bonding. Hydrogen bonding between the abundant glutamine amide groups is probably the most important of these, but ionic bonding and hydrophobic interactions also have a role. The gliadin molecules are assumed to have a modifying influence on the viscoelastic properties of the dough. It is now well established that the relative proportions of HMW and LMW proteins, the glutenins and gliadins, are a major factor in the bread-making character of a particular flour. In general, a high proportion of glutenins results in doughs that require more mixing, are stronger and give loaves of greater volume, and hence a more open, lighter crumb. A good bread-making flour is unsuitable for biscuits (except crackers) and many types of cake and pastry. Made with strong flour, some types of biscuit would be hard rather than crisp and also tend to shrink erratically during baking. However, these are problems for the commercial baker rather than the domestic cook.

Traditionally, the bread-making properties of a flour have been found to be improved by prolonged storage. Autoxidation of the polyunsaturated fatty acids of flour lipids (see Chapter 4) results in the formation of hydroperoxides, which are powerful oxidising agents. One consequence is the bleaching of the carotenoids in the flour, giving the bread a more attractive, whiter crumb. However, the most important of the beneficial effects of ageing are on loaf volume and crumb texture. During the first 12 months the flour is stored, there is a steady increase in loaf volume and the crumb becomes finer and softer. However, longer storage times result in worsening baking properties. For many years it has been common practice to simulate the ageing process by treating flour with oxidising agents, either at the mill or as additives in the bakery. These have included chlorine dioxide (applied as a gaseous treatment by the miller), benzoyl peroxide [di(benzenecarbonyl)peroxide, (5.11)], ammonium or potassium persulfate (5.12) and potassium bromate or iodate. However, none of these is now permitted within the European Union.^{xvi}



The elimination of these oxidising processes has been made possible by the introduction of ascorbic acid (see Chapter 8) as an improving agent. This has come about largely by the adoption of the Chorleywood Bread Process (CBP) in commercial bread-making, discussed in detail later in this chapter. Its acceptance has undoubtedly been enhanced by the status of ascorbic acid as a vitamin, giving it a whiter-than-white image with consumers, even though none of its vitamin activity survives the oven. The consequent use of unbleached flour has led to the appearance of bread with a distinctly yellow tinge to its crumb, but this is now equated with naturalness rather than a lack of purity as once would have been the case.

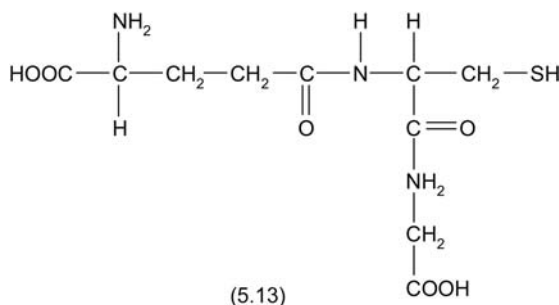
A satisfactory dough is one which can accommodate a large quantity of gas and retain it as the protein “sets” during baking. The achievement of such a dough requires more than simple mixing of the ingredients and mechanical work must also be applied. In traditional bread-making, the kneading of the dough provides some of this work, but the remainder comes from the expanding bubbles of carbon dioxide evolved during fermentation of the yeast. At the molecular level these processes, collectively referred to

^{xvi}Prior to being banned in 2000, chlorine was used in the manufacture of commercial “high ratio” flours used for certain types of cakes, notably American-style muffins. These were made using very high proportions of water and sugar. The chlorine treatment damaged the protein membrane around the starch granules, allowing them to imbibe and retain the extra water. A similar effect is now achieved by subjecting the flour to an intense dry heat treatment. The oxidising agent azodicarbonamide ($\text{H}_2\text{NCON=CONH}_2$) is no longer permitted in the UK, but it remains in use in the USA – for example, in the flour used for burger buns.

as dough development, are not well understood and the following account is unlikely to represent the final word on the subject.

It is generally believed that, during development, the giant glutenin molecules are stretched into linear chains that interact to form elastic sheets around the gas bubbles. The HMW glutenin subunits are involved in a number of different reactions as the formation and development of the dough proceeds. Initially, before the addition of water, the central domains of the protein molecules are bound to one another by the hydrogen bonding referred to earlier. As the proteins hydrate and water molecules become attached, increasing numbers of these inter-chain bonds will be broken. The result, shown in Figure 5.21, is that loops are formed and these are believed to account for much of the dough's elasticity. The β -spiral structure is assumed to be the other source of elasticity in dough and may, in fact, be more important in determining the texture of the crumb after baking.

The polypeptide chains of both gliadins and glutenins will tend to become aligned alongside each other during the early stages of mixing the dough. This gives much more opportunity for hydrogen bond formation and the resistance of the dough to mixing shows a sharp increase. Other reactions involve the sulfhydryl groups of the proteins. Under mechanical stress, exchange reactions between neighbouring sulfhydryl groups allow the glutenin subunits to adopt a more extended arrangement. Exchange reactions (Figure 5.22) require the involvement of an LMW sulfhydryl compound. Glutathione (γ -glutamylcysteinylglycine, GSH, (5.13)), which is found in flour in sufficient quantities ($10\text{--}15\text{ mg kg}^{-1}$), has been identified as having this role. It occurs naturally in flour in three forms: the free form (GSH), an oxidised dimer (GSSG) and bound to a protein molecule (PSSG).



Traditional baking processes require up to three hours of fermentation before the dough is ready for the oven and a great deal of research has taken place to find ways of accelerating the process. After a number of attempts both in the UK and USA, it was at the laboratories of the Flour Milling and Baking Research Association (at Chorleywood, Hertfordshire, UK) that the first completely successful process was developed. Known internationally as the CBP, it depends on the use of ascorbic acid (AA) as a flour improver, coupled with high-speed mixing of the dough. When the flour, water and other ingredients are mixed, the AA is oxidised to dehydroascorbic acid (DHAA, see Chapter 8) by ascorbic acid oxidase, an enzyme present naturally in flour:

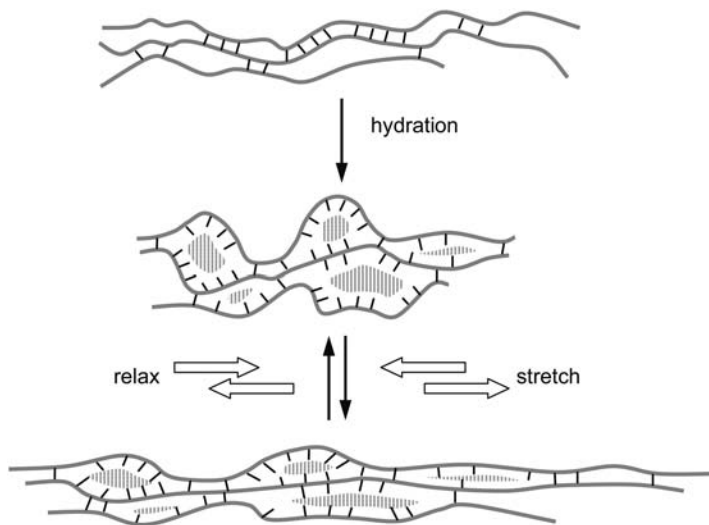


Figure 5.21 Formation of loops in glutenin subunits when flour is mixed with water and their contribution to the elasticity of the dough. There is evidence that the extended sections of the polypeptide chains change their conformation from a β -spiral to a β -pleated sheet.

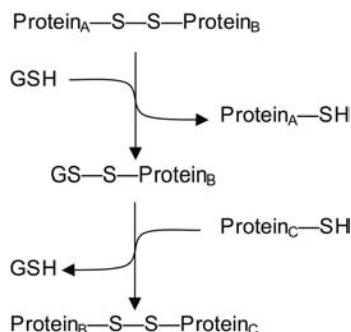
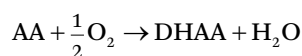
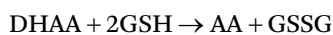


Figure 5.22 Disulfide exchange reactions in dough. The principal cysteine residues involved in reactions with GSH are those in the LMW subunits. These subunits are suspected of forming links between the extended glutenin macropolymers.



The dehydroascorbic acid, an oxidising agent, owes its effectiveness to the presence in flour of a second enzyme, which catalyses the conversion of glutathione to its oxidised dimeric form:



This is, of course, inactive in the disulfide exchange reactions during dough development. The elimination of long fermentation periods has made the CBP popular with bakers, although it requires special mixers capable of 400 rpm and careful control of the

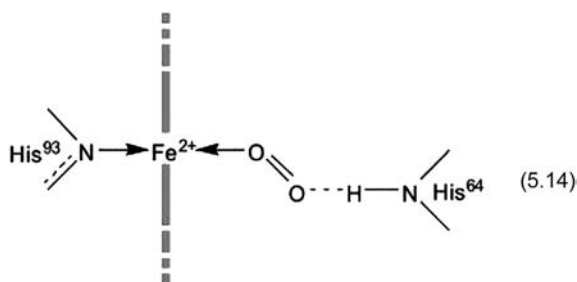
energy imparted to the dough, 11 Watt hours (4×10^4 J) per kg of dough being required. The resulting bread is not easily distinguishable from that produced by traditional bulk fermentation methods. However, it has often been observed that the flavour of CBP bread is inferior, partly because the yeast has had less time to contribute ethanol and other metabolic by-products to the dough. Another factor is the tendency of bakers to under-bake their bread, which means that less of the Maillard reaction takes place as the crust forms. For bakers this has two advantages. The absence of a thick crust makes the loaf easier to slice by machine, but, even more importantly, it means that more water is left behind in the loaf, allowing less flour to be used to produce a loaf of the same weight.

5.6 Special Topics

5.6.1 Myoglobin and Free Radicals

Figure 5.15 showed the basic structure of the three forms of myoglobin found in meat: myoglobin itself; oxymyoglobin with the iron atom present as the Fe^{2+} ion; and metmyoglobin with the iron in the oxidised Fe^{3+} state. The actual mechanism of the reactions involved in the formation of the oxymyoglobin and metmyoglobin is, however, more complex than implied in Figure 5.15.

The iron in the haem part of the molecule depends on the surrounding protein to moderate its reaction with oxygen. In isolation, the Fe^{II} in haem would simply be oxidised irreversibly by molecular oxygen (O_2). To carry out its biological role of binding oxygen reversibly, however, this must be prevented. It is achieved by the occupation of the fifth coordination position of the iron atom by the nitrogen atom of the imidazole ring of histidine-93, together with the hydrophobic environment of the “haem pocket” of the protein. The oxygen adduct is believed to be further stabilised by the formation of a hydrogen bond with the imidazole ring of histidine-64 (5.14).



There is a general consensus that the actual structure of the oxygen adduct represents an equilibrium between the three structures on the left of the sequence shown in Figure 5.23. It is probable that only a very small proportion of the myoglobin exists in the covalent form at any one time. In the equilibrium between the dioxygen and ionic forms, increasing temperature favours the formation of the ionic ferric superoxide.

In common with other free radicals, the superoxide anion is extremely unstable, but in its short life it is still capable of wreaking havoc. With the exception of the obligate anaerobe bacteria, *i.e.* those which cannot survive in the presence of oxygen, all other organisms have a pair of enzymes, superoxide dismutase (SODM, pronounced

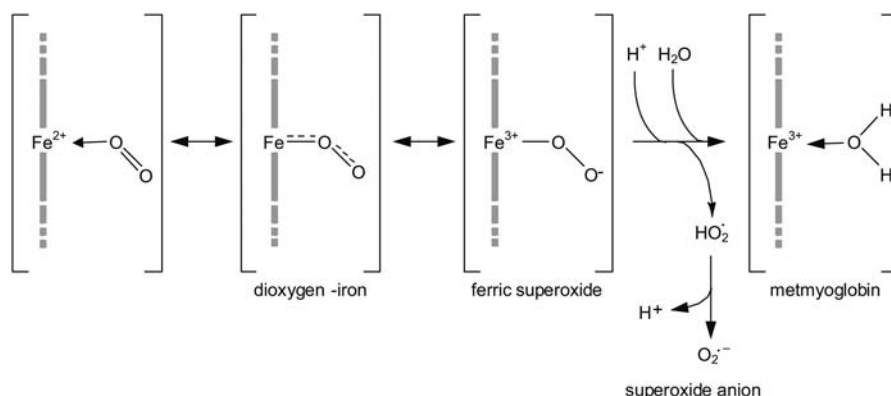
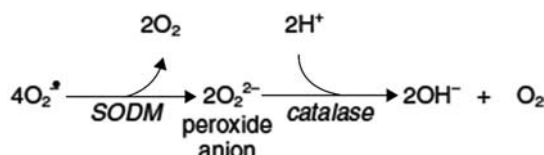


Figure 5.23 Conversion of oxymyoglobin to metmyoglobin and formation of the superoxide anion. The grey bars represent the porphyrin nucleus seen edge-on.

as though associated with Gomorrah) and catalase, whose joint role is to destroy this dangerous by-product of oxygen metabolism:



5.6.2 Wheat Genes and Chromosomes

The large number of different, but closely related, gliadins and glutenin subunits found not only in the single species, *T. aestivum*, but even within a single cultivar (variety) of wheat are a consequence of the special genetic character of the modern bread wheat plant. The nuclei of the vast majority of animal and plant cells (excluding, of course, the germ cells required for sexual reproduction) contain one double set of each chromosome, *i.e.* their cells are *diploid*. The cell nuclei of *T. aestivum* are exceptional in having three copies of each set (referred to as genomes AA, BB and DD) of six paired chromosomes and we therefore refer to bread wheat as *hexaploid*. This is the consequence of normal hybridisation being immediately followed by the doubling of chromosomes, rather than conventional cross-breeding between wheat ancestors. The ancestors of bread wheat are believed to have included relatives of a number of modern species, for example:

<i>Triticum tauschii</i>	Goat-faced grasses	Diploid	DD
<i>Triticum speltoides</i>	Goat-faced grasses	Diploid	BB
<i>Triticum monococcum</i>	Einkorn wheat	Diploid	AA
<i>Triticum turgidum</i>	Emmer wheat	Tetraploid	AABB

Modern durum wheat (*Triticum durum*), used in pasta, is a *tetraploid* relative of emmer wheat. Archaeologists believe that a hexaploid ancestor of modern bread wheat had arisen in the eastern Mediterranean area by 7500 BC. *T. aestivum* had a much larger grain size and a higher protein content than its ancestors, and the impact of this event on early agriculture and human history was considerable. Perhaps through the impact

of human intervention on its evolution during cultivation, the species shed some of its other wild grass characteristics. In particular, the central stem of the ear, the rachis, is much less brittle in modern wheats than in wild emmer wheat. As a result, the grains are not shed spontaneously (essential for a wild grass) and the ears remain intact until they are threshed. Of course, this also makes wheat dependent on human cultivation for seed distribution and its ability to compete with weeds.

The genome and chromosome locations of the genes for most gliadins and HMW glutenin subunits are shown in Table 5.10.

5.6.3 Further Details of Gluten Proteins

The polypeptide chains of ordinary globular proteins are often organised into *domains*. These are usually regions that fold up together to form compact units in which some of the biological activity is localised. Although very little is known for certain about the three-dimensional structure of gluten proteins, it is common to divide their sequences into three (or more) domains. The central domain (usually II) is dominated by the β -spiral structure, whereas in the domains at the extremities (usually I and III) the polypeptide chain is more globular in character, with a great deal of α -helix and other less well-defined structures (Figure 5.24).

The different classes of gluten proteins have different consensus sequences. For example, the consensus sequence P-Q-Q-P-F-P-Q-Q- is found in ω -gliadins, and several others (including G-Q-Q-, P-G-Q-G-Q-Q- and G-Y-Y-P-T-S-L-Q-Q-) are found in HMW glutenin subunits. In most departures from the consensus involving a change of amino acid (as opposed to an insertion or deletion), the replacement amino acid has similar physical properties and there are only minimal changes in the overall structure of the protein. In the case of α -gliadin_A, two short sections of which are shown in Figure 5.25, the full DNA sequence has been established and we know that of the seven changes in these two sections, five (at positions 8, 14, 56, 58 and 66) are the result of single base changes in the codon for the amino acid (Figure 5.25). The regular repeats of these consensus sequences inevitably leads to long sections of regular secondary structure, such as β -spiral shown in Figure 5.20 that characterises these proteins.

5.6.4 Alternative Proteins for Human Food

In recent years, concerns have been growing about the global environmental impact of our increasing demands for animal proteins (meat, fish and milk). Alongside these concerns are issues arising from our increasing demands for cereal proteins (for

Table 5.10 Loci of the gliadin and glutenin genes.

	Chromosome	Genome		
		AA	BB	DD
HMW glutenin subunits	1	1 and 2*	6, 7, 8, 9, 13, 14, 15, 16, 17, 19, 21 and 22	2, 3, 4, 5, 10, 11 and 12
Gliadins	1	ω and γ		
Gliadins	6	α and β		

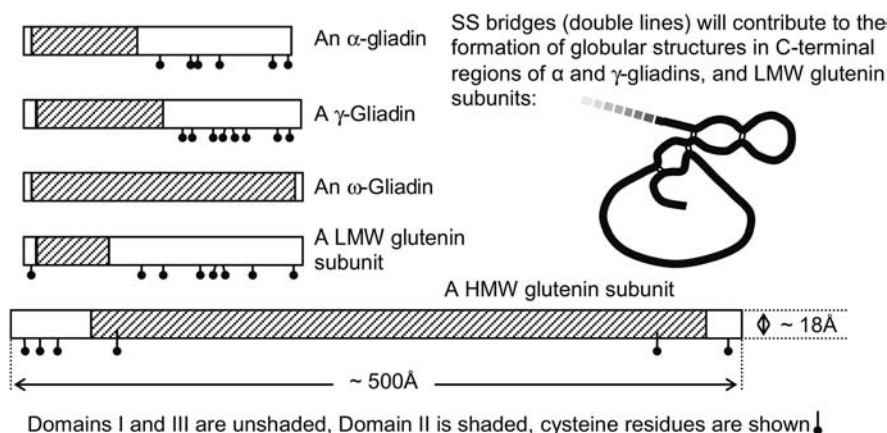


Figure 5.24 Domain structure of gluten proteins. The C-terminal regions of α - and γ -gliadins are believed to contain intramolecular SS bridges between some of their cysteine residues, which results in these molecules being more globular in shape. The central domains are dominated by repeated sequences, such as those shown in Figure 5.25, and these tend to result in the polypeptide chain taking on the β -spiral configuration. In the HMW subunits, the central domain appears to consist entirely of a single β -spiral, giving these molecules a dumbbell shape, with a central rod-like spiral region terminated by globular regions consisting largely of α -helix and containing most or all of the cysteine residues. However, as discussed in Section 5.5.5, the central region is flexible and is involved in the formation of loops as the gluten becomes hydrated.

Residues 4–14	Pro ⁴	Val ⁵	Pro ⁶	GIN ⁷	↔	Leu ⁸	GIN ⁹	Pro ¹⁰	↔	AsN ¹² ↓	GIN ¹¹	Pro ¹³	Ser ¹⁴
Observed codon		CAG				TTG	CAG				CAG		TCT
Consensus codon		GTG				TTT	CCA				CAA		TAT
The consensus dodecapeptide	Pro	GIN	Pro	GIN	Pro	Phe	Pro	Pro	GIN	GIN	Pro	Tyr	
Consensus codon	CAG		CAG							CTA			
Observed codon	CTG		CTG										
Residues 56–68	Leu ⁵⁶	GIN ⁵⁷	Leu ⁵⁸	GIN ⁵⁹	Pro ⁶⁰	Phe ⁶¹	Pro ⁶²	Pro ⁶⁴	GIN ⁶⁵	Leu ⁶⁶	Pro ⁶⁷	Tyr ⁶⁸	
							GIN ⁶³ ↑						

Figure 5.25 Masked repetitions in the amino acid sequence of α -gliadin_A. Two typical sections of the sequence are compared with the postulated consensus sequence. The shading shows where the residues are identical. There are two insertions, asparagine at position 12 and glutamine at position 63, and two deletions in these sections, between positions 7 and 8 and positions 62 and 63. The DNA codons for the positions involved in mutations are italicised.

bread-making) and animal feedstuffs, such as soya. The author of this book is not qualified to contribute to the politics or economics of these debates, but food chemistry may throw some light onto some of the possible alternative sources of protein for human diets.

(a) Plant-based milk-like products

Although the role of milk, of either bovine or human origin, in the nutrition of young humans cannot be challenged, this is not true of its use as a beverage or food ingredient for adult humans. Human intolerance of the lactose in milk is not uncommon (see Chapter 2, Section 2.2) and a small proportion of human infants are allergic to one or more of the proteins in cow's milk (see Chapter 10, Section 10.5). For others, the possible role of milk fats in heart disease *etc.* and the appeal of vegetarian or vegan diets have prompted an interest in milk-like products of purely plant origin.

A wide range of plant materials are now used for the manufacture of milk-like products. Although the details vary, the manufacturing processes generally involve wet or dry milling and water extraction to obtain proteins, carbohydrates, vitamins, *etc.* in solution or as a stable suspension or emulsion. Heat treatments corresponding to pasteurisation and fortification with added vitamins, *etc.* then follow. With the exception of soya, the protein contents of these plant-derived milk-like products are significantly lower than that of cow's milk, as shown in Table 5.11. Manufacturers often claim that their products are “rich in essential amino acids”, but none of the published data suggest that plant sources can provide *all* of the essential (*i.e.* indispensable) amino acids in proportions close to human requirements, or the full range of micronutrients, vitamins and trace minerals found in cow's milk.

The environmental costs of cattle farming are widely recognised. As ruminants, cattle generate large quantities of methane, an important greenhouse gas, and also consume large quantities of water, either directly for drinking or to sustain the crops on which they are fed. However, as shown in Table 5.11, the production of plant-based milk-like products also require substantial amounts of water for both the irrigation of crops and the extraction processes.

(b) Insects

Insects are not usually thought of as a normal component of human diets in Europe and North America, although many other larger invertebrates, such as lobsters, squid, oysters and snails, are widely consumed. However, insects have always been an important supplementary source of dietary protein in much of Asia and Africa and are often

Table 5.11 Protein contents of plant-derived milk-like products compared with cow's milk. The data were obtained from manufacturers' advertising material. Estimates of the volume of water required for the production of some of these milks are also shown,^a but these figures are, in spite of their apparent precision, only rough estimates.

Source	Protein (g L ⁻¹)	Water (L) required for the production of:	
		1 L of product	100 g of protein
Bovine milk	31.5	620	1968
Soya (<i>Glycine max</i>)	28.8	28	972
Almond (<i>Prunus dulcis</i>)	7.6	371	2049
Oat (<i>Avena sativa</i>)	10.4	48	462
Coconut (<i>Cocos nucifera</i>)	12.8		
Rice (<i>Oriza sativa</i>)	2.8	270	9643
Hemp (<i>Cannabis sativa</i>)	8.3		

^aJ. Poore and T. Nemecek, Reducing food's environmental impacts through producers and consumers. *Science*, 2018, **360**, 987.

regarded as a highly desirable delicacy. Christian, Jewish and Islamic traditions all include references to the use of locusts and other insects for food. It is usually the soft-bodied larvae or pupae of insects (*e.g.* beetles, ants, weevils, moths and butterflies) that are consumed, although swarms of adult locusts are harvested for human consumption in parts of Africa.

It is becoming widely recognised that insects are an excellent source of protein and other nutrients. The enormous number of different species of insects that are used as food in different parts of the world makes it impossible to generalise their nutritional quality in terms of amino acid composition. However, the biological functions of these proteins, as enzymes, muscle and membrane components, leads to the expectation that they may have a better balance of amino acid composition than the plant storage proteins on which many humans currently depend.

Gathering insects in the wild is inevitably an inefficient and seasonal process, although it remains the most common way of harvesting them. However, the commercial rearing of insects (*e.g.* mealworms, the larval stage of the mealworm beetle, *Tenebrio molitor*) has been shown to be far more efficient in terms of global warming potential (kg CO₂ per kg protein) and land use (m² per kg protein) than producing beef, milk, pork or chicken. Unfortunately, cultivated insects still have to be fed. Although the feed conversion efficiency of insects may be little better than that achieved by conventional agriculture, rearing insects may well prove to be an efficient mechanism for reutilising wastes from industrial-scale food operations, such as vegetable processing, catering operations and domestic/household situations. The absence of undesirable residues, such as toxic metals, *etc.* from the insect's feed must also be assured.

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6 Colours

Colour is very important in our appreciation of food. Parents who may well deplore their children's selection of the most lurid items at the sweetshop will later be found in the greengrocer's applying identical criteria to their own selection of fruit. Since we can rarely expect to sample the taste of food before we buy it, appearance is really the only guide we have to quality other than the reputation or our past experience of the particular market stall, shop or manufacturer.

Ever since food preservation and processing began to move from the domestic kitchen to the factory, there has been a desire to maintain the colours of processed and preserved foods as close as possible to the colours of the original raw materials. Some foodstuffs acquire their recognisable colour as an integral part of their processing – for example, the brown of bread crust and other baked products, considered in Chapter 2, and the pink of cured meats, mentioned in Chapter 5. In this chapter, we will be particularly concerned with the most colourful foodstuffs, fruit and vegetables, in their raw state (to which the term “natural” might be applied) and the effects on them of culinary and processing operations. Domestic cooks, restaurant chefs and industrial-scale food manufacturers all rely on colour to confirm, or perhaps merely imply, the inclusion of a particular fruit or vegetable in their recipe. For more than 200 years purely artificial colours have often been used to achieve a similar effect. In many cases this is to avoid the expense, or technical difficulty, of actually using any of the particular fruit or vegetable at all.

Public concern over use of unnatural colouring agents in food began to appear in the mid-nineteenth century, but in the UK legislators did not take a serious interest until well into the twentieth century. Over the last 50 years or so the public have become increasingly concerned about the food industry's use of synthetic colours and pressure to eliminate them has greatly increased in recent decades. One response to this pressure has been the development of food colours derived from natural sources. The sources of some of these are foodstuffs already familiar to us, such as beetroot (*Beta vulgaris*, beets in the USA), or rather exotic, such as black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens*). The realisation that many plant pigments may have health benefits for

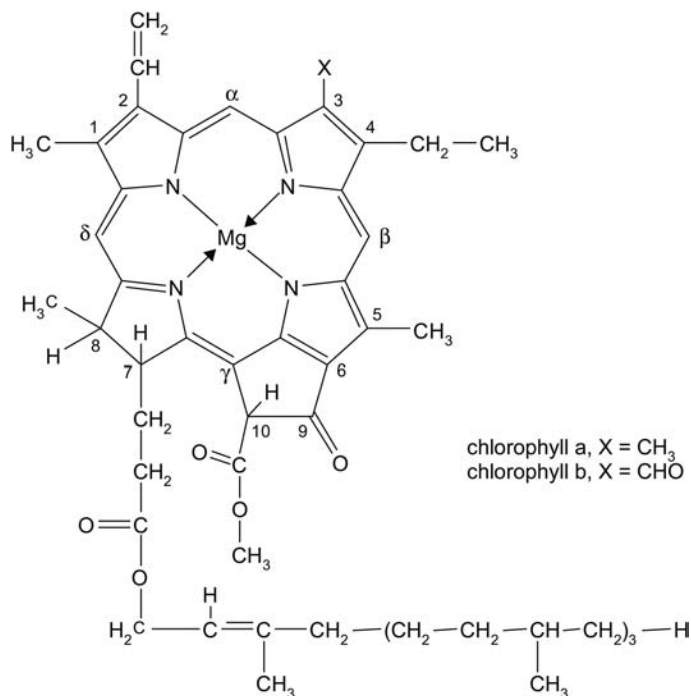
consumers has provided an added incentive for this development. In recent years food scientists have had to widen their search for sources of natural colours to microorganisms that would not usually be regarded as food. As will be discussed in this chapter, these colours are posing interesting questions for consumers and legislators to resolve.

6.1 Chlorophylls

The chlorophylls are the green pigments of leafy vegetables. They also give the green colour to the skin of apples and other fruit, particularly when unripe. Chlorophylls are, in fact, the functional pigments of photosynthesis in green plants. They occur alongside a range of carotenoid pigments in the membranes of the chloroplasts, the organelles that carry out photosynthesis in plant cells. The pigments of the chloroplast are intimately associated with other lipophilic components of the membranes, such as phospholipids, as well as the membrane proteins. Algae and photosynthetic bacteria also contain a number of different types of chlorophyll, but the higher plants that concern us contain only the types known as chlorophyll a and b, in an approximate ratio of 3:1. The structures of chlorophyll a and b are shown in Figure 6.1. They are essentially porphyrins, similar to those in haem pigments such as myoglobin, apart from the substituents, the presence of magnesium rather than iron and the formation of a fifth ring by the linkage of position 6 to the γ -methine bridge. By analogy with myoglobin, we can deduce that the porphyrin ring will associate readily with the hydrophobic regions of chloroplast membrane proteins. The extended phytol side-chain facilitates close associations with carotenoids and the membrane lipids.

The absorption spectra of chlorophyll a and b are shown in Figure 6.2. The absorption maxima and extinction coefficients are not easily defined because they vary with the organic solvent used and the presence of trace amounts of moisture. These variations make the accurate determination of chlorophyll levels in leafy tissues by spectrophotometry rather difficult. Another difficulty is that, in solution, especially if methanol is present, there is a tendency for oxidation or isomerisation to occur, with replacement of the hydrogen at position 10 with $-\text{OH}$ or $-\text{OCH}_3$.

It is the stability of chlorophyll in vegetable tissues, or its lack of it, which is of particular interest to food chemists. Chlorophyll is lost naturally from leaves at the end of their active life in the plant. Their breakdown accompanies a general breakdown of the chloroplast membranes, but the carotenoids are more stable. This gives autumn leaves – and vegetables that are no longer fresh – a residual yellowish colour. Several different reactions have been implicated in the breakdown of chlorophyll, including cleavage and opening of the porphyrin ring and removal of the phytol residue by the enzyme chlorophyllase, but there is little hard evidence pointing at one mechanism to the exclusion of others. The enzyme chlorophyllase is denatured by blanching. When green vegetables are blanched prior to freezing, during ordinary cooking or in canning, there is evidence that the phytol side-chain is lost, giving the corresponding chlorophyllide a and b. The most significant event, however, is the loss of the magnesium ion. This occurs most readily under acid conditions, with the Mg^{2+} ion being replaced by protons (hydrogen ions) to give pheophytin a and b. Pheophytins are a dirty brown



Figures 6.1 Structure of chlorophyll. The double bonds of the porphyrin ring system are shown arbitrarily; in fact, the π -electrons in such systems are completely delocalised. The C20 isoprenoid alcohol esterified to the propionyl substituent at position 7 is known as phytol.

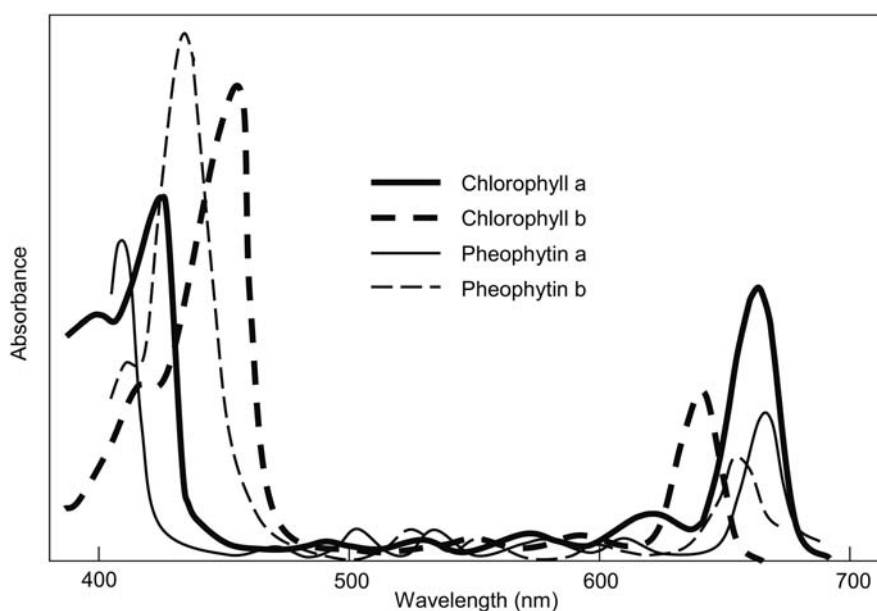


Figure 6.2 Absorption spectra of the chlorophylls and pheophytins.

colour (see Figure 6.2) and are familiar as the dominant pigments in vegetables, such as cabbage, which have been overcooked. The acidity of the contents of plant cell vacuoles makes it difficult to avoid pheophytin formation – for example, during the vigorous heating involved in canning peas. One approach is to keep the cooking water slightly alkaline by the addition of a small quantity of sodium bicarbonate. This is successful in retaining the colour, but the alkaline conditions have an unhappy effect on texture and flavour, and the vitamin C content also suffers.

Some chlorophyll loss is inevitable during the canning of peas and has traditionally been compensated for by the addition of artificial colouring. Copper(II) sulfate was occasionally (and dangerously) used for this purpose in the early years of the twentieth century, but this was later universally replaced by synthetic dye mixtures, such as tartrazine plus Green S (see Section 6.7). In recent years, these too have been superseded as public disquiet with synthetic food colours has led to their replacement with more acceptable, if less effective, chlorophyll derivatives. In the eighteenth and nineteenth centuries green vegetables, such as cabbage, were preserved, for consumption in later months, by boiling followed by storage in vinegar (pickling). It might be expected from previous paragraphs that this would lead to the destruction of chlorophyll and that the pickles would have a most unappetising colour. In fact, this problem hardly ever arose because copper pans were used to boil the vegetables. The acetic acid in the vinegar dissolved Cu^{2+} ions, which readily took up the places in chlorophyll vacated by the Mg^{2+} ions. The resulting chlorophyll derivative had a strong bluish green shade, thus maintaining an acceptable green colour for the pickled vegetables. In the 1850s the British public began to be concerned over this practice of “coppering” vegetables and, by about 1860, the practice had been abandoned (see Chapter 11, Section 11.2.2). As far as chutney-type mixed pickles are concerned, we have grown to expect their brown colour and caramel is now added to enhance the brown colour of the pheophytins already present.

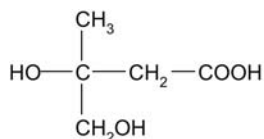
Growing doubts about the safety of synthetic dyestuffs, especially tartrazine, have encouraged food chemists to examine the possible use of chlorophyll derivatives as alternatives. Chlorophyll itself presents difficulties. Not only is it unstable, but its insolubility in water makes it difficult to incorporate in all but fatty foods (where greenness is rarely required). However, a derivative known as sodium copper chlorophyllin is now being used. This is a sodium salt of chlorophyllin (*i.e.* chlorophyll from which the phytol side-chain has been removed) and has had the magnesium replaced by copper. Various plant materials are used as sources of chlorophyll by pigment manufacturers, including grasses, alfalfa (*Medicago sativa*), spinach (*Spinacea oleracea*) and stinging nettles (*Urtica dioica*). The product has an acceptable blue–green colour, is moderately soluble in water and, most importantly, it is able to survive the heating involved in canning. The amount of copper it contains is far too small to represent a toxicity hazard.

Chlorophylls and chlorophyllins, and their copper complexes, are permitted food additives in Europe, Australia, New Zealand, Canada and Japan. In Europe, and elsewhere, the use of colourants of any type in raw or unprocessed foods such as green vegetables is forbidden. Olives (*Olea europaea*) for table consumption are processed in various ways, including heat treatment and fermentation, and their green colour is regarded as an important indicator of quality. The use of any artificial colours with olives is therefore widely forbidden. Illegally used synthetic dyestuffs, such as Food

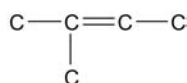
Green S, are relatively easy for food analysts to detect, but methods are now becoming available that can discriminate between the olive's natural chlorophylls and added copper chlorophyll derivatives.

6.2 Carotenoids

Carotenoid pigments are responsible for most of the yellow and orange colours of fruits and vegetables. They are found in the chloroplasts of green plant tissues alongside chlorophyll and in the chromoplasts of other tissues, such as flower petals. In chloroplasts, carotenoids are associated with the membrane proteins of the photosynthetic machinery. They occur as crystals in the chromoplasts of carrots and tomatoes, whereas in some other orange-coloured tissues, such as paprika and mangoes (*Mangifera indica*), the carotenoids are dissolved in lipid droplets. Chemically, carotenoids are classified as terpenoids, derived in nature from the metabolic intermediate mevalonic acid (6.1). This is the natural precursor of the basic terpenoid structural element, the isoprene unit (6.2). Terpenoids with one, two, three or four isoprene units (hemi-, mono-, sesqui- and diterpenoids, respectively) are well known, but the steroids, which are triterpenoids (*i.e.* containing 30 carbon atoms), and the carotenoids, the only known tetraterpenoid compounds, are the most important to food chemists.

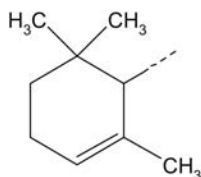


(6.1)

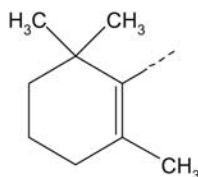


(6.2)

The carotenoids are divided into two main groups: the carotenes, which are hydrocarbons; and the xanthophylls, which contain oxygen. The structures of the most important carotenoids in foodstuffs are shown in Figures 6.3 and 6.4. The simplest carotene is lycopene and the numbering system illustrates clearly how the molecule is constructed from two diterpenoid subunits linked “nose-to-nose”. Other carotenes are formed by cyclisation at one or both the ends of the chain. One of two possible ring structures results, the α -ionone (6.3) or the β -ionone (6.4). The acyclic end-group occurring in lycopene and γ -carotene is sometimes referred to as the ψ -ionone structure.



(6.3)



(6.4)

The xanthophylls arise initially by hydroxylation of carotenes and most plant tissues contain traces of cryptoxanthins, the monohydroxyl precursors of the dihydroxyl xanthophylls, such as zeaxanthin and lutein (Figure 6.4). Subsequent oxidation leads to

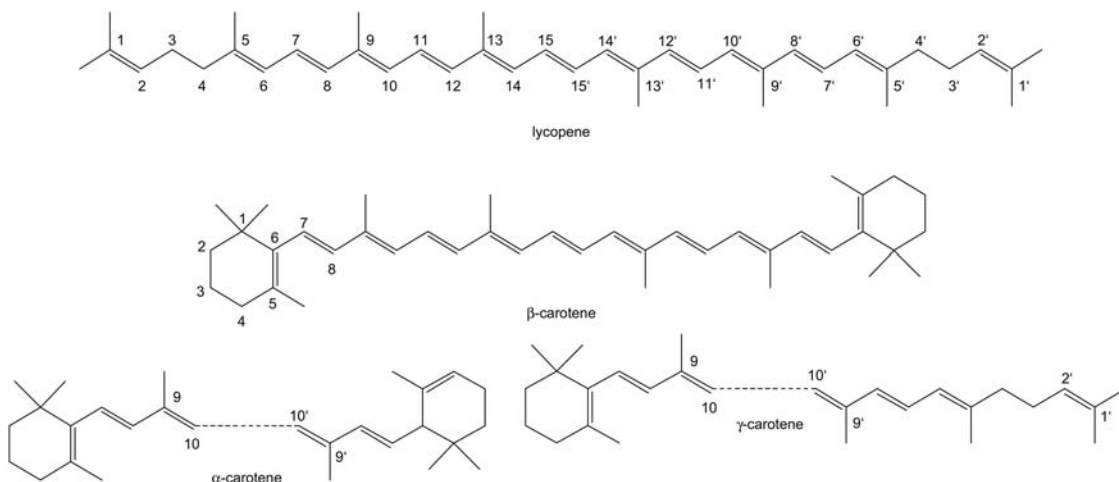


Figure 6.3 Carotene structures. In the conventional numbering system used here, the backbone carbon atoms are numbered from 1 to 15 and 1' to 15'. The terminal and branch methyl groups along the chain are numbered 16 and 16', etc. The central region common to all carotenoids (11 to 11') has been omitted from the formulae of α - and γ -carotene and in Figure 6.4. In all naturally occurring carotenoids, all the double bonds in the central acyclic region are in the *trans* conformation. As was discussed earlier (see Figure 4.1), this ensures that the overall shape of the carotenoid molecule is straight and able to integrate readily with the hydrocarbon chains of membrane lipids.

the formation of epoxides such as antheraxanthin. Neoxanthin is an example, rare in nature, of an allene, in which one carbon atom carries two double bonds.

The apocarotenoids are a small group of xanthophylls and carotenes in which fragments have been removed from one or both ends of the chain. Three examples are shown in Figure 6.5. With the exception of the two acidic apocarotenoids, which form water-soluble salts under alkaline conditions, carotenoids are only freely soluble in non-polar organic solvents. The absorption spectra of carotenoids are generally fairly similar, the nature of the solvent causing almost as much variation in λ_{max} and the extinction coefficient as occurs between individual carotenoids. The absorption spectra shown in Figure 6.6 confirm the observation that xanthophylls are the dominant pigment in yellow tissues, whereas carotenes give orange and orange-red shades or, in the case of carotene, a bright red.

The distribution of the carotenoids between the different plant groups and types of food material shows no obvious pattern. Among the leafy green vegetables, the carotenoid content follows the general pattern of all higher plant chloroplasts, with β -carotene dominant and the xanthophylls lutein, violaxanthin and neoxanthin all prominent. Zeaxanthin, α -carotene, cryptoxanthin (3-hydroxy- β -carotene) and antheraxanthin also occur in small amounts. The quantity of β -carotene in leaf tissue is usually between 200 and 700 $\mu\text{g g}^{-1}$ (dry weight).

A wider range of carotenoids is found among different types of fruit. Only rarely are β -carotene and its immediate xanthophyll derivatives, cryptoxanthin and zeaxanthin, predominant. The levels of β -carotene in the flesh of apricots (*Prunus armeniaca*) can

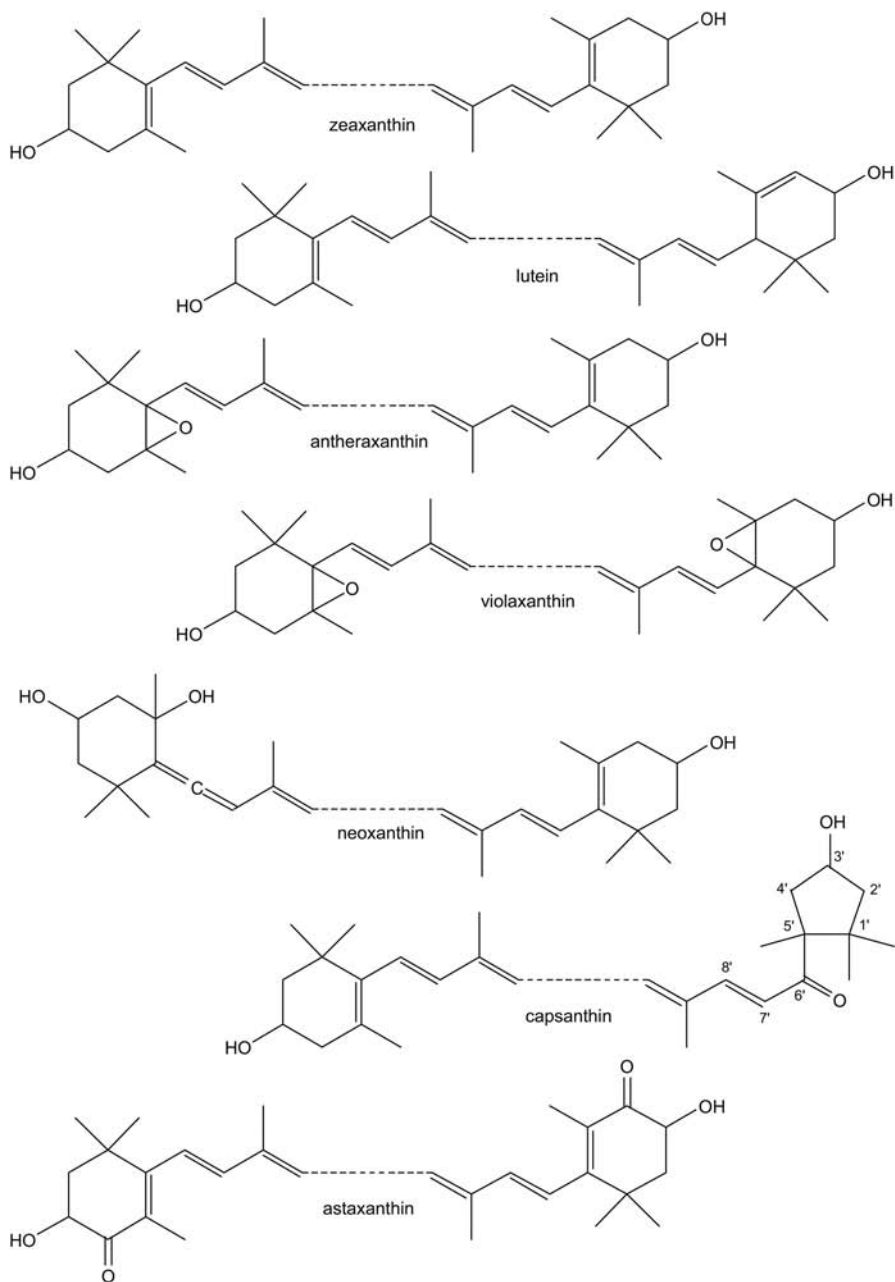


Figure 6.4 Xanthophyll structures.

vary widely, from around 200 to 2000 or more mg per 100 g, depending on the variety, growing cultivation conditions, *etc.* Mangoes (*M. indica*) and persimmons (*Diospyros* spp.) are also notable for their β -carotene content. Lycopene is the major carotenoid in tomatoes. Orange juice contains varying proportions of cryptoxanthin, lutein, antheraxanthin and violaxanthin, together with trace amounts of their carotene precursors.

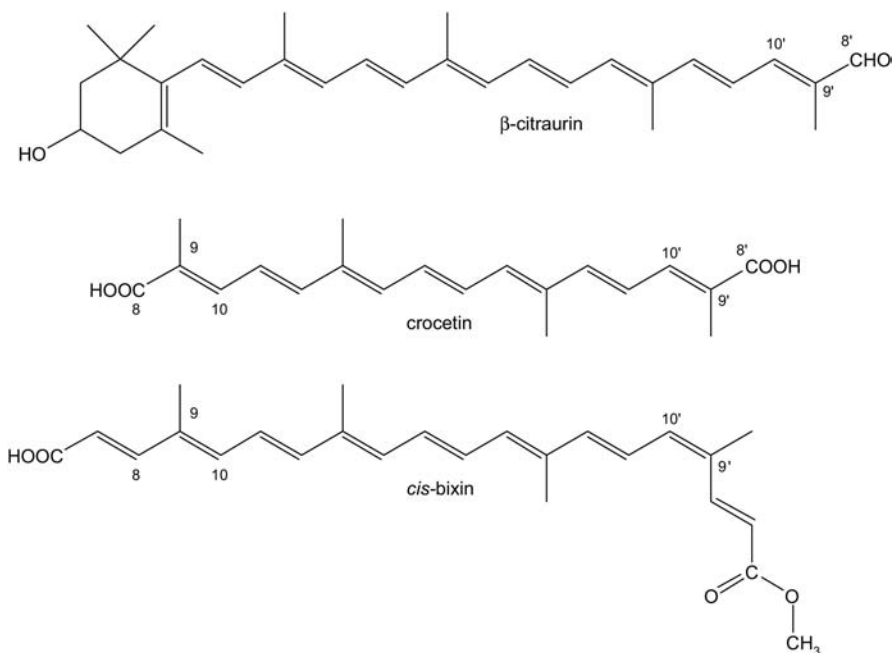


Figure 6.5 Apocarotenoid structures.

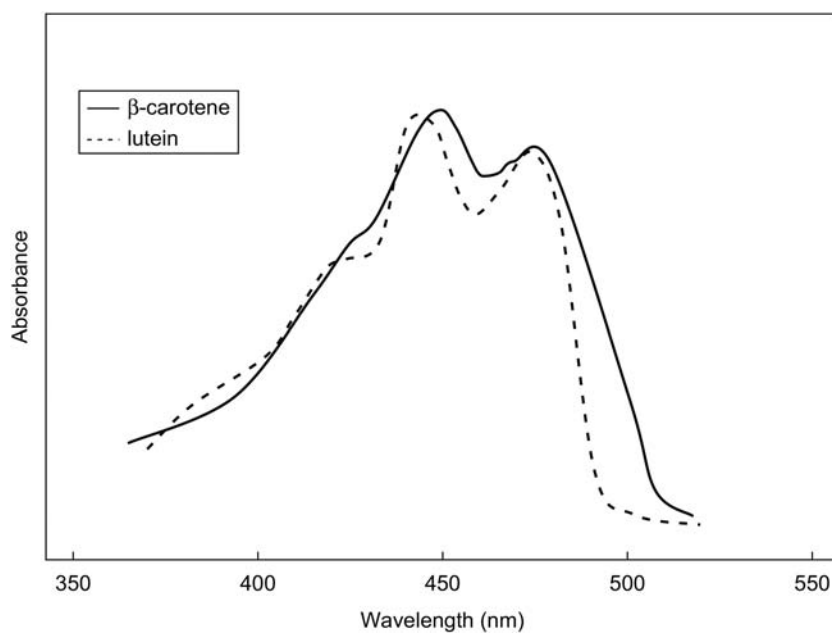
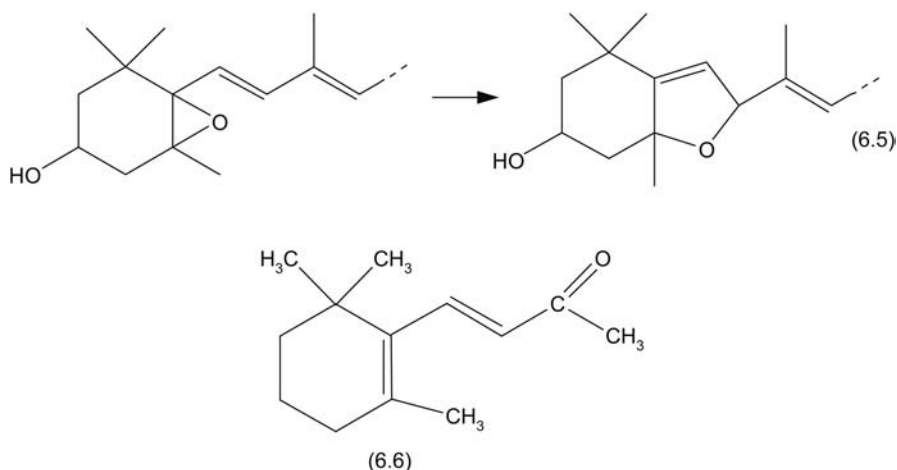


Figure 6.6 Absorption spectra of carotenoids.

The apocarotenoid β -citraurin (see Figure 6.5) has also been reported. The acidic conditions during the processing of juice promote some spontaneous conversion of the 5,6- and 5',6'-epoxide groups into 5,8- and 5',8'-furanoid oxides (6.5). Oxidation and bleaching occur rapidly in the presence of oxygen, particularly in dried foods such as dehydrated diced carrots. Hydroperoxides resulting from lipid breakdown (see Chapter 4, Section 4.3.2) are very effective in bleaching carotenoids. The final breakdown of β -carotene leaves a residue of β -ionone (6.6), a volatile compound that gives sun-dried (and partially bleached) hay its characteristic odour.



Some carotenoids are restricted to a few or even a single plant species; the capsanthin of red peppers is a good example. The classic occurrence of carotenoids is, of course, in the carrot. Here β -carotene predominates, together with a proportion of α -carotene. The carotenoids in the usual varieties of carrot comprise only 5–10% xanthophylls, located mostly in the yellow core. The total carotene content of carrots is 60–120 $\mu\text{g g}^{-1}$ fresh weight, but some varieties contain $>300 \mu\text{g g}^{-1}$.

The nutritional functions of dietary carotenoids are beginning to receive greater attention from food scientists and the contribution of different foodstuffs to the supply of particular carotenoids in the human diet needs to be recognised. Although leafy green vegetables also contain significant amounts of β -carotene, carrots remain its most important source in the temperate regions of the northern hemisphere, such as Europe. In tropical and subtropical regions, however, mangoes are the major source of β -carotene. Leafy green vegetables are the predominant source of lutein in most diets and tomatoes provide the bulk of the lycopene.

Carotenoids continue to be popular as food colourants and many of them are now officially sanctioned for this use in Europe, including the UK (see Table 6.1). The two apocarotenoids, crocetin and bixin, have been used in a limited range of foods for many years. Crocetin is the major pigment of the spice saffron (from the saffron crocus, *Crocus sativus*), in which it occurs in the form of a glycoside, with each of its carboxyl groups esterified by the disaccharide gentiobiose. Saffron is extremely expensive because it requires about 150 000 stigmas from the flowers of the autumn-flowering *C. sativus* to produce 1 kg of dried spice. Crocetin is not currently classified as a food

Table 6.1 Carotenoids commonly used as food colourants in Europe. Extracts containing crocin or crocetin are treated as spices rather than colourants and are not classified as food additives in the European Union.

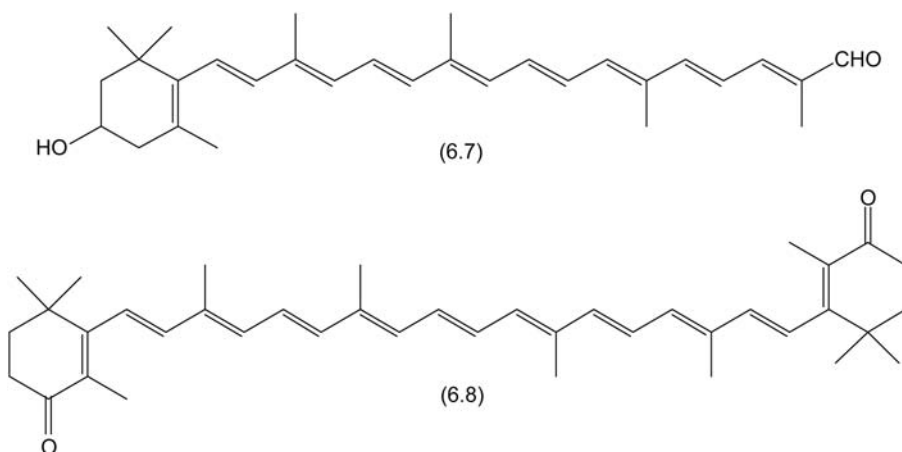
Pigment	E No.	Commercial source
Mixed carotenes and β -carotene	E160a	Palm oil, carrots and algae (<i>Dunaliella</i> sp.)
<i>Cis</i> -bixin, <i>cis</i> -norbixin	E160b	Annatto
Capsanthin	E160c	Red peppers (<i>Capsicum annum</i>)
Lycopene	E160d	Tomato
β -Apo-8'-carotenal	E160e	Chemical synthesis
Ethyl ester of β -apo-8'-carotenal	E160f	Chemical synthesis
Lutein	E161b	Marigold (<i>Tagetes erecta</i>), alfalfa
Canthaxanthin	E161g	Chemical synthesis
Crocin and crocetin		<i>Crocus sativus</i>

additive (hence no E number) because it is always used in the form of saffron, which is officially regarded as a food ingredient, the primary function of which is as an aromatic flavouring.

cis-Bixin (see Figure 6.5) is the major constituent of the colouring annatto, which is the resinous coating on the seeds of *Bixa orellana*, a large shrub grown in a number of tropical countries. Norbixin is a water-soluble version obtained by alkaline hydrolysis, which gives the dicarboxylic acid. The high temperature of the hydrolysis results in considerable isomerisation of the *cis* double bond and the end-product contains both *cis* and *trans* isomers. Annatto probably has the longest history of use as natural food colour in the UK and is the traditional colouring used in red cheeses such as Red Leicester. It is specifically forbidden for use in spices such as curry powder in case the red colour gives a false impression of quality. Palm oil contains high levels of β -carotene (13–120 mg per 100 g) and is also used to colour margarine.

Lycopene, lutein and β -carotene extracted from natural sources are commercially available for use as food colourants in essentially aqueous environments such as yoghurts, cloudy or translucent soft drinks and confectionery. Very small crystals of the carotenoid are dispersed either in a liquid carrier, such as glycerol, or a inert powdered solid compatible with food. The dramatic fall in the acceptability of synthetic food colours, such as tartrazine, discussed later in this chapter, has provided a considerable stimulus for the development of these carotenoid-based colourants. Several carotenoids have been chemically synthesised commercially, including β -carotene, lycopene, β -apo-8'-carotenal (6.7) and canthaxanthin (6.8). Synthetic lycopene can be used as a food colour in the USA, but not currently in Europe, where it is only permitted as a dietary supplement. Their commercial preparations are increasingly used in a wide range of products, including margarine, cheese, ice cream and some baked goods, such as cakes and biscuits. In Europe, canthaxanthin is only permitted for direct use in one product, Strasbourg sausage ("*Saucisses de Strasbourg*"). It is, however, allowed as an additive in certain feedstuffs for animals and fish intended ultimately for human consumption, such as farmed salmon and chickens, in which it improves the colour of the flesh and the egg yolk, respectively. Canthaxanthin is permitted in the USA for general use in food products for human consumption. Astaxanthin, extracted from cultures of the marine alga *Haematococcus pluvialis*, is also used on a large scale in the feed for

farmed salmon. This algal species is regarded as a significant source of the astaxanthin found in the flesh of wild salmon.

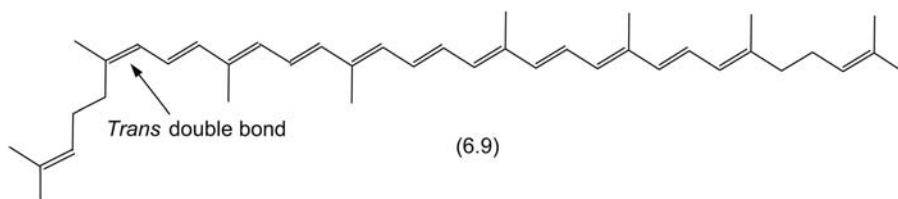


Although carotenoids are only synthesised by plants and microorganisms, they are also found in some animal tissues. Egg yolk owes its colour to the two xanthophylls, lutein (750–800 mg per 100 g) and zeaxanthin, with only a small proportion of β -carotene. These carotenoids, and the smaller amounts that give the depot fat of animals its yellowish hue, are derived from vegetable material in the diet. The dark greenish-purple pigment of the lobster carapace (and the shells of other shellfish) is a complex of protein combined with astaxanthin. When lobster is boiled, the protein is denatured and the colour reverts to the reddish shade more typical of a carotenoid. Astaxanthin is also the natural source of the pink colour of salmon flesh. Algae at the bottom of the food chain leading to these marine animals are responsible for the initial formation of the tetraterpenoid raw material for astaxanthin synthesis.

Carotenoids are generally fairly stable in their natural environment, but they are much more labile when food is heated or when they are extracted into solution in oils or organic solvents. On heating in the absence of air, there is a tendency for some of the *trans* double bonds of carotenes to isomerise to *cis*, forming what are sometimes termed “neocarotenes”. Although carotenes containing one or more *cis* double bonds have been detected in trace amounts in photosynthetic plant tissues, this is most often the result of high-temperature food processing. There is a loss of colour intensity as the number of *cis* double bonds increases. The difference in colour between canned and fresh pineapple is a well-known example of this effect. The provitamin A activities of 13-*cis*- β -carotene and 9-*cis*- β -carotene, resulting from high-temperature processing, are about half that of the usual 100% *trans* isomer.

The implications of lycopene isomerisation have attracted considerable attention from nutritionists. Traces of lycopene isomers with *cis* rather than *trans* double bonds can be detected in plant tissues. However, once removed from the stabilising influence of cell membranes, heating in the presence of cooking oils can lead to isomerisation of as much as 50% or more of the lycopene. Isomers with *cis* double bonds at positions 5 (6.9), 9, 13 or 15 are most abundant. These isomers dissolve more readily in cooking oils and are also more readily absorbed into the bloodstream from cooked foods such as lasagne and

similar Mediterranean dishes rich in tomatoes. The *cis* isomers predominate in the lycopene that accumulates in human tissues. There is now a considerable body of epidemiological evidence indicating an inverse relationship lycopene intake and prostate cancer risk. It is widely assumed that the biological activity of dietary lycopene is derived from its antioxidant properties and it appears that not only is lycopene a particularly effective antioxidant, but also that its “unnatural” *cis* isomers are more active than the naturally occurring *trans* isomers. These same isomers may also be a factor in the benefits of tomato-rich Mediterranean diets in reducing the incidence of cardiovascular disease.



The role of carotenoids containing at least one β -ring (collectively referred to as provitamin A) as the precursors of vitamin A is well established. Vitamin A activity is possessed by substances that are the precursors of retinal, the non-protein component of the visual pigment of the retina. The enzyme-catalysed formation of retinal from carotenoid precursors is illustrated in Figure 6.7. Subject to the availability of sufficient vitamin A or its precursors in the diet, the level of retinol in the tissues is physiologically regulated and high dietary levels (*i.e.* at the upper end of the normal nutritional range) do not increase the concentration of retinol in the tissues or the bloodstream. Higher serum β -carotene levels have been associated with lower risks of various types of cancer, notably of the lung, in a number of epidemiological studies.ⁱ Vitamin A is discussed further in Chapter 8.

Two common xanthophyll carotenoids, lutein and zeaxanthin, are the major source of the yellow colour of the cells in the macula lutea of the eye. This is a small area at the centre of the retina where the photosensitive cells (the rods and cones) are most tightly packed. The yellow pigments are believed to act as filters to protect the photosensitive cells from damage by ultraviolet light. Age-related macular degeneration (AMD) is a major cause of sight loss in elderly people and has been attributed to inadequate levels of dietary xanthophylls. There is growing support for the possibility that dietary supplementation with lutein and zeaxanthin may offer some protection against AMD, although the intake of lutein that has been recommended (at least 10 mg per day) may be difficult to achieve by diet alone.

Epidemiological studies, in which the occurrence of a particular disease within a population is studied in relation to the proportions of particular foodstuffs or nutrients in

ⁱUnfortunately, the obvious response to these observations, *i.e.* giving high-dose β -carotene supplements (20–30 mg daily) to smokers, the people most likely to be affected by lung cancer, has the reverse effect. Over several years, significant increases in the rates of lung cancer were found among smokers who received such supplements. The likely explanation of this apparent anomaly is that the combination of elevated β -carotene concentrations and the oxidative stress induced by smoking gives rise to significant concentrations of other products in addition to retinal from the cleavage of β -carotene, notably β -apo-13-carotenone (see inset in Figure 6.7). These have been shown to interfere with the role of retinal, vitamin A, in the regulation of gene expression (see R. M. Russell, *J. Nutr.*, 2004, **134**, 262S; A. Eroglu *et al.*, *J. Biol. Chem.*, 2012, **287**, 15886). Among non-smokers, β -carotene supplements did not lead to an increase in the incidence of lung cancer.

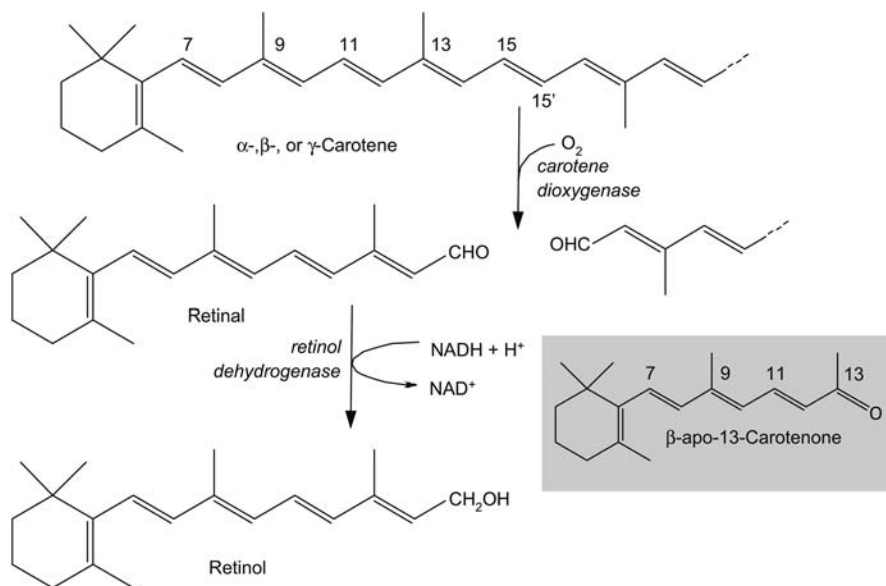


Figure 6.7 Formation of retinol and retinal. The inset formula of β -apo-13-carotenone is referred to in footnote *i*.

the diet, have suggested that the consumption of fruit and vegetables is associated with a reduced incidence of many types of cancer. This effect is most marked in the case of cancers of the gastrointestinal tract (such as those of the mouth, oesophagus and stomach) and the respiratory system (*e.g.* larynx and lung). Although attention has been focused on β -carotene, it has been recognised that other plant constituents, such as other carotenoids, ascorbic acid, flavonoids and fibre, may all be involved. The possibility that β -carotene and other carotenoids, along with vitamins C and E, might have an antioxidant function and therefore a bearing on the incidence of coronary heart disease is a separate issue, which was briefly considered in Chapter 4.

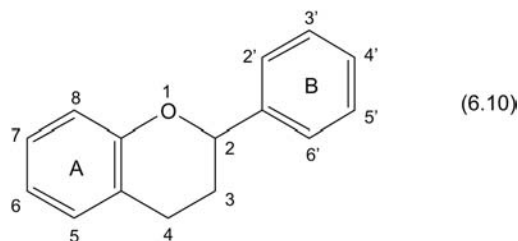
6.3 Anthocyanins

The pink, red, mauve, violet and blue colours of flowers, fruit and some vegetablesⁱⁱ are caused by the presence of pigments known as anthocyanins. They are usually located in the vacuoles of plant cells, kept apart from the enzymes and the other delicate structures found in the cytoplasm. Chemically, anthocyanins are a class of glycoside (see Chapter 2, Section 2.1) consisting of an anthocyanidin (the aglycone component) carrying a sugar unit. The anthocyanidins are flavonoids,ⁱⁱⁱ which are polyphenolic molecules

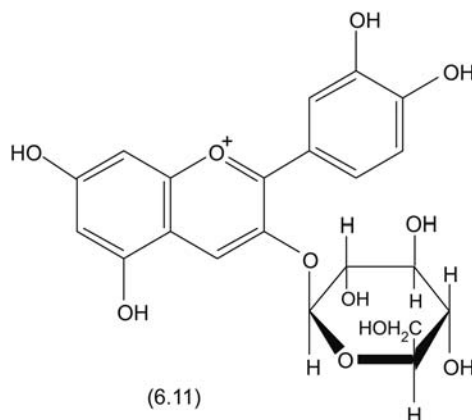
ⁱⁱThroughout this book, the terms “fruit” and “vegetable” are used in the culinary rather than the strictly botanical sense. Thus tomatoes and aubergines, botanically fruit, are treated as vegetables because they are usually eaten in a savoury context, as well as being found in the vegetable section of the supermarket, whereas rhubarb, definitely not a fruit to a botanist, is almost always eaten as part of dessert dishes and is listed here with other fruit.

ⁱⁱⁱOften incorrectly spelt *flavanoids*.

based on the flavan nucleus (6.10). The central oxygen-containing ring is sometimes referred to as a pyrilium ring.



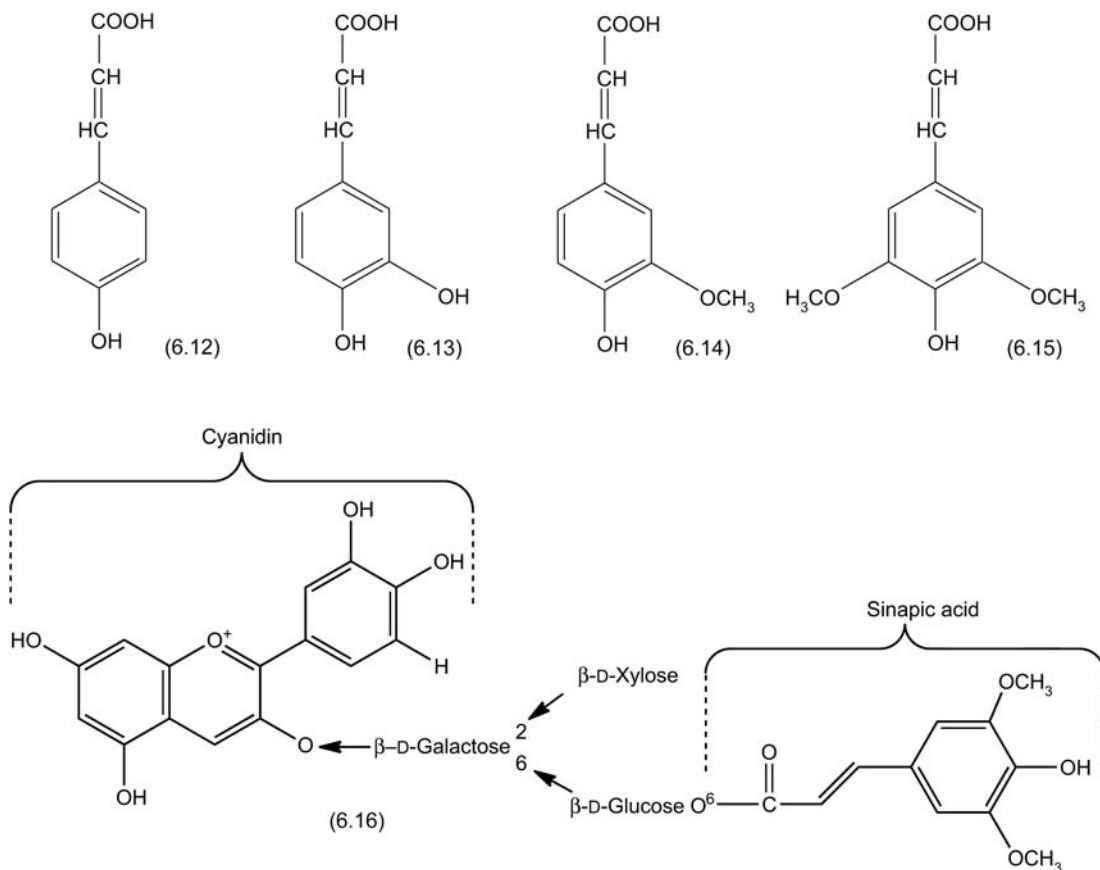
Six different anthocyanidins occur widely in nature, differing in the groups attached at the 3' and 5' positions on the B-ring of the flavan nucleus (Figure 6.8). The sugar residues of anthocyanins are usually attached at position 3 of the flavan nucleus, more rarely at position 5. Glucose often occurs additionally at position 5 and, more rarely, at positions 7, 3' or 4'. Apart from glucose, the monosaccharides most often found are galactose, rhamnose and arabinose. Some unusual disaccharides also occur, such as rutinose [α -L-rhamnosyl-(1 $\rightarrow$ 6)-D-glucose] and sophorose [β -D-glucosyl-(1 $\rightarrow$ 2)-glucose]. The complete structure (6.11) of a relatively simple example, cyanidin-3-glucoside, which occurs in a number of different edible fruits, illustrates the key features of the anthocyanin pigments.



	R ¹	R ²	λ_{max}^*
pelargonidin	– H	– H	503
cyanidin	– OH	– H	517
peonidin	– OCH ₃	– H	517
delphinidin	– OH	– OH	526
petunidin	– OCH ₃	– OH	526
malvinidin	– OCH ₃	– OCH ₃	529

Figure 6.8 Structures of the commonly occurring anthocyanidins and the absorption maxima (λ_{max}) of the corresponding 3-glucoside anthocyanins at pH 3. The form of the anthocyanidins shown here is termed the flavylium cation, but, as we shall see, other forms occur depending on the pH.

More complexity can arise from the esterification of the sugar residue at position 3 of the flavan nucleus with phenolic compounds, such as *p*-coumaric acid (6.12) or its derivatives caffeic (6.13), ferulic (6.14) or sinapic (6.15) acids, resulting in acylated anthocyanins. These are particularly associated with vegetables, although they are also found in grapes. The sugar residue of the principal black carrot anthocyanin (6.16) consists of a branched trisaccharide of D-galactose, D-xylose and D-glucose esterified with sinapic acid.



As indicated in Table 6.2, cyanidin is by far the most widespread anthocyanidin found in fruit and vegetables. However, the distribution of cyanidin and the other anthocyanidins among the species represented in Table 6.2 suggests that there is no link between the taxonomic classification of a plant and the identity of its anthocyanidins.

It may not be obvious why the numerous phenolic compounds found in plants, anthocyanidins being only one example, so often occur as glycosides. In the case of anthocyanins, the presence of the glycosyl group (*i.e.* the sugar) has only a small effect on their colour. The most likely explanation appears to be that phenolic hydroxyl groups interact readily with the tyrosine hydroxyl groups on the surface of proteins. The bulky sugar molecules tend to block this interaction, thereby preventing the unwanted inhibition of the cell's enzymes (see Chapter 7, Section 7.1.5, where the related phenomenon of astringency is described).

The black grape anthocyanins are particularly interesting. Of the six anthocyanidins, only pelargonidin is not found in grapes and there is much more variety in the pattern

Table 6.2 Anthocyanins found in fruit and vegetables. In the cases of red onions and red cabbage, the cultivars of the source materials were not specified by the original investigators.

Fruit or vegetable	Principal anthocyanins
Blackcurrant (<i>Ribes nigrum</i>)	3-Glucosides and 3-rutinosides of delphinidin and cyanidin
Blackberry (<i>Ribes fruticosus</i>)	3-Glucosides and 3-rutinosides of cyanidin
Raspberry (<i>Rubus ideaus</i>)	3-Glucosides, 3-sophorosides and 3-rutinosides of pelargonidin and cyanidin
Elderberry (<i>Sambucus nigra</i>)	3,5-Diglucosides and 3-sambubiosides ^a of cyanidin
Strawberry (<i>Fragaria</i> spp)	3-Glucosides of pelargonidin and cyanidin, 3-arabinosides of pelargonidin
European grape (<i>Vitis vinifera</i>)	3-Glucosides, 3-acetylglucosides and 3-coumarylglucosides of malvidin, peonidin, delphinidin, petunidin and cyanidin
Aubergine (<i>Solanum melongena</i>)	3-Rutinoside of delphinidin
Black carrot (<i>Daucus carota</i> ssp. <i>sativus</i> var. <i>atrorubens</i>)	3-Xylosyl(glucosyl)galactosides of cyanidin acylated with sinapic, ferulic or coumaric acids
Red cabbage (<i>Brassica oleracea</i> var. <i>capitata</i> f. <i>rubra</i>)	3-Sophoroside-5-glucodides of cyanidin unacylated or acylated with sinapic, ferulic or <i>p</i> -coumaric and malonic acids
Red onion (<i>Allium cepa</i>)	3-Glucosides, 3-malonylarabinosides and 3-malonylglucosides of cyanidin
Radish (<i>Raphanus sativus</i>)	3-Sophoroside-5-glucodides of pelargonidin, mono- or diacylated with cinnamic and malonic acids

^aSambubiose is β -D-xylosyl-(1 $\rightarrow$ 2)- β -D-glucose.

of glycosylation and acetylation than in most plants. The classic European grape species is *Vitis vinifera*, which contains only 3-monoglucosides. In non-European countries and the USA, species such as *Vitis riparia* and *Vitis rupestris* and their hybrids with *V. vinifera* are grown. All these species, but not *V. vinifera*, contain both 3-monoglucosides and 3,5-diglucosides. The diglucosides can readily be detected by a chemical test and can also be separated from the monoglucosides by chromatography. Detection of diglucosides in a red wine is therefore conclusive evidence of a non-European origin.

As shown in Figure 6.8, increasing substitution in the B-ring (identified in Formula 6.10) moves the absorbance maximum of anthocyanidins towards the red end of the spectrum, but these changes are not sufficient to account for the great range of colours that anthocyanins impart to flower petals and fruit. The pH and the presence of other substances have a much greater influence on their colour than the nature of the ring substituents. Anthocyanins form hydrogen-bonded complexes with the colourless flavonoids also common in plant tissues. The basic anthocyanidin structure shown in Figure 6.8 is the flavylium cation form, which predominates at low pH; this is the only significant form present at pH 1. As the pH increases, a proton is lost, a water molecule is acquired and the carbinol pseudo-base is formed (see Figure 6.9). The pK_a value (*i.e.* the pH at which the ionisation is half complete) for this transition is about 2.6. The red-coloured forms of the anthocyanins are the flavylium cations and the carbinol pseudo-bases are colourless, so there is a gradual loss of colour intensity with increasing pH, although strong blue colours are obtained at high pH values (Figure 6.10).

Work with purified anthocyanins has clarified the pattern of other ionisations, including those at higher pH. Above pH 3, the small residual proportion of the anthocyanins in the flavylium cation form begins to lose protons, forming first the non-ionised

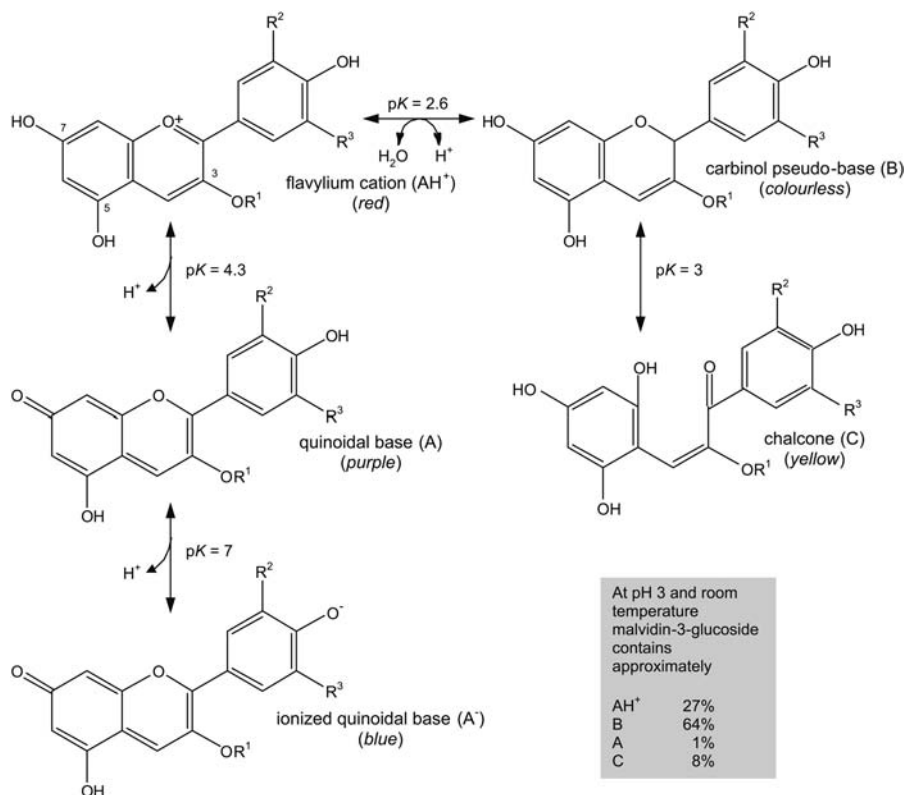


Figure 6.9 Transitions in the structure of anthocyanins with changes in pH. The presence of sugar residues at positions 5 or 7 will obviously influence the formation of quinoidal forms.

quinoidal base, which is weakly purple in colour, and then the ionised quinoidal base, which is deep blue. At the same time, the carbinol pseudo-base gives rise to a small proportion of the colourless chalcone forms. After extended periods at elevated pH, fairly high proportions of chalcones are present.

Although these equilibria have been described in some detail for purified anthocyanins, it must be remembered that the situation is neither as straightforward nor as well understood in fruit juices and other natural plant materials. Anthocyanins readily interact with the other colourless flavonoids that abound in plant tissues. These associations, which depend on hydrogen bonding between hydroxyl groups, enhance the intensity of the colour, modify the $\lambda_{\max}$ values, and tend to stabilise the blue quinoidal form.^{iv}

Once isolated from their parent plant tissues, the acylated anthocyanins of vegetables are remarkable for their much more intense colours at neutral pH and their greater stability to heating. These two properties make them particularly suitable for use as

^{iv}The enhanced stability of the acylated anthocyanins has been ascribed to an association between the aromatic ring of the acyl group and the central pyrilium ring of the flavylum cation. This stacked arrangement is believed to stabilise the quinoidal form, inhibiting its conversion into the carbinol base.

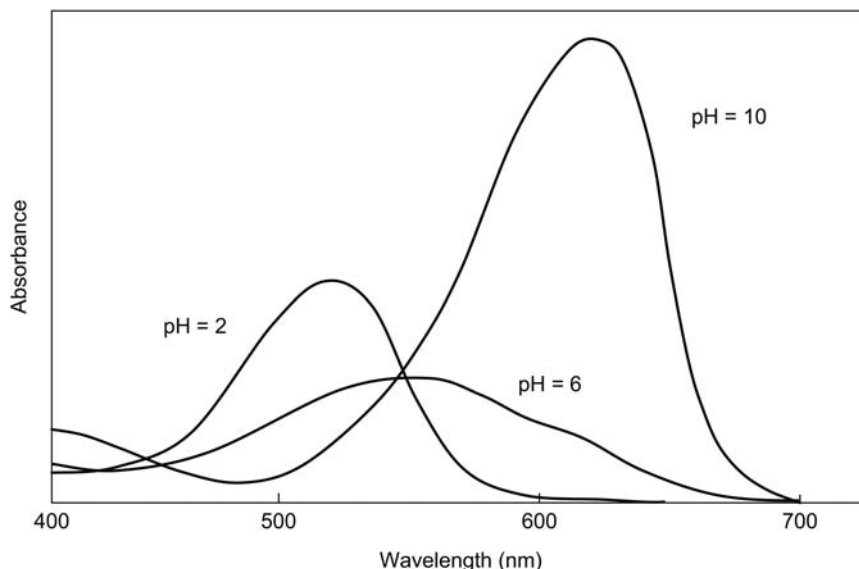


Figure 6.10 Effect of pH on the absorption spectrum of an anthocyanin. (Redrawn from R. Brouillard, in *Anthocyanins as Food Colours*, ed. P. Markakis, Academic Press, New York, 1982.)

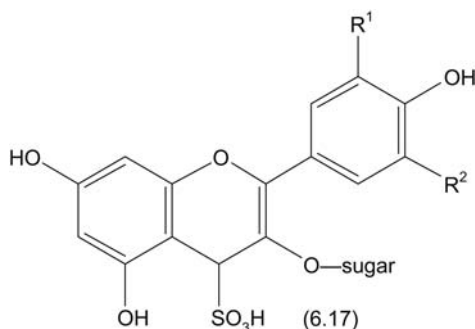
food colourants and several, notably the anthocyanins isolated from black carrots, are now being widely used in manufactured food products. It appears that intra-molecular interactions between the aromatic rings of the acyl group and the flavan nucleus are responsible for the stabilisation.

Blue is the one primary colour that is rarely associated with food, especially in Europe. Its almost taboo status is reflected in the traditional use of blue glass bottles for poisons, disinfectants *etc.* A notable exception is the use of the bright blue flower petals of the butterfly pea (*Clitoria ternatea*) to colour some traditional rice dishes and beverages in South East Asia. The butterfly pea's various anthocyanins, known as ternatins, provide an extreme example of the type of colour stabilisation mentioned in the previous paragraph. The most abundant of these, ternatin A, consists of a delphinidin unit that carries two short chains of alternating *p*-coumaric acid (6.12) and glucose units. These are assumed to interact with, and stabilise, the ionised quinoidal base structure of the delphinidin unit. At about pH 2, butterfly pea extracts have the typical bright red colour of anthocyanins, but a small increase to pH 3 turns the extracts purple. They are a stable bright blue colour at the neutral pH values typical of most rice-based dishes.

Anthocyanins are also able to complex metal cations, but it is not clear how important these are to the colour of fruit. Occasional unusual colour developments in canned fruit are undoubtedly due to interactions between the anthocyanins of the fruit and the metal (iron or tin) of the can when the internal lacquer has failed. The unexpected appearance of a pink coloration in canned pears has been attributed to the formation of iron or tin complexes with leucoanthocyanins (see Figure 6.17), which are normally colourless. The blue ionised quinoidal base (see Figure 6.9) form of anthocyanins, which prevails at neutral pH values, is not usually very stable, breaking down to colourless

products. However, a complex of aluminium ions and red cabbage anthocyanins in the presence of sodium bicarbonate provides a stable blue colour suitable for food use at pH values between 5.5 and neutrality and became available commercially in 2013 (US Patent No. 7279189, 2007).

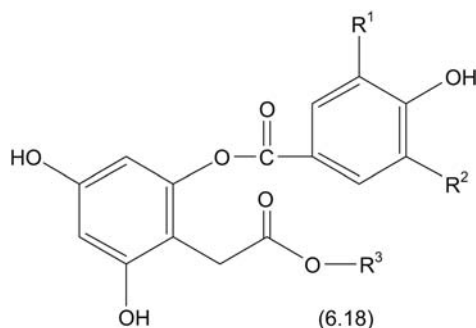
Another important reaction of anthocyanins occurs with sulfur dioxide. Sulfur dioxide, usually in the form of sulfite (SO_3^{2-}) or metabisulfite ($\text{S}_2\text{O}_5^{2-}$), is routinely used as an antimicrobial preservative in wine and fruit juices (see Chapter 9). At high concentrations (1–1.5%) these cause irreversible bleaching^v of anthocyanins, but at lower concentrations (500–2000 ppm) they react with the flavylium cation to form a colourless addition compound, chroman-4-sulfonic acid (6.17). The lack of colour in the addition compound is explained in a similar way to that of the carbinol pseudo-base (see Figure 6.9). Acidification, or the addition of excess acetaldehyde (ethanal), will remove the sulfur dioxide. The reaction with sulfites provides the basis for an insufficiently well-known technique for dealing with red wine spills on white table linen: adding a few drops of white wine, which always contains sulfur dioxide, to the stain. Unfortunately, this is only effective with stains of very young red wines, but may provide an excuse to open another bottle.



The anthocyanins in red wine undergo a number of reactions with some of the colourless flavonoids also originally present in the grape skins and seeds, such as catechins (better known as a component of tea). The formation of a link between the 4-position of the anthocyanin and the 8-position of the catechin leads to the formation of procyanidins, which are more strongly coloured compounds and are less affected by changes in pH or by sulfur dioxide. By the end of the fermentation period, as much as a quarter of the anthocyanins may have formed oligomers with other flavonoids. The brown colour characteristic of older red wines is the result of extensive polymerisation of the anthocyanins and other flavonoids. This is accompanied by a mellowing of the flavour because it is these tannins that give young red wine its characteristic astringency. The anthocyanins are fairly stable in most food processing operations, especially when the low pH of the fruit is maintained. Occasionally, however, the ascorbic acid naturally present can cause problems. In the presence of iron or copper ions and oxygen, the oxidation of ascorbic acid to dehydroascorbic acid is accompanied by the formation

^vMaraschino cherries, used for garnishing cocktails and some desserts, are bleached with sulfur dioxide and then dyed with synthetic colours.

of hydrogen peroxide. This oxidises the anthocyanins to colourless malvones (6.18), a reaction implicated in the loss of colour in canned strawberries.



Anthocyanins are the red food colour of choice in a wide variety of foodstuffs. There is a lengthening list of plant sources of anthocyanins being developed commercially that are taking over from synthetic red food colours. Some are red-coloured varieties of vegetables, such as red cabbage, black carrot and purple sweet potato (*Ipomoea batatas*). Fruit sources include black chokeberry (*Aronia melanocarpa*), elderberry and black grapes. Hibiscus flowers^{vi} (*Hibiscus sabdariffa*) and the skins of radishes are unlikely sources of anthocyanins that are also finding commercial applications. A particular impetus for this takeover, in Europe and elsewhere, has been the response of consumers to the outcome of a study instigated by the UK Food Standards Agency and undertaken at the University of Southampton into the possible involvement of synthetic food colours, including the red colours carmoisine and Allura Red, in children's health. This issue is discussed later in this chapter. Anthocyanins are of limited value in dairy products because their colour at less acid pH values is either weak or an unappealing blue, or even purplish green if other yellow pigments are present. The development of stable blue colours based on red cabbage anthocyanins complexed with aluminium may well lead to the resolution of this difficulty.

6.4 Betalaines

The characteristic red–purple pigments of beetroot,^{vii} *Beta vulgaris*, used to be described as “nitrogenous anthocyanins”. However, it is now established that these pigments, and those of the other members of the eight families usually grouped together as the order Centrospermae, are distinct from the anthocyanins and never occur with anthocyanins in any member of these eight families. The betalaines are divided into two groups: the betacyanins, which are purplish-red in colour (λ_{max} 534–555 nm); and the less common yellow betaxanthins (λ_{max} about 480 nm). The betalaines of *B. vulgaris* are the only ones of food significance and this account will therefore be largely restricted to these. Their structures are shown in Figure 6.11.

^{vi}Roselle in the USA.

^{vii}Also known as “red beet”.

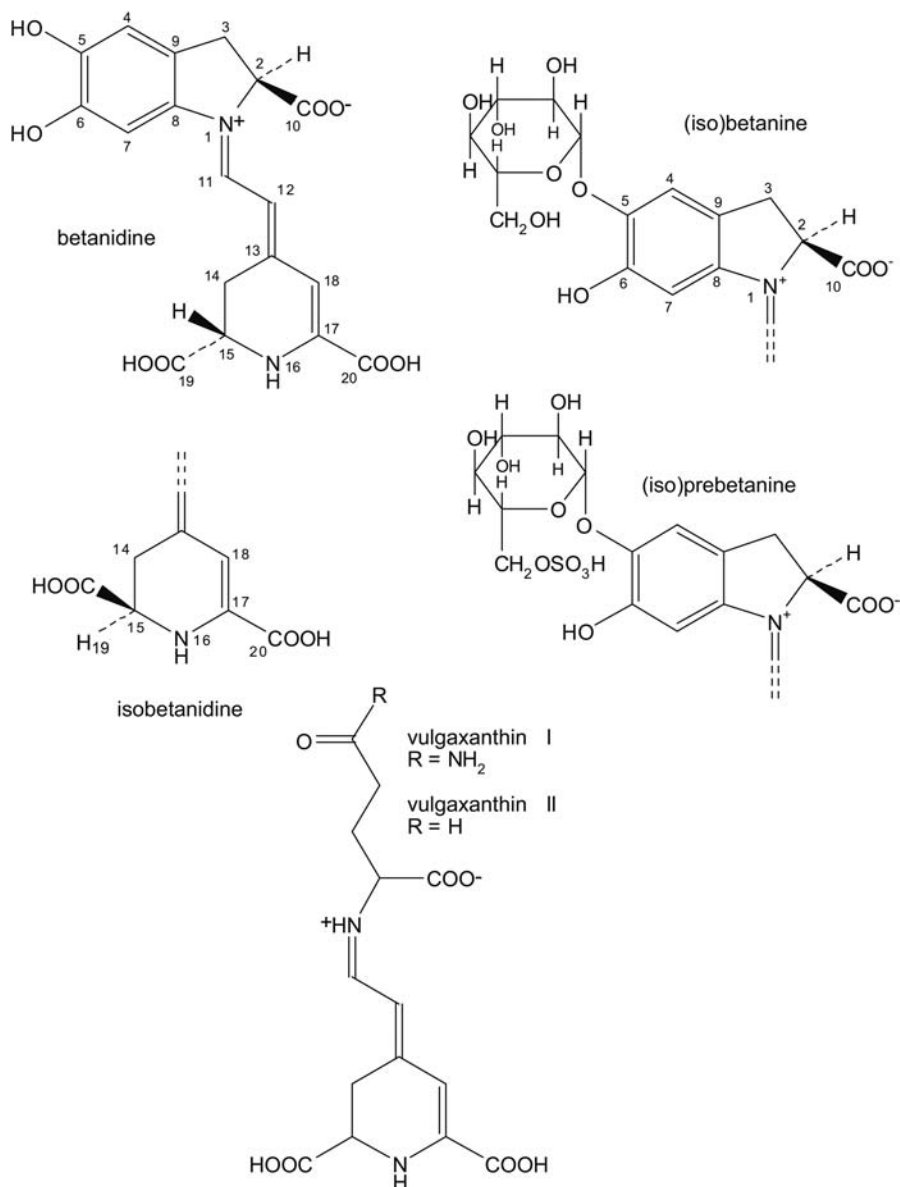


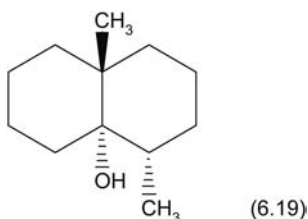
Figure 6.11 Betalaine and vulgaxanthin structures. The significance of “*iso*” and “*pre*” is explained in the text.

The betacyanins account for about 90% of beetroot betalaines and occur at around 120 mg per 100 g of fresh beetroot. They are all either glycosides or the free aglycones of betanidine or its C-15 isomer. In beetroot, *iso* forms account for only 5% of the total betacyanins. About 95% of the betanidine and isobetanidine carry a glucose residue at C-5. A very small proportion of these glucose residues are esterified with sulfate to give the prebetanins. The betaxanthins in beetroot are represented by the vulgaxanthins, which are characterised by the lack of an aromatic ring system attached to N-1 and by

the absence of sugar residues. A great diversity of betaxanthins are found in the *Centrospermae*, but only the two known as vulgaxanthin I and II are found in beetroot, in roughly equal proportions.

Although of similar colour to the anthocyanins, betacyanins differ in that their colour is hardly affected by pH within the range usually encountered in foodstuffs. The reason for this is clear from the structure. Changes in the pattern of ionisation of an anthocyanin can be seen from Figure 6.9 to cause considerable changes in the arrangement of the double bonds. However, the state of ionisation of the carboxyl groups in betacyanin has no such effect. Below pH 3.5, the absorption maximum of betanin solutions is at 535 nm, between pH 3.5 and 7.0 it is at 538 nm and at pH 9.0 it increases to 544 nm. Betacyanins are fairly stable under food processing conditions, although heating in the presence of air at neutral pH causes them to break down to brown compounds. Concentrated beetroot extracts (containing around 0.3% pigment, the remainder being almost entirely sugar) are becoming popular as colourants for dairy and dessert products. Beetroot extracts are widely permitted food ingredients or additives around the world, designated as E162 in Europe. Unfortunately, their instability to heat at neutral pH means that they are unsuitable for colouring cakes, *etc.*

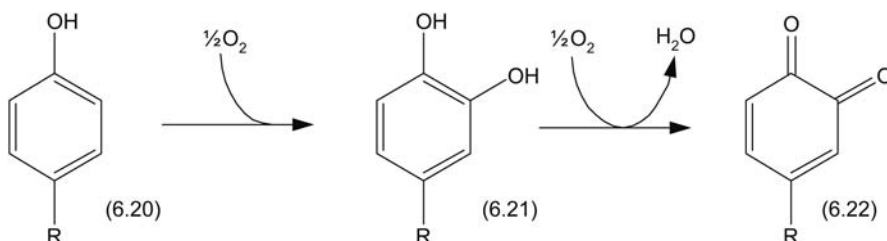
The processing of beetroot for use as a food colour poses some interesting problems. The betalaines are natural substrates for the enzyme phenolase (see Section 6.5), which causes rapid browning of the pigment in the presence of oxygen, but early heating is sufficient to prevent this. Beetroot is naturally high in nitrates (see Table 10.4) and sugars, but both of these can be eliminated by allowing yeast to grow in the crude beet extract and then removing the yeast cells by filtration or centrifugation. The characteristic earthy odour of beetroot, which might limit the usefulness of its derivatives in delicately flavoured dessert products, is caused by geosmin (6.19). Fortunately, this is distilled off when beetroot juice is concentrated.



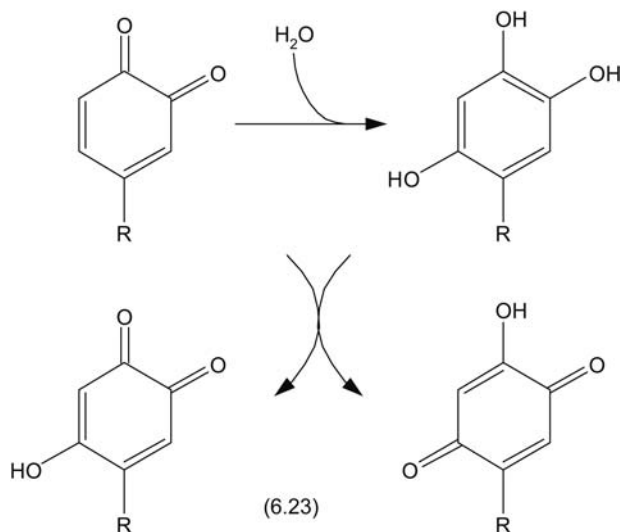
6.5 Melanins

Although we do not necessarily regard them as desirable pigments in our fruits and vegetables, this is a convenient point to consider the brown melanin derivatives formed when plant tissues are damaged. Everyone is familiar with the way in which pale-coloured fruit and vegetables, such as apples, bananas and potatoes, quickly turn brown if air is allowed access to a cut surface. The browning occurs when the polyphenolic substances that are usually contained within the vacuole of the plant cells are oxidised by the action of the enzyme phenolase, which occurs in the cytoplasm of plant cells. Tissue damage caused by slicing or peeling, fungal attack or bruising will

bring the enzyme and substrate together. The enzyme phenolase (originally known as polyphenol oxidase) occurs in most plant tissues. Phenolase is an unusual, but not unique, enzyme in that it catalyses two very different types of reaction. The first of these, its cresolase activity, results in the oxidation of a monophenol (6.20) to an *ortho*-diphenol (6.21). The second, catecholase activity, oxidises the *o*-diphenol to an *o*-quinone (6.22).

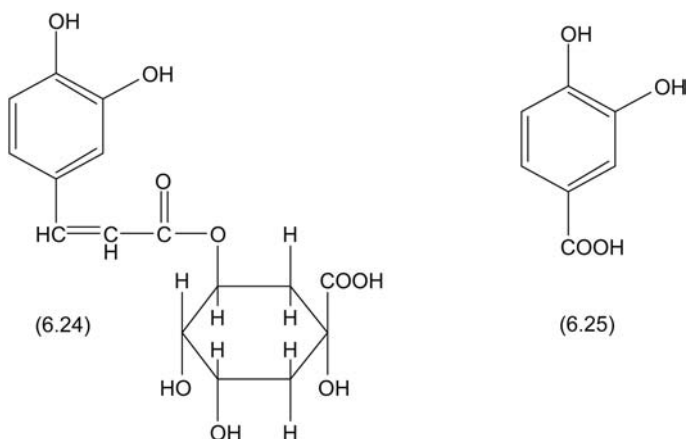


o-Quinones are highly reactive and readily polymerise following their spontaneous conversion into hydroxyquinones (6.23).



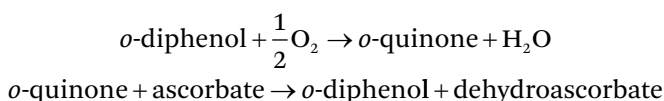
A related enzyme, tyrosinase, is found in mammalian skin. Its only substrate is the amino acid tyrosine. The reaction in this case leads to the formation of the melanin pigments in skin and hair. The brown pigments that develop on the cut surface of fruit and vegetables may well be regarded as unsightly by consumers, but they do not detract from their flavour or nutritional value. In the plant, the products of phenolase action, particularly the quinones, are very important as antifungal agents. The *o*-quinones interact with the tyrosine hydroxyl groups on the surface of fungal enzymes in much the same way as the reactions shown in (6.23). This can cause the inhibition of these enzymes and reduces the ability of fungi to penetrate tissues that have suffered minor physical damage. The relative resistance to fungal diseases of different varieties of onions and apples has been correlated with their phenolase activity and their quinone content. The most important substrate for phenolase in apples, pears and potatoes is chlorogenic

acid (6.24) and, in onions, protocatechuic acid (6.25), but very little is known about the structures of the polymerised quinones derived from them.



Efforts are made to prevent phenolase action during fruit and vegetable processing, both in the home and in the factory. Making sure that the fruit and vegetables are blanched as soon as possible after (or preferably before) any tissue-damaging operations will reduce phenolase action to a minimum. Reducing contact with air by immersion in water is also common practice. Some of the most effective specific inhibitors of phenolase activity are chelating agents, which act by depriving the enzyme of the copper ion that is a component of its active site. However, the substances used in analytical laboratories, such as diethylthiocarbamate, cyanides, hydrogen sulfide or 8-hydroxyquinoline, are hardly appropriate for food use.

Although less specific, and less potent, the organic acids citric (6.12) and malic acid (6.13) have advantages. Not only are they present in fruit juices, their acidity helps to keep the pH well below the optimum for phenolase action (about pH 7). Immediately after being peeled, and especially if a strong sodium hydroxide solution (lye) has been used to assist this, fruit such as peaches are often immersed in neutralising baths containing dilute acids, often supplemented with ascorbic acid or sulfites. Ascorbic acid is not only valued as a vitamin, but also as a chelating agent and antioxidant. It interacts directly with quinones to prevent browning and, if sufficient is present, it can “mop up” the oxygen in closed containers such as cans:



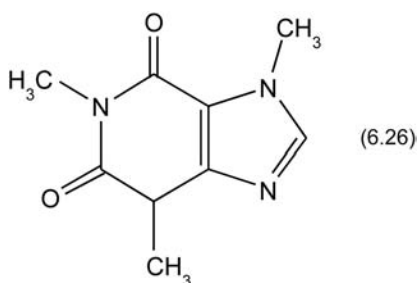
Although it seems likely that manufacturers of dehydrated potato powders include ascorbic acid in their products to ensure their whiteness rather than for the benefits accruing from vitamin C, the motive does not belittle their nutritional value, particularly for people who rely on such convenience foods in place of fresh fruit and vegetables.

Sulfur dioxide also has a specific, if not fully explained, inhibitory action against phenolase. A sulfite concentration sufficient to maintain a free SO_2 concentration of

10 ppm will completely inhibit phenolase. Being a reducing agent, sulfite has the additional benefit of preserving the ascorbic acid levels, but it is not without its disadvantages because it tends to bleach anthocyanins and to hasten can corrosion.

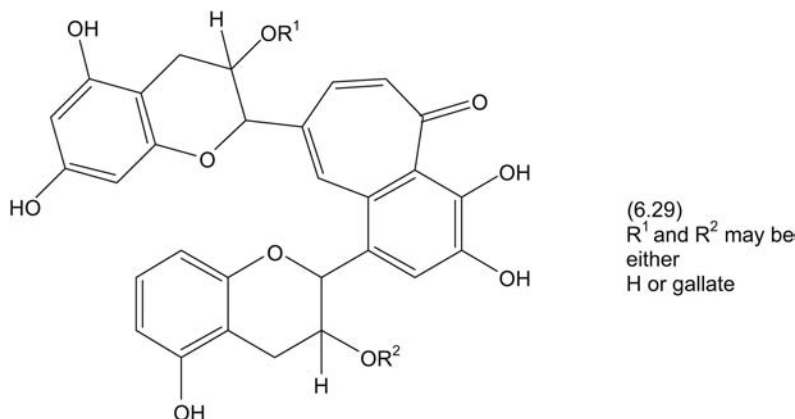
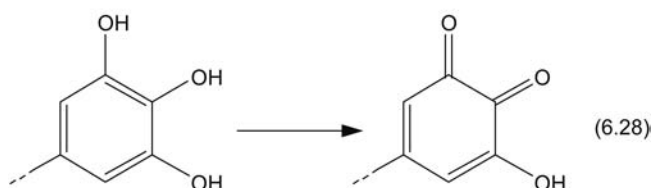
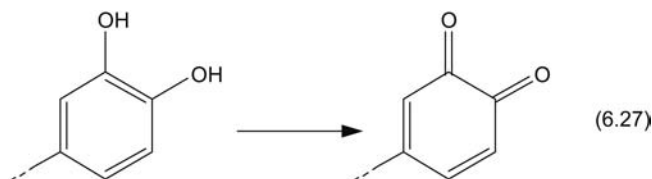
6.5.1 Tea

The enzymic oxidation of polyphenolic substances is a highly desirable feature of one particular foodstuff: tea. The tea we drink is a hot water infusion of the leafy shoots of the tea plant, *Camellia sinensis*, which are processed in various ways depending on the type of tea. The tea drunk most often in Europe and North America is known as black tea, to distinguish it from green tea, also known as Chinese tea. The leaves of the tea plant contain enormous quantities of polyphenolic materials, >30% of their dry weight. Caffeine (6.26), the stimulant also found in coffee and cola-type drinks, amounts to about 3–4% of the dry weight of the leaf. Caffeine is considered in more detail in Chapter 10. The freshly plucked leaves, usually just the bud plus the first and second leaves (the “flush”), are allowed to lose about one-fifth of their water content after they are macerated, a process known as “withering”. This physical damage to the leaves allows the phenolase in the cytoplasm of the leaf cells to make contact with the polyphenolic substances contained in the cell vacuoles. The macerated leaves are then left at ambient temperature for a few hours to ferment, during which a high proportion of the polyphenolic substances are oxidised and polymerisation occurs. The leaves are then “fired”, *i.e.* dried at temperatures up to 75 °C, which inactivates the phenolase and brings the fermentation reaction to a halt. This gives the product we recognise as tea. Green tea, by contrast, is fired before the leaves are macerated and the phenolase is inactivated before significant fermentation can occur. An intermediate type, Oolong tea, is fermented for only about 30 minutes before firing.



Almost all of the polyphenolic substances in tea are flavonoids, either catechin or its derivatives (Figure 6.12). Of these, epigallocatechin gallate is by far the most abundant, about 9–13% of the dry weight of the unfermented leaves.

The initial effect of phenolase action is to convert the diphenols into the corresponding *o*-quinones (6.27 and 6.28). The quinones are highly reactive and readily form a wide variety of dimers during the fermentation period. Most of these interact further to produce polymeric thearubigins, but one class of dimer, the theaflavins (6.29), comprise about 4% of the soluble solids of black tea. Being bright red in colour, they make a major contribution to the “brightness” of the tea colour.



The brown thearubigins are the principal pigments of black tea infusions. They contribute not only the bulk of the colour, but also the astringency, acidity and body of the tea. The various thearubigins extracted with hot water have a wide range of molecular weights. Although the average corresponds to oligomers of four or five flavonoid units, molecules consisting of as many as 100 flavonoid units may be encountered in tea infusions. Degradation studies have shown that all the principal flavanols shown in Figure 6.12 are represented in these oligomers. Although there is no certainty as to the nature of the links involved in the polymerisation, a proanthocyanidin structure is most likely (Figure 6.13). The proanthocyanidins themselves are dimers and occur in small amounts in many fruit juices, where they contribute to astringency.

The proportions of the different classes of polyphenolics present in brewed tea vary considerably with the variety of tea, the proportion of leaves to water, and the brewing temperature and time. The data in Table 6.3 are reasonably representative. Data on the thearubigin content of brewed tea are sparse, but the indications are that the most common varieties of black tea, such as Ceylon and Assam, give brews containing 20–35 mg thearubigins per g dry tea leaf.

There has been growing interest in the potential health benefits of drinking tea. Tea undoubtedly provides a valuable source of polyphenolic compounds, and thus antioxidants, in the diets of many communities around the world. Considerable benefits in

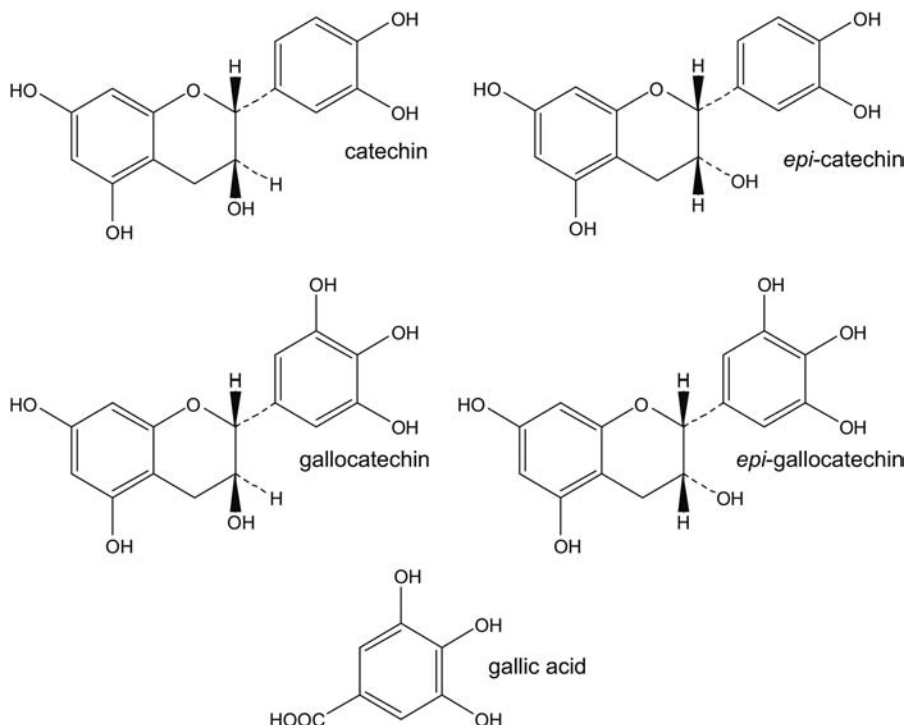


Figure 6.12 Gallic acid and the principal flavonoids of the unfermented tea flush. The prefix *epi-* refers to the optical configuration at the 3-position of the flavonoid ring system. *Gallo-* indicates three hydroxyl groups attached to adjacent carbon atoms in the aromatic ring. Both *epi*-gallo catechin and *epi*-catechin also occur as gallates, in which case gallic acid is esterified at the 3-position.

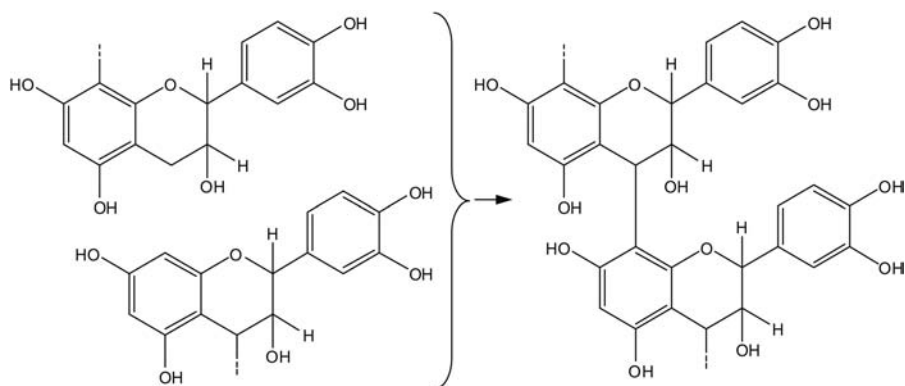


Figure 6.13 Polymerisation of tea flavonoids to form a proanthocyanidin structure.

relation to both cardiovascular disease and cancer have been attributed to tea, especially green tea. Laboratory studies have shown that many components of both black and green tea infusions inhibit the growth of tumour cells of numerous types, including the gastrointestinal tract. However, the evidence from epidemiological studies of different tea-drinking populations around the world remains inconclusive (*e.g.* see the 2011

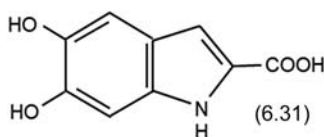
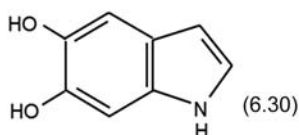
Table 6.3 Typical flavonoid content in tea extracted with boiling water for five minutes, expressed as mg flavonoid per g dry tea leaf (based on data presented by M. Friedman *et al.*, *J. Food Sci.*, 2005, **70**, C550). The data for the four classes of catechins (marked by asterisks) represent the sum of the catechins and their respective *epi*-isomers.

Flavonoid	Tea variety				
	Assam	Ceylon	Darjeeling	Oolong	Green
Catechins*	2.9	0.6	4.2	8.2	1.0
Gallocatechins*	1.2	Trace	4.5	5.7	Trace
Catechin gallates*	2.8	5.6	21.5	4.5	3.9
Gallocatechin gallates*	2.8	8.0	71.3	26.7	13.3
Theaflavin	0.6	0.8	0.7	0	0
Theaflavin gallates	1.1	1.3	0.7	0	0
Theaflavin digallates	3.0	3.1	3.8	0	0

review by Yuan, Sun and Butler detailed in the Further Reading section). With regard to cardiovascular disease, the situation is more promising (*e.g.* see the 2011 review by Deka and Vita), especially with regard to green tea. Readers will find additional information in the first of the Special Topics at the end of this chapter.

6.5.2 Squid Ink

Another group of polyphenolic substances have come to the attention of food chemists in recent years: the eumelanins, the black pigments in squid ink. Different species of squid are abundant in marine environments worldwide and are harvested for human consumption. Like their relatives the cuttlefish and octopus, squid, are well known for their ability to eject large quantities of dense black fluid from specialised glands to camouflage them from attacks by predators. Squid ink, often referred to as “sepia”, has been traditionally been used for writing, dyeing textiles and as a black food colouring. What is marketed commercially as “squid ink” is actually obtained from the common cuttlefish, *Sepia officinalis*, and it is this that has been most studied. In addition to its characteristic black pigment, eumelanin, squid ink contains a variety of other components that can add flavour. Squid ink is most commonly used in Italian and Japanese food dishes. Eumelanin is a co-polymer of 5,6-dihydroxyindole (6.30) and 5,6-dihydroxyindole-2-carboxylic acid (6.31) units, both believed to be derived from the amino acid tyrosine *via* a series of enzyme-catalysed and spontaneous reactions.

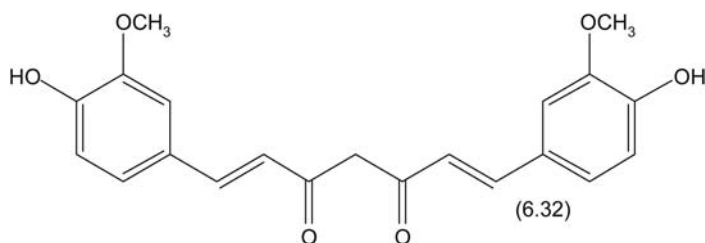


6.6 Other Natural Food Colourants

Much of the consideration of colour so far in this chapter has been devoted to the colour of foods contributed by substances present in them naturally. The application of some of these natural colours as colourants for other foods has already been mentioned. This section is concerned with several important food colourants that do not fit into any of the major pigment classes. Most of these are permitted additives in countries around the world, but, where appropriate, the E numbers and C.I. numbers (defined in the following section) have been given.

6.6.1 Turmeric

Turmeric, the powdered dried root of the turmeric plant, *Curcuma longa*, has been used for centuries both as a spice and as a food colour. In addition, it has found use in the Far East as a textile dye. It is best known for its role in Asian cookery as an essential component of curry powder. As well as its colour, it also contributes a characteristic earthy, slightly bitter taste. It is a traditional ingredient in the English pickle known as piccaililli. Curcumin (6.32), turmeric's principal pigment, constitutes 3% of the powdered spice. The oil-soluble pigment can be obtained by solvent extraction. In use as food colour it is yellow, but with a slightly greenish cast. In recent years curcumin (E100, C.I. 75300) has become popular for colouring dessert products such as ice cream, as well as savoury products and other manufactured foods.



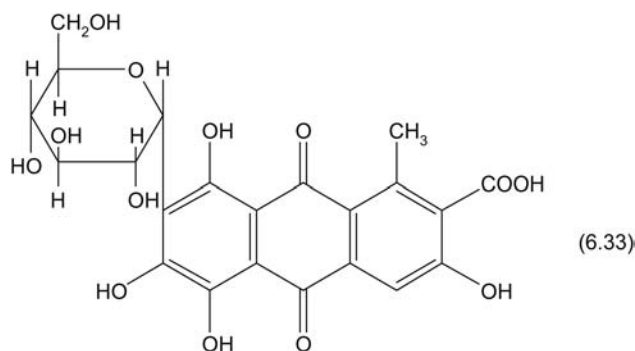
Turmeric has always been recognised in India as having medicinal properties, but interest has increased in its use in “alternative” medicine. There is little doubt that it can be an effective antioxidant and anti-inflammatory agent, but the doses required, 1–4 g daily, can only be achieved by the consumption of concentrated curcumin, not the spice as used in a food context. It seems extremely unlikely that its antioxidant properties justify its inclusion in shampoo, cosmetics *etc.*

6.6.2 Cochineal

Plants do not have a monopoly in providing natural colouring materials. The name “cochineal” refers to a group of red pigments obtained from various insects similar to aphids, most notably *Dactylopius coccus* (previously known as *Coccus cacti*), which belongs to the superfamily Coccidoidea. In the form of the dried and powdered female insects (about 100 000 are required per kg of pigment), cochineal has been an object of commerce since ancient times. The insects are harvested from cactus plants

of the prickly pear, or *Opuntia*, family in various parts of the world, including Central and South America and the Canary islands. Apart from its use as a food colour (commercially described as carmine, E120, C.I. 75470), it has been used as a dyestuff for textiles, most famously in the past for the red coats of British military uniforms, and as a heart stimulant. Although varieties of cochineal have been “cultivated” in many parts of the world, the bulk of production now comes from Peru.^{viii} One limitation on the use of cochineal is that its insect sources means that, for Jewish consumers, it is not kosher and, for Muslim consumers, not halal. Vegetarians also rule out the use of cochineal.

The principal component of cochineal is carminic acid (6.33), an anthraquinone glycoside with an unusual link between the glucose and the aglycone. Cochineal itself gives a purple colour, but textile dyers discovered that it gave a strong scarlet dye with tin salts. The deep red pigment used as a food colour, variously referred to as “carmine”, “carmines of cochineal” or simply “cochineal” is obtained by first extracting the carminic acid from the crude cochineal powder with hot water. The extracts are then treated with aluminium salts to produce brilliantly coloured complexes of uncertain structure known as “lakes”. These can be precipitated out with ethanol to give a water-soluble powder. Despite cochineal’s high cost, the increasing unpopularity of chemically synthesised dyestuffs may lead to a revival in its use.

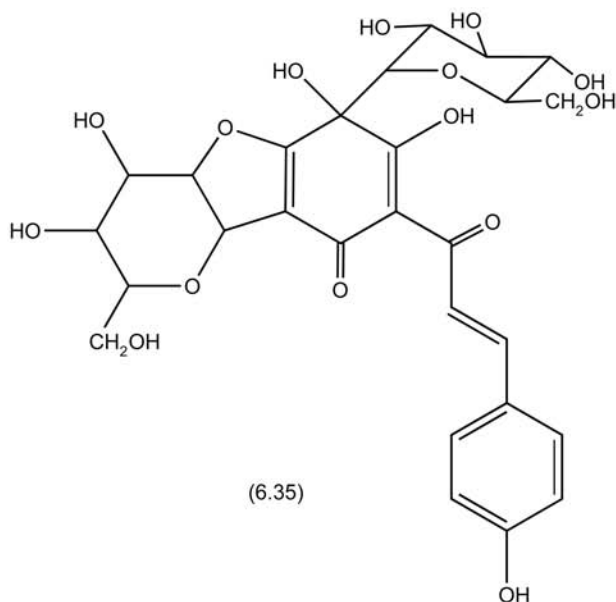
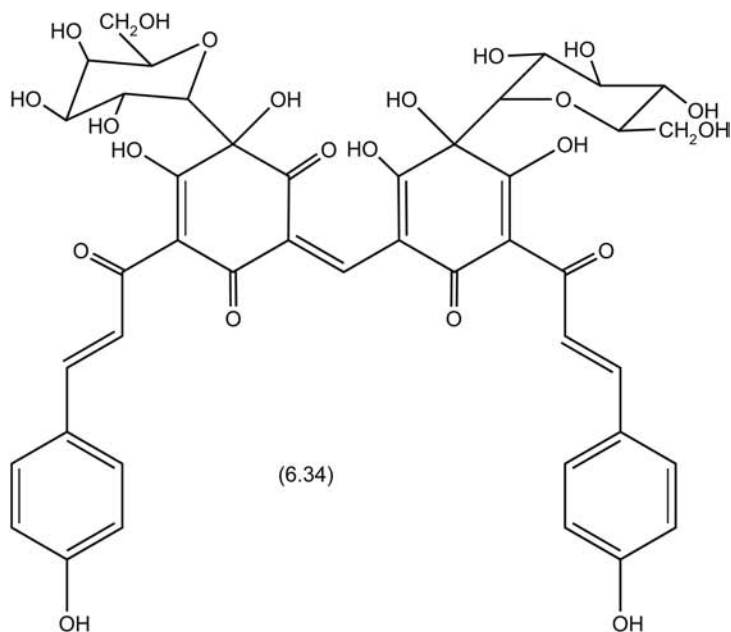


6.6.3 Safflower

Like cochineal, safflower is a colouring material with a long history of use in both food and textiles (particularly wool), having been cultivated for these purposes by the ancient Egyptians. The thistle-like safflower plant (*Carthamus tinctorius*) grows readily in arid regions and is now mostly cultivated, as a valuable oilseed source, in India, the USA and Mexico. The petals of the colourful flowers, sometimes known as “false” or “bastard” saffron, contain a red pigment, carthamin (6.34) and its yellow precursors, safflor yellow A (6.35) and safflor yellow B. All three pigments are soluble in water. Carthamin is fairly unstable, being susceptible to light, oxygen and low pH values. The yellow pigments are more stable, but still tend to fade in bright light. In chemical terms they

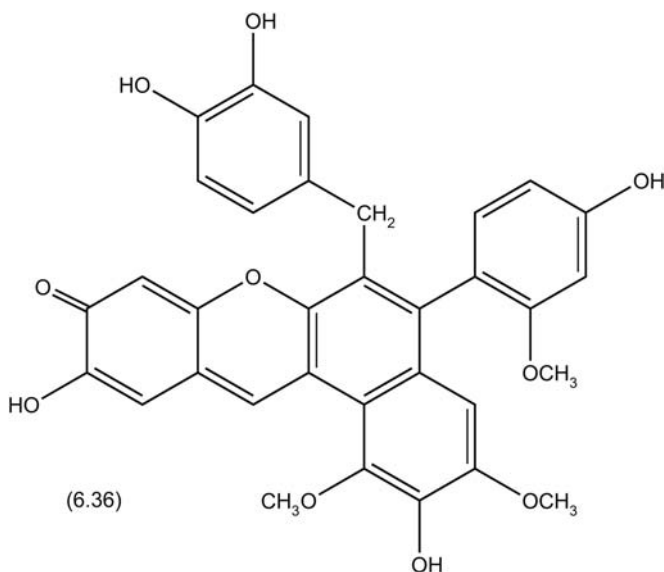
^{viii}Several other particularly stable red dyes used in antiquity were, and still are, extracted from insects and also have an anthraquinone structure, notably lac (from India and South East Asia) and kermes (from southern Europe).

share with cochineal a rather unusual linkage between their glucose residues and the aglycone portion of the molecule. The aglycone component is structurally related to the chalcone form of anthocyanin pigments (6.11). Like saffron, dried safflower petals are not regarded as food additives in European legislation, but as food ingredients that happen to have a colouring action. They are used in the manufacture of a number of traditional British soft drinks and are recommended as a natural replacement for quinoline yellow (see Table 6.6).



6.6.4 Sandalwood

Like safflower, sandalwood is a traditional coloured spice now receiving more attention with the decline in popularity of artificial food colours. The heartwood of the large sandalwood or red sanders tree, *Pterocarpus santalinus*, which grows in southwest India, is ground to a dark red powder that is often used directly as a spice. In Europe, its traditional applications have been in seafood products, notably pickled herrings, where its sweet-spicy flavour is as important as its colour. The major pigments, santalin A (6.36) and B, are not soluble in water, but are soluble in ethanol. Ethanol extracts are also used as a food colour. Like saffron and safflower, it is treated by most regulatory authorities as a colouring foodstuff rather than a food additive.



6.6.5 Malt Extract and Caramel

Malt extract is now being marketed to provide a brown colour in various products, including soft drinks and soup mixes. In the first stage of normal beer-making, partial germination of the barley, known as “malting”, is accompanied by the synthesis of the enzymes that will be required to convert starch into fermentable sugars. Germination, and enzyme synthesis, is brought to a halt by increasing the temperature. Inevitably, this heating leads to the Maillard reaction (see Chapter 2) taking place, giving the brewer an opportunity to manipulate the colour, and flavour, of the final product. The most strongly heated and roasted malt can be extracted with water to produce, after concentration, syrups or powders rich in brown pigments. These extracts inevitably contain the sugars (*e.g.* glucose, maltose and higher oligosaccharides) that were generated during the initial stages of barley germination. These are regarded as a useful addition to the overall flavour of many food products.

6.7 Synthetic or Artificial Food Colourants

The use of artificial, non-natural colours in food has a long, but far from glorious, history. Wine has always been a target for the unscrupulous. In the eighteenth and nineteenth centuries, burnt sugar and a range of rather unpleasant vegetable extracts were commonly added to give young red wines the appearance of mature claret. However, for sheer lack of scruples, the confectionery trade had no equal. In 1857, a survey of adulterants to be found in food revealed that sweets (*i.e.* confectionery for children) were commonly coloured with, for example, lead chromate, mercuric sulfide, lead oxide (red lead) and copper arsenite. Legislation, and the availability of the newly developed aniline dyestuffs, eventually eliminated the use of metallic compounds, together with some of the more dubious vegetable extracts.

The new synthetic dyestuffs had many advantages over natural colours. They tended to be much brighter, more stable and cheaper, and a wide range of shades was available. It did not take long before some of these dyes also turned out to have some toxic properties, although the effect was mainly on those engaged in their manufacture rather than on consumers.^{ix} Since that time, there has been a steady increase in the range of dyestuffs available, but at the same time we have become increasingly aware of their potential toxicity. Thus in 1957 there were 32 synthetic dyestuffs permitted for use in food in the UK. By 1973, 19 of these had been removed from the list and three added. In 1979, 11 of those remaining were granted E numbers, together with several others not permitted in the rest of the European Community.

This situation has now been simplified and Table 6.4 includes the E numbers of the synthetic colours currently permitted in the UK, European Union and elsewhere. (It seems to be unlikely that the withdrawal of the UK from the European Union will result in changes to the numbers used in the UK, although pressure to change the “E” to some other letter may arise.)

Three of the red colours listed as permitted in the European Union are in fact restricted to particular applications: amaranth (E123) in aperitifs (*i.e.* vermouth) and fish roe; erythrosine (E127) in cocktail (*i.e.* Maraschino) and other candied cherries; and Lithol Rubine BK (E180) in waxy cheese rinds. Similarly, Orange B (C.I. 19235) and Citrus Red No. 2 (C.I. 12156) in the USA are restricted to citrus fruit peels and frankfurter skins, respectively. In Japan, three red colours, Acid Red 52 (45100), Rose Bengal (45440) and Phloxine (45410), are permitted, but apparently rarely used, in food.

The European system of E numbers forms the basis of the International Numbering System (INS) for Food Additives as defined by the Codex Alimentarius (Latin for “Book of Food”), the international food standards of the World Health Organization and the United Nations Food and Agriculture Organization. The INS number for a particular food additive is identical to its E number, apart from lacking the “E”. In the USA, the

^{ix}There are some historical references suggesting that the laxative effects of phenolphthalein, the popular laboratory pH indicator, were first discovered in Hungary in 1902, when wine to which it had been added, supposedly to enhance its colour, had this unanticipated effect on its consumers. In fact, its pK_a value of 9.2 means that it would be completely colourless at the pH of wine.

Table 6.4 Synthetic food colours permitted in Europe and elsewhere (in 2020). In most jurisdictions there are limits on the amounts of these colours that may be used and some, italicised here and detailed in the text, are restricted to particular foods. Other limitations in the uses of particular colours are detailed in the footnotes. “Y” means “Yes”.

Common name	C.I. number	EU or INS number	USA number	Permitted in					
				EC and UK	USA	Canada	Australia and New Zealand	Japan	South America ^a
Tartrazine	19140	E102	FD&C Yellow No. 5	Y	Y	Y	Y	Y	Y
Quinoline Yellow	47005	E104	D&C Yellow No. 10	Y			Y		Y
Sunset Yellow FCF	15985	E110	FD&C Yellow No. 6	Y	Y	Y	Y	Y	Y
Carmoisine	14720	E122		Y			Y		Y
Amaranth	16185	E123		Y ^b		Y	Y	Y	Y
Ponceau 4R	16255	E124		Y			Y	Y	Y
Ponceau SX	14700	125				Y			
Erythrosine	45430	E127	FD&C Red No. 3	Y ^c	Y	Y	Y	Y	Y
Red 2G	18050	128							Y
Allura Red AC	16035	E129	FD&C Red No. 40	Y	Y	Y	Y	Y	Y
Citrus Red No. 2	12156				Y ^d				
Patent Blue V	42051	E131		Y					Y
Indigo Carmine	73015	E132	FD&C Blue No. 2	Y				Y	Y
Brilliant Blue FCF	42090	E133	FD&C Blue No. 1	Y	Y		Y	Y	Y
(Food) Green S	44090	E142		Y	Y		Y		
Fast Green FCF	42053	143	FD&C Green No. 3					Y	Y
Black PN	28440	E151		Y	Y				Y
Chocolate Brown HT	20285	E155		Y	Y				Y

^aThe Mercosur countries: Argentina, Brazil, Paraguay and Uruguay.

^bAperitifs and fish roe only.

^cMaraschino cherries only.

^dCitrus peel only.

synthetic colours that are permitted are identified by a simple numbering system preceded by the letters “F”, “D” and “C”, which indicate whether they are permitted for use in food, drugs (*i.e.* pharmaceutical products) and cosmetics, respectively.

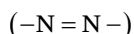
Many other countries elsewhere in the world have brought in legislation similar to that in Europe, but readers will not be surprised that the authorities in different countries often have differing views on which colours are, or are not, permitted. This means that importers and exporters of food products from one country to another need to be especially vigilant. The differences between the lists from different jurisdictions, such as those in Table 6.4, are not necessarily the result of different interpretations of the data from toxicity tests or the susceptibility of public authorities to commercial pressures. For example, Food Green S (C.I. 44090, E142) is permitted in Europe, but not in the USA. The opposite is true of Fast Green FCF (C.I. 42053, FD&C Green No. 3). The reason that Food Green S is not permitted in the USA is simply that at the time when food manufacturers were seeking approval from the Food and Drug Administration for colours to be certified (*i.e.* approved), there was widespread satisfaction with the not dissimilar properties of Fast Green FCF, which had previously been in use for many years. There was no incentive to engage in the considerable expenditure and effort to additionally seek approval for Food Green S.

The chemical structures of a representative selection of synthetic food colourants are shown in Figure 6.14. In addition to their common names, their correct systematic name (according to the rules of the International Union of Pure and Applied Chemistry, IUPAC) are also shown. The complexities of their chemical structures are reflected in these formal names, which hardly lend themselves to incorporation into ordinary sentences, either written or spoken. The use of the common names in Figure 6.14 and Table 6.4 is not necessarily straightforward because most dyestuffs have a number of other familiar or commercial names. For example, Carmoisine, disodium 4-hydroxy-3(4-sulfonato-1-naphthylazo) naphthalene-1-sulfonate, is known by at least 26 other names, including Acid Brilliant Rubine 2G, Acid Red14, Azorubin S, Brilliant (or Brilliant) Carmoisin O, Food Red 3 and Solar Rubine. This problem of dyestuff terminology has been with us for a very long time. In 1925 a joint committee of the Society of Dyers and Colourists (of Great Britain) and the American Association of Textile Chemists and Colorists set out to resolve the problem by establishing the Colour Index in which all known artificial colours (including those used for cosmetics, textiles and paint) are listed and given a reference number. These C.I. numbers are also included in Table 6.4. Other difficulties arise from the fact that, in some cases, the commercial name applies to a mixture rather than a single substance. The common names of many dyestuffs include suffixes derived from their intended application. Thus “FCF” indicates “For Colouring Food” and the “FK” of Brown FK, is an abbreviation of “for kippers”, for which this now prohibited dye was once used.^x Brown FK lacks a C.I.

^xEven before its complete withdrawal from permitted status in 2011, Brown FK was restricted to the UK where it was only allowed to be used for colouring kippers (hence the “FK” suffix, “for kippers”), a type of smoked herring. A combination of manufacturing difficulties, uncertainty over its safety and the realisation that no food manufacturers were actually using it led to its official withdrawal. For many years now the natural carotenoid pigment, annatto, has been used to colour kippers and smoked haddock (*Melanogrammus aeglefinus*).

number because its commercially available form consisted of an ill-defined mixture of 2,4-diamino-5-(*p*-sulphophenylazo)-toluene and a number of chemical derivatives.

These food colours are all characterised by elaborate aromatic ring systems. They are all freely soluble in water, a property generally conferred by their sulfonic acid groups. The chromophore of the azo dyes is the azo group:



conjugated with aromatic systems on either side to give colours in the yellow, orange, red and brown range. Three linked aromatic systems provide the chromophore of the

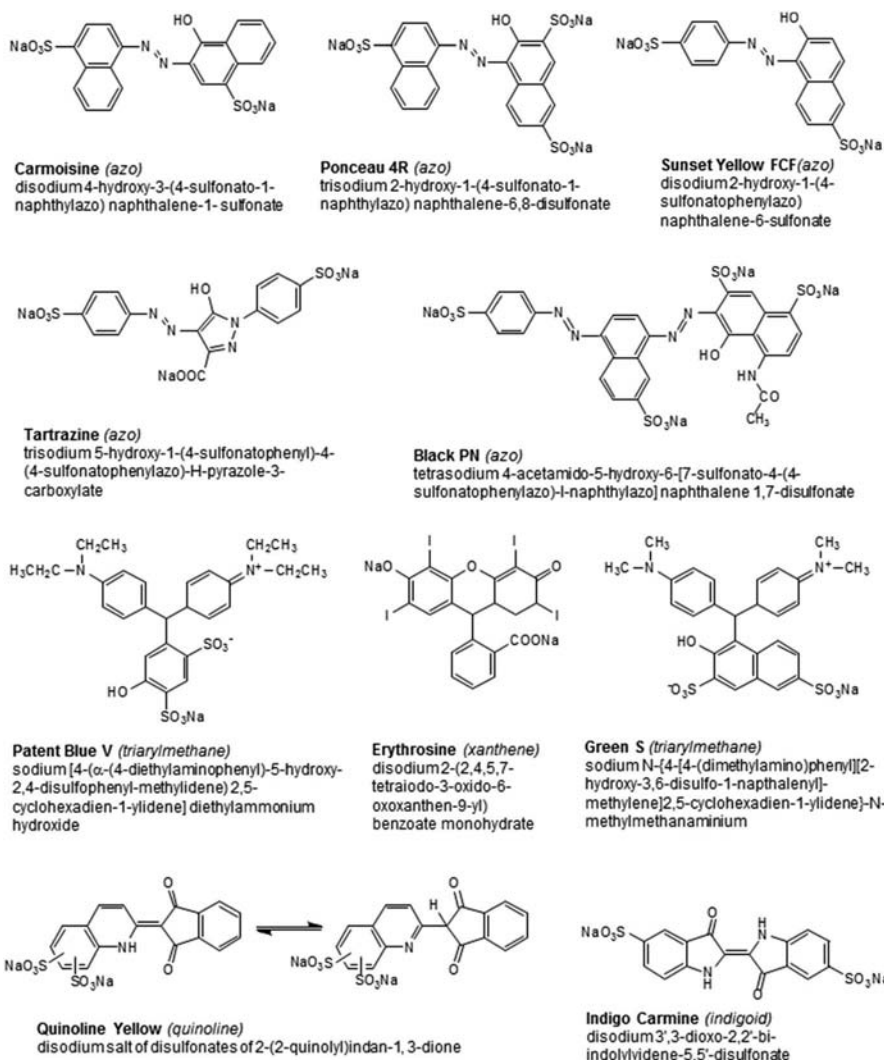


Figure 6.14 Chemical structures and chemical names of a selection of the synthetic food colourants currently used in Europe. The chemical class to which each colourant belongs is shown in *italics*.

triarylmethane dyes, which, unlike azo dyes, are anionic; they are characteristically bright green, violet or blue. Xanthene dyes are also anionic and have a similar range of hues to the triarylmethane dyes; erythrosine, the only example permitted in food, has a brilliant red shade. Resonance hybrids are important in the colours of all these dyes. Indigo Carmine in particular has many possible zwitterionic arrangements in addition to those shown in Figure 6.14. The absorption spectra of a number of the dyes are shown in Figure 6.15. The application of synthetic colours in food processing demands attention to other factors apart from the hue and intensity required. For example, high cooking temperatures may pose problems in sugar confectionery and baked goods. In soft drinks to be sold in clear glass or plastic bottles, one may have to contend with light fastness (Food Green S and Indigo Carmine are particularly vulnerable). The presence of sulfur dioxide, ascorbic acid or a low pH may also cause problems.

There is a continuing decline in the use of synthetic colours in food. It appears that consumers are now becoming content with less vibrantly coloured confectionery. In the past, the widest use of food colours was in nominally fruit-containing products, including desserts, confectionery and ice cream. Canned peas were traditionally coloured with a mixture of Food Green S and Tartrazine. As mentioned earlier, natural chlorophyll does not survive the canning process. The once popular low-cost canned vegetable called processed peas consisted of peas that had been allowed to mature and dry in the field before harvest. All traces of chlorophyll would have been lost even

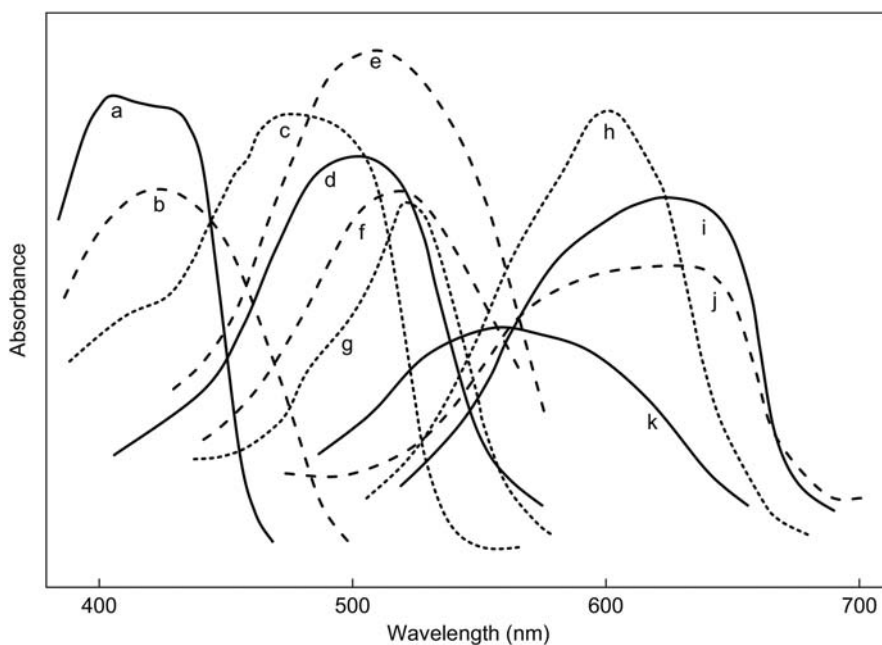


Figure 6.15 Absorption spectra of synthetic food colours (at pH 4.2 in a citrate buffer). a, Quinoline Yellow; b, Tartrazine; c, Sunset Yellow FCF; d, Ponceau 4R; e, Carmoisine; f, Amaranth; g, Erythrosine; h, Indigo Carmine; i, Patent Blue V; j, Food Green S; and k, Black PN.

before they were subjected to the high temperatures of canning in brine containing food colours. The colours of chocolate-flavoured products were often enhanced with Chocolate Brown HT or with mixtures of Sunset Yellow and amaranth or Food Green S and tartrazine. The two colours most often used in the past for meat products are no longer permitted for such use in Europe, where erythrosine is now restricted to cocktail (Maraschino) and candied cherries. Red 2G, which had been popular for use in products such as sausages or meat pastes likely to be exposed to light, was banned in Europe in 2007. Although Red 2G was not believed to be a carcinogen in its own right, evidence had accumulated that showed that, once consumed, it could be broken down to yield aniline, a recognised carcinogen.

In general, synthetic food colours such as the azo dyes are added at a rate of 20–100 mg kg⁻¹. Rather higher levels of caramels (1000–5000 mg kg⁻¹) are required to achieve a useful degree of colouring. Some products, particularly some types of confectionery or predominantly fatty foods, contain very little free water in which the dyestuff can be dissolved. In these cases insoluble lakes are used, in which the colour is adsorbed onto a hydrated alumina substrate. The powdered lake is then milled down to a particle size of about 1 mm to avoid speckling. The intensity of colour obtained using a given amount of lake colour increases as the particle size is reduced. Current regulations in the UK forbid the addition of any colouring to raw or unprocessed foods such as meat, fish and poultry, fruit and vegetables, tea and coffee (including “instant” products), wine, milk or honey. The use of added colours in foods for babies and infants is also prohibited.

6.8 Inorganic Food Colourants

There is one small group of food colours that do not fit readily into any of the groups considered so far in this chapter. These are the inorganic substances listed in Table 6.5. Although, with the exception of carbon black (also known as vegetable carbon), they are not obtained from plant, animal or microbial sources, they are still regarded as “natural”. Carbon black is obtained by heating plant materials to high temperatures in the absence of air. Virtually any plant material could be used, including wood or coconut shells, but peat is preferred because it has a very low ash (*i.e.* mineral) content. Apart from in liquorice confectionery, carbon black is little used today, except occasionally in cake icing. It does not blend well with other colours, tending to result in greyness rather than darkening.

Although we are familiar with the use of white pigments in paint, cosmetics *etc.*, a role for white pigments in food products may not be so obvious. In fact, powdered white pigments are often used to enhance the appearance of white confectionery. The inclusion of a white pigment will also brighten the appearance of other colours. Titanium oxide occurs widely as the minerals ilmenite (which also contains iron oxide, approximately FeTiO₃) and rutile (TiO₂). Fairly drastic industrial chemical processes have to be applied to these to give the pure white pigment. Titanium oxide’s whiteness depends on the very high light scattering capability of the crystals, a result of their high refractive index. Light scattering is at a maximum when the crystals are 200–350 nm in size, *i.e.* approximately half the wavelength of visible light. The inertness of titanium oxide

Table 6.5 Inorganic food colours permitted in Europe.

Name	EU number	C.I. number
Carbon black	E153	77266
Calcium carbonate	E170	77220
Black iron oxides and hydroxides	E172 (i)	77499
Red iron oxides and hydroxides	E172 (ii)	77491
Yellow iron oxides and hydroxides	E172 (iii)	77492
Aluminium	E173	
Silver ^a	E174	
Gold ^a	E175	

^aOnly permitted for the surface decoration of sugar confectionery.

at the pH values encountered in biological materials has, until recently, assuaged toxicological concerns. However, there has been a growing recognition that such *nanoparticulate* materials might present hazards at the subcellular level, irrespective of their chemical characteristics, and from 2021 titanium oxide ceased to be a permitted food additive in Europe. Alternatives, such as rice flour, are of limited value because they do not provide the opacity required in many applications.

Calcium carbonate is most often used in food products as a source of carbon dioxide in raising agents, but it can also be used as a white pigment. Its obvious tendency to dissolve in even mildly acidic food environments limits its use. Microencapsulation, coating particles with an impervious material, may provide a solution. There are a number of potential materials that could be used to encapsulate calcium carbonate crystals, notably polysaccharide gums, which are already permitted food additives. Various forms of iron oxides/hydroxides are manufactured by the controlled oxidation of the pure metal in the presence of water. They are insoluble in water under the normal conditions of food processing or consumption and also offer total heat and light stability. Their shades tend to be rather dull and they are most commonly used in pet foods.^{xi}

6.9 Restrictions on the Use of Colours in Foodstuffs

For some years, now concern has been expressed in the media, and by sections of the medical profession, about the safety of food additives, in particular synthetic food colourings. The stringency of the toxicity testing procedures now applied in Europe, and elsewhere, leads to the conclusion that the synthetic food colours now permitted are at least as safe as any of the “natural” components of our diet. (The author is only too well aware of the diversity of interpretations that can be placed on this adjective when food is being discussed.) However, it cannot be denied that most food testing procedures concentrate on the issue of carcinogenicity. Studies with laboratory animals are well suited to obtaining quantitative data for Acceptable Daily Intakes (ADI)^{xii} in this field. The possibility that a food additive may provoke an allergic response, or some other manifestation of intolerance, can be much more difficult to quantify.

^{xi}Although cats and dogs are almost certainly indifferent to the colour of their food, most of their human owners are not.

^{xii}The ADI is a measure of the amount of a substance, such as a food additive, which can be consumed over a lifetime without an appreciable health risk, usually expressed in mg (of the substance) per kg of body weight per day.

Among the food colours, tartrazine is widely regarded as the most suspect, and there is some evidence that between 0.01 and 0.1% of the population may show intolerance towards this dye. Intolerance usually manifests itself as one or more of the symptoms of allergic reactions, such as eczema and asthma (see Chapter 10, Section 10.5), and is most often encountered in those who already show a reaction to certain other groups of “chemicals”, notably the salicylates (including aspirin) and benzoates.

The possibility of food colours being involved in disorders such as hyperactivity in children has been given a great deal of attention by the popular press, but until the early years of the twenty-first century had tended to be dismissed by most medical authorities. However, in 2007, studies carried out at the University of Southampton provided new evidence for the involvement of at least some food colours in the incidence of hyperactivity (correctly termed attention deficit hyperactivity disorder, ADHD) in susceptible children. The double-blind study used two different mixtures of food colours plus the preservative sodium benzoate in a fruit drink vehicle, along with a placebo. The trial was designed so as not to allow the participants (a total of 297 children) or their families to identify which particular additive(s) might be responsible for any effects observed. The colours included in the study were Sunset Yellow, Ponceau 4R, tartrazine, carmoisine, Allura Red (all azo dyes) and Quinoline Yellow, a choice designed to reflect likely consumption by children at that time. There were marked differences in the responses of individual children, some showing no effect on behaviour at all, but others showing definite adverse effects. However, the average effects in both groups of children were significant and judged to be worthy of further study.

The results of this study were then evaluated by a panel set up by the European Food Standards Authority, who concluded that the weight of evidence was insufficient to provide a basis for banning the six colours outright or altering the current ADI figures.^{xiii} The UK authorities responded to this apparent setback in two ways. In 2010, they made a regulation that additional labelling information is required when one or more of these colours is used in food and drink. The name or E number of the colour has to be accompanied by the wording “may have an adverse effect on activity and attention in children”. Food and drink manufacturers who stated that they no longer used any of the six colours in their products were also encouraged to add their names to a published list. The combined effect of these two measures was the rapid, and near total, disappearance of these colours from food products in the UK, especially those (such as confectionery and soft drinks) most likely to be consumed by children. The publicity attached to these measures in the UK had repercussions in many countries around the world and the use of these colours is now greatly reduced.

The elimination or unpopularity of so many artificial food colours has forced food manufacturers to explore their replacement with “natural” alternatives. Many of these have already been mentioned in the sections on chlorophylls, carotenoids, *etc.* However, these replacement colours often have severe limitations in terms of heat stability or pH, which restricts the range of food products in which they can be used. Table 6.6

^{xiii}The full text of the European Food Standards Authority opinion is available at: www.efsa.europa.eu/EFSA/efsa_locale1178620753812_1178694648892.htm (accessed 2013). In the USA, the Food and Drug Administration reached a similar conclusion in 2004.

lists the natural alternatives that have been recommended by several different manufacturers of food colours and the UK Food Standards Agency (*Guidelines on Approaches to the Replacement of Tartrazine, Allura Red, Ponceau 4R, Quinoline Yellow, Sunset Yellow and Carmoisine in Food and Beverages*, Food Standards Agency, 2011).

Although natural colours have obvious attractions to consumers who have concerns about the safety of synthetic food colours, it should be noted that, in the European Union, there are a considerable number of foods in which the only colours permitted are those of the “natural” type (Table 6.7). In this context, it is usual to include the caramels as natural colours. An interesting alternative to the manufactured caramels (Classes I–IV, E150a–d) is the use of malt extract as a brown colour. Another variation of this process been marketed in the USA since 2018. Intensive cooking of a variety of vegetable and fruit concentrates, naturally rich in sugars, yields, *via* the Maillard reaction, brown materials that can be used as food colourants. Unlike traditional caramel production, their manufacture does not involve any additional acids, alkali, sulfite or ammonia and the products’ “no added chemicals” credentials amply compensate for the lower intensity of the brown colour.

Growing unpopularity and the ever-increasing costs of safety testing are making the appearance of new synthetic food colours extremely unlikely. It must be remembered that it is not only the food colour itself that must be shown not to cause death or disease.

Table 6.6 Recommended natural colours for the replacement of the six artificial colours identified as potentially harmful by the University of Southampton study.

Synthetic colour	Suggested natural alternatives
Tartrazine (E102)	Curcumin, annatto, ^a carotenes, lutein, safflower with copper chlorophyllin
Quinoline Yellow (E104)	Curcumin, lutein, carotenes, safflower with copper chlorophyllin
Sunset Yellow FCF (E110), Carmoisine (E122)	Capsanthin, cochineal, annatto, ^a carotenes Cochineal, anthocyanins (grape skin) ^b alone or with carotenes, betanin ^a
Ponceau 4R (E124)	Anthocyanins (black/purple carrot or elderberry), ^b betanin, cochineal
Allura Red (E129)	Betanin, anthocyanins (black/purple carrot or elderberry), ^b cochineal

^aRestricted to dairy products.

^bNot suitable for dairy products and some flour confectionery.

Table 6.7 Foods restricted in the European Union to the use of natural colours. Note that neither blue nor green colours of any type may be added to blue cheeses such as Stilton, Roquefort or Gorgonzola.

Foodstuffs	Permitted colours
Beer, cider, vinegar and spirits	Caramels (E150a–d)
Butter	Carotenes (E160a)
Margarine <i>etc.</i>	Carotenes (E160a), annatto (E160b), curcumin (E100)
Cheese	As margarine, plus paprika (E160c) and the chlorophylls (E140, E141) for Sage Derby, cochineal (E120) for red marbled cheeses
Pickled vegetables	Chlorophylls, caramels, carotenes, betanin (E162), anthocyanins (E163)
Extruded, puffed and/or fruit-flavoured breakfast cereals	Caramels, carotenoids (E160a–c), betanin (E162), anthocyanins (E163), cochineal, betanin, anthocyanins
Many types of sausages and pates	Carotenoids (E160a,c), curcumin, cochineal, caramels, betanin

Any impurities that arise during its manufacture, and any breakdown products that might arise during food processing, cooking or digestion, all have to be considered.

6.10 Molecular Basis of Colour

Many readers will be happy to accept that certain substances are coloured without wishing to understand what it is about the molecular structure of such substances that imparts this particular property. Such readers can ignore the next few pages with a clear conscience. Others, however, will want to know a little more, possibly to understand why the colours of some food materials are what they are, and how they are subject to change.

Materials or objects appear coloured because they absorb some of the incident light that shines on or through them. This means that light of certain wavelengths is absorbed more than others. The light that subsequently reaches our eyes, having been reflected or transmitted, is therefore richer in light from some parts of the visible spectrum than from others. To appreciate the molecular basis of colour, we need to understand, first, how molecules absorb light energy and, second, what determines the wavelengths, or the colour, of the light they absorb.

Table 6.8 shows the relationship between the wavelength of light and its colour. Although, for most purposes, physicists and chemists regard light as having the properties of waves, light can also be thought of as a stream of energy consisting of particles. These particles are called “quanta”. The amount of energy possessed by a quantum is related to the wavelength of the light. The shorter the wavelength, *i.e.* the nearer to the violet end of the visible spectrum, the more energetic the quantum.

The energy in the form of light that a coloured molecule absorbs cannot simply be soaked up and vaguely converted into heat. Each single quantum must be used

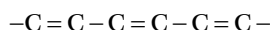
Table 6.8 The visible spectrum. Substances or objects that absorb light of the wavelength in the left-hand column have the (complementary) colour shown in the right-hand column. For example, the yellow carotenoid pigments strongly absorb light in the purple/blue region (420–480 nm; see Figure 6.6). We describe the response of the human eye to the mixture of all the other visible wavelengths that the carotenoids do not absorb (480–800 nm) as yellow or orange.

Colour	Wavelength (nm)	Complementary colour
Violet	400	Yellow
Blue	450	
Green	500	Orange
Yellow	550	Red
Orange	600	Violet
Red	650	Blue
	700	Green

individually to provide exactly the right amount of energy to facilitate a particular change (or transition) in the molecule. The quantum will only be absorbed if its energy content matches the energy demand of a transition that the molecule can undergo. The transitions that match the range of energies associated with visible light quanta involve the electrons that form the bonds between the atoms of the molecules of the absorbing substance. Of the many theoretically possible types of transition, only those associated with the π -electrons of conjugated double bonds are common among the coloured compounds in food. A single bond between two carbon atoms consists of one pair of shared electrons that form one σ -bond. The four bonding electrons comprising a double bond occur as one σ -bond and one π -bond. In the normal or ground state of σ - or π -bonds, the two electrons are regarded as spinning in opposite directions, shown as ($\uparrow\downarrow$). However, if an appropriate quantum of energy is absorbed by the molecule, then one of the bonds may undergo a transition to an excited higher energy state in which both electrons are spinning in the same direction ($\uparrow\uparrow$). Such transitions are known as σ to σ^* and π to π^* transitions. The σ to σ^* transitions require quanta of much higher energy levels than those of visible or ultraviolet light and are therefore irrelevant to the question of colour.

By contrast, the π to π^* transitions require less energetic quanta and are relevant to colour. Once an electron has undergone transitions like these, it usually returns immediately to its original state and the energy it has absorbed is released as heat. In some molecules, the return of the electron to its original state can lead to the release of a quantum of energy sufficient to appear as light, although obviously at a longer wavelength than that originally absorbed. This is the phenomenon of fluorescence. The wavelength at which a substance absorbs most radiation, the position of the highest peak in the absorption spectrum, is referred to as the λ_{max} value.

When the bonds linking a chain of carbon atoms are alternately single and double, *e.g.*



then the double bonds are described as *conjugated*. All the coloured organic substances described in this chapter have an abundance of conjugated double bonds. The π -electrons of conjugated systems interact and, in doing so, particular pairs of π -electrons lose their association with a particular carbon-carbon bond. In this state they are said to be *delocalised* and require much less energy to undergo a π to π^* transition. In fact, the more double bonds are conjugated together in a molecule, the less energy is required for the π to π^* transition and therefore the radiant energy absorbed by the process is of increasing wavelength. This is clearly illustrated by the sequence of carotenoid structures shown in Figure 6.16. Only when a carotenoid has its full complement of conjugated double bonds, as in lycopene, is visible light absorbed. Of course, the exact λ_{max} values we observe, and the precise details of the absorption spectrum, are affected by other structural elements in the molecule, such as the involvement of the double bonds in rings and also by interactions with external agencies such as solvents.

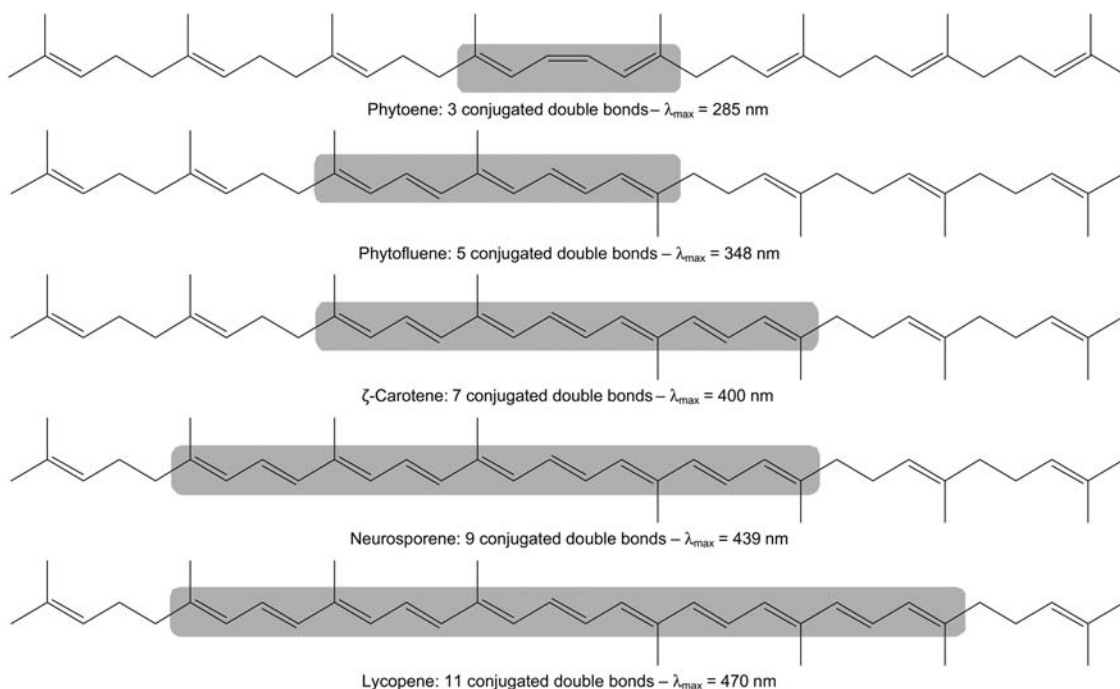


Figure 6.16 Relationship between the structure of the carotenoid precursors and their λ_{max} values. The chromophore regions, with their systems of conjugated double bonds, are shaded. This sequence, including the conversion of the central double bond from *trans* in phytoene to *cis* in phytofluene, happens to correspond to the biosynthetic pathway for the formation of carotenoids in plants. β -Carotene, and the other carotenoids containing rings, are derived from neurosporene.

The need for at least seven conjugated double bonds for a substance to absorb visible light is also nicely demonstrated by the anthocyanins (see Figure 6.9). The flavylium cation (red) has a total of eight double bonds, but when an increase in pH converts it into the carbinol pseudo-base, then the conjugation in the middle of the system is lost. A molecule is colourless when there are less than four double bonds in any one conjugated system. The bleaching action of sulfur dioxide (6.17) has a similar basis. By contrast, the ionisation of the carboxyl groups in betanidine with change in pH causes no change in its eight conjugated double bonds and there is no colour shift (see Figure 6.11). The term “chromophore” is used to denote the region of a molecule where delocalised electrons are responsible for the absorption of light. For example, the porphyrin ring system is the chromophore in chlorophyll. The sandalwood pigments, *e.g.* santalin A (6.34) provide another relevant example of this effect.

Some food pigments contain metal atoms, for instance the chlorophylls (see Section 6.1), myoglobin and its derivatives (see Chapter 5, Section 5.5.4) and vitamin B12 (see Chapter 8, Section 8.5). The structures of all these pigments have elaborate systems of fused rings providing large numbers of conjugated double bonds, and hence delocalised electrons. However, the electrons from the metal atom also become involved, with a considerable effect on the colour. For example, the loss of the magnesium from green

chlorophyll gives us brown pheophytin. The colour of myoglobin in meat depends on whether the iron is in the Fe^{II} or Fe^{III} state and which other substances are also coordinately bonded to the iron atom.

6.11 Special Topics

6.11.1 Flavonoids, Tannins and Health

The anthocyanins in fruit and the catechins in tea are important members of the flavonoid group of plant components. They are all based on the flavan nucleus (6.10), but differ in the state of oxidation and the substituents in the central oxygen-containing ring. Tannins are polymerised flavonoids. Flavonoids are difficult to categorise in terms of their function in food (and therefore their placement in this book) because they contribute colour, flavour or “mouth feel” in different situations.^{xiv} Significant degrees of antioxidant and anti-tumour activity and the converse, carcinogenicity, have also been attributed to flavonoids and it is their effect on human health that has brought them wider attention.

As shown in Figure 6.17, flavonoids can be grouped into classes that differ in the structure of the central ring. The A-ring almost invariably carries hydroxyl groups at positions 5 and 7, and these hydroxyls often carry sugar residues. Similarly, the B-ring carries one, two or three hydroxyl groups. When double bonds in the central ring provide a conjugated link between the aromatic systems of the A- and B-rings, then the flavonoids absorb visible light, as indicated by the approximate absorption maxima (λ_{max}) shown in Figure 6.17.

The definition of tannins is imprecise and usually refers to polyphenolic substances, *i.e.* substances containing aromatic rings carrying multiple hydroxyl groups, which can form links with proteins. These links usually result in the protein being rendered insoluble, as in the tanning of leather. Most often, the polyphenolic substances concerned are flavonoids, especially where two or more flavonoids are condensed together *via* their 4- and 8-positions. These oligomeric flavonoids are also known as condensed tannins or, more precisely, proanthocyanidins. Proanthocyanidins often contain flavin-3,4-diols (leucoanthocyanidins), but flavin-3-ols (catechins) and anthocyanidins also commonly occur. The oligomers may contain as many as ten flavonoid units and have molecular weights in the range 500–3000.

The possible beneficial effects of flavonoids, including tannins, on the health of consumers continues to receive considerable attention, focusing particularly on red wine and tea, but also on brightly coloured fruit in general. A complication is that both adverse and beneficial effects have been reported, and the results of epidemiological studies are less encouraging than the results of laboratory or clinical experiments.

Also on the negative side, the habit of chewing betel nuts, which have a very high content of condensed tannins, has been implicated in the high incidence of mouth and oesophageal cancer in the Far East. The risk of oesophageal cancer in the Far East has also been linked to drinking tea, but it is now accepted that the culprit is not the

^{xiv}What scientists refer to as “mouth feel” is popularly described as “body” when applied to drinks.

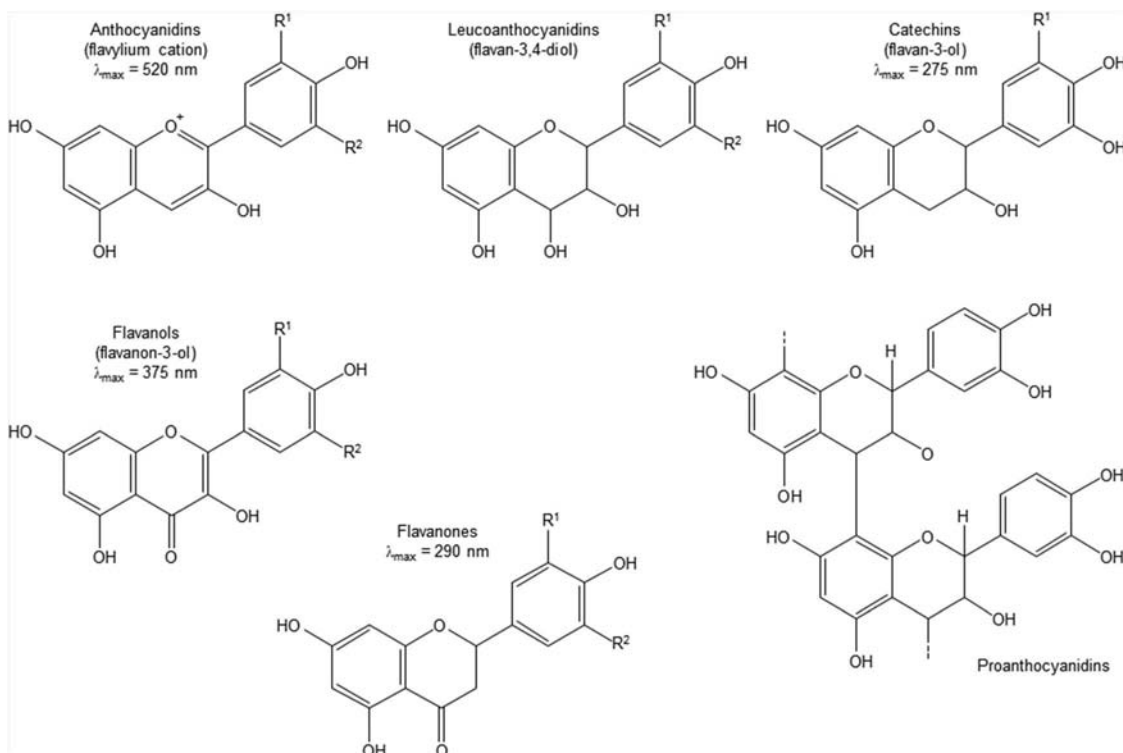


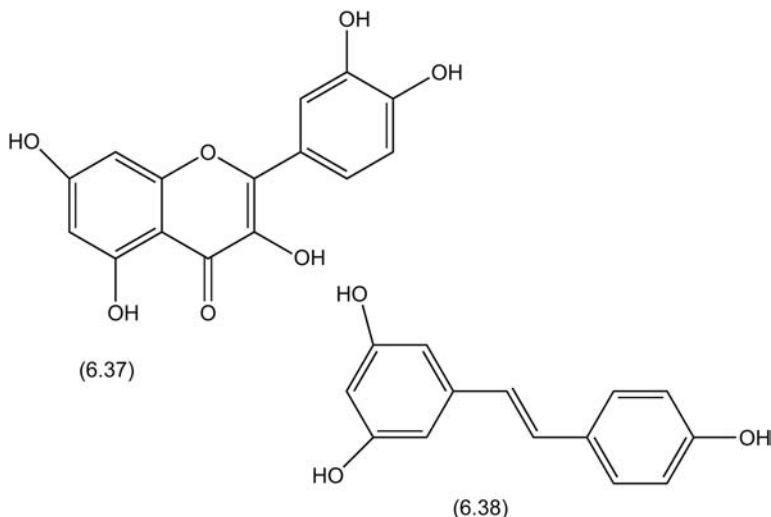
Figure 6.17 Basic structure of the major groups of flavonoids. Where available, typical values for the absorption maxima are shown. Except in the anthocyanins, where further details are shown in Figure 6.8, R¹ and R² are either H or OH. The proanthocyanidin illustrated contains two flavan-3-ol units, but proanthocyanidins commonly include flavan-3,4-diol and anthocyanidin units.

tea itself, but cell damage caused by routinely swallowing very hot tea ($>55^\circ\text{C}$). Different studies of stomach cancer have shown contradictory results, both increased and decreased risks being linked to tea consumption. Various flavonoids have been shown to possess anti-tumour activity in laboratory experiments, but useful conclusions cannot be drawn in the absence of unequivocal epidemiological data.

By contrast, dietary flavonoids have well-recognised positive effects on arterial disease. The initial interest in their possible benefits arose from the observation that French and Italian people eat diets (popularly referred to as “Mediterranean diets”) that include a lot of fat, but that they have far less heart disease than might be expected. A reduced mortality rate from coronary heart disease has been linked to the intake of wine, a good source of flavonoids ($1\text{--}3 \text{ g L}^{-1}$ in red wine, 0.2 g L^{-1} in white wine), as well as plenty of fresh fruit and vegetables. However, the total mortality rate does not show the same association, presumably due to the adverse effect of other factors, such as a high alcohol intake. The rates of coronary heart disease are also low in the Far East, where the consumption of green tea is high.

Two potential mechanisms have been implicated in these beneficial effects. First, flavonoids – including the quercetin (6.37) found in wine, tea, onions and apples and

the catechins in tea – are recognised as effective antioxidants. Many laboratory experiments and some clinical studies indicate that they can be effective inhibitors of the oxidation of low-density lipoproteins, an important adverse factor in the development of atherosclerosis. Unfortunately, there is a considerable gulf between demonstrating antioxidant activity *in vitro* in laboratory experiments and showing the same substances to be capable of reaching the relevant human tissues and exhibiting antioxidant activity once they arrive. It is unfortunate that many of those who advocate the consumption of colourful, expensive and/or exotic fruit^{xv} on the basis of their apparent antioxidant content are either unaware of, or untroubled by, this gulf. A second potential mechanism is the inhibition of platelet aggregation, another important stage in coronary heart disease, by wine flavonoids. Quercetin is particularly effective, but the catechins are not. Resveratrol (*trans*-3,5,4'-trihydroxystilbene, 6.38), a non-flavonoid present in wine (about 4 g L⁻¹ in red wine, 0.7 g L⁻¹ in white wine), appears to have similar activity and has attracted plenty of attention. However, the link between laboratory experiments and measurable effects on human health is far from established.



6.11.2 Measurement of Colour

The measurement of colour is a major issue in many fields of food science and, strictly speaking, is a subject for physicists rather than chemists. However, in the real world, it is almost invariably the analytical chemists who are required to cope with this aspect of food science. The aspect of colour being measured depends on the circumstances. Very often we need to be able to express the hue of a food product, ingredient or raw material in quantitative terms. To describe a tomato as red may be adequate for artistic or culinary purposes, but expressing the concise difference in colour between a tomato and a strawberry to someone who has never seen either fruit would place impossible demands on even our greatest poets. In addition to the hue, we may find that the

^{xv}Often referred to in the popular press as “superfoods”.

intensity of the colour is expressed using terms such as “lightness” or “darkness” – for example, in matters of fruit juice quality.

Chemists confronted by this problem usually reach for a spectrophotometer to obtain an absorption spectrum, like those shown in Figures 6.2 and 6.6. This approach tends to fail because food materials are often solids or turbid liquids. This difficulty can be overcome by adapting the instrument to record the light reflected by the material rather than that transmitted through it, giving a reflectance spectrum. However, this does not solve the real problem with absorption or reflectance spectra, which is that they present too much information. The human eye uses the cone cells of the retina to perceive the colour of an object. There are three different types of cone cell, each responding best to light of different wavelengths (445, 535 and 570 nm, respectively). There is considerable overlap between the responses of the different types of cone cell. This means that light of a given wavelength, or mixture of wavelengths, will stimulate at least two and possibly all three types of cone cells. Our perception of the colour of light is based on the ratio between the three responses, combined with the contribution of the rod cells. The rod cells are about 10 000 times more sensitive than the cone cells and have peak sensitivity at 505 nm. It should therefore be possible to describe any colour perceived by the human eye simply in terms of such ratios. In fact, the wavelengths used in colour definition using scientific instruments are more evenly distributed across the visible spectrum and correspond to the primary colours, red, green and blue. It is well known that an appropriate mixture of red, green and blue light will appear white, and it might be assumed that any colour could be similarly achieved. However, this turns out to be not quite true.

In 1931, the Commission Internationale de l’Eclairage (CIE) introduced the concept of theoretical primary colours, known, respectively, as X, Y and Z. Although no real lamp could produce them, they were able, theoretically, to match any shade. Their spectra are shown in Figure 6.18. The red, green and blue curves are labelled X, Y and Z, respectively. An important characteristic of the Y curve is that it matches the response of the eye to the total amount of light energy, in other words, it represents the response of the rod cells of the retina. The amounts of the three theoretical primary colours required to produce a given colour can be calculated from its spectrum and the curves in Figure 6.18 and are known as its tristimulus values. The ratios between these are given by:

$$x = X / (X + Y + Z), y = Y / (X + Y + Z) \text{ and } z = Z / (X + Y + Z)$$

Since $x + y + z = 1$, any colour can be described by just two of them – for example, by x and y .

Figure 6.19 shows the famous horseshoe-shaped CIE chromaticity diagram, in which x is plotted against y . On this plot, the quantities x and y for a colour are called its chromaticity coordinates. The theoretical red primary is located at $x = 1, y = 0$, blue at $x = 0, y = 0$ and green at $x = 0, y = 1$. The outer curved line shows the positions of monochromatic colours at different positions in the spectrum from 380 to 770 nm. The straight line closing the horseshoe represents the various purplish shades resulting from mixtures of red and blue. The colours approach white towards the centre of the horseshoe.

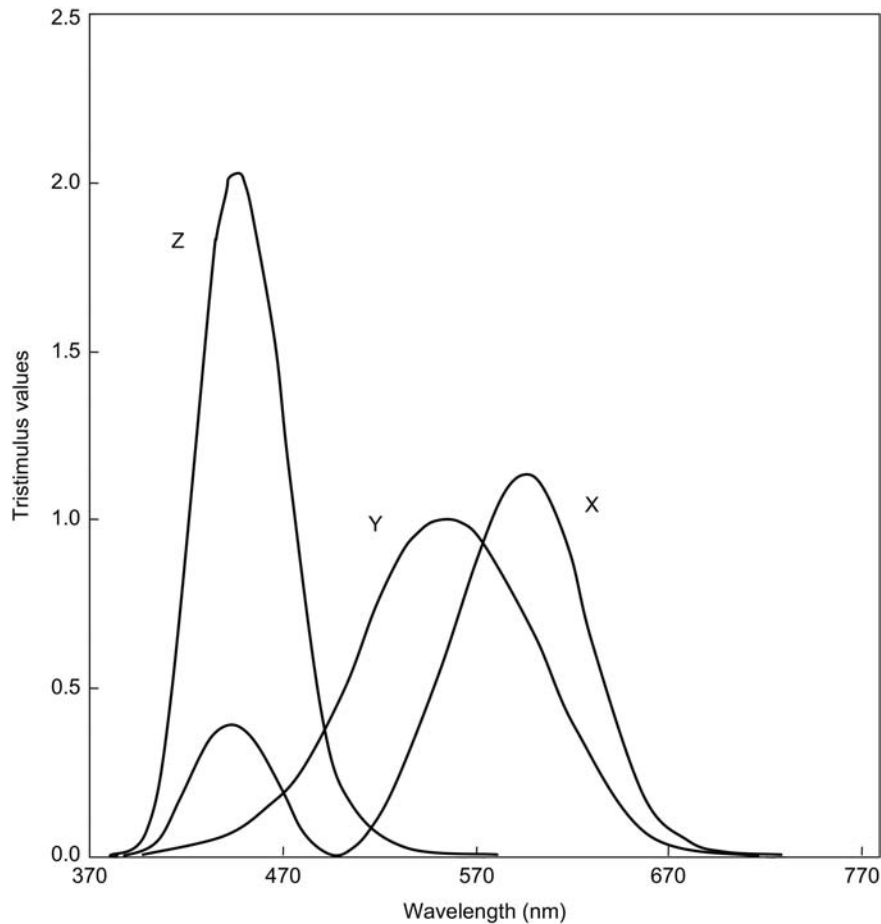


Figure 6.18 Spectra of the theoretical primaries of the CIE system. The subsidiary peak on the X curve results from the mathematical ingenuity used to ensure that all shades can theoretically be matched, as explained by Billmeyer and Saltzmann (see Further Reading).

The CIE system is linked closely to theoretical concepts of colour perception, but has several practical drawbacks. One of these is the status of black. To handle this properly, the diagram would have to be three-dimensional, with values for the tristimulus value, Y, expressing the total amount of light, plotted perpendicular to the plane of the paper. Another problem is that our perception of degrees of difference between colours are not uniform over the whole diagram. Both problems are dealt with better by the Munsell and Hunter systems.

The Munsell system (Figure 6.20a) has a similar structure, but it describes colour in terms of the three attributes hue, value and chroma. Five basic hues are distributed around the circumference of a circle. The vertical axis of the circle defines the value, or lightness, from black below the circle, through grey at the centre, to white above the circle. The distance from the centre to the circumference gives a figure for the chroma, or saturation. It is important to remember that it is extremely unlikely that the colours

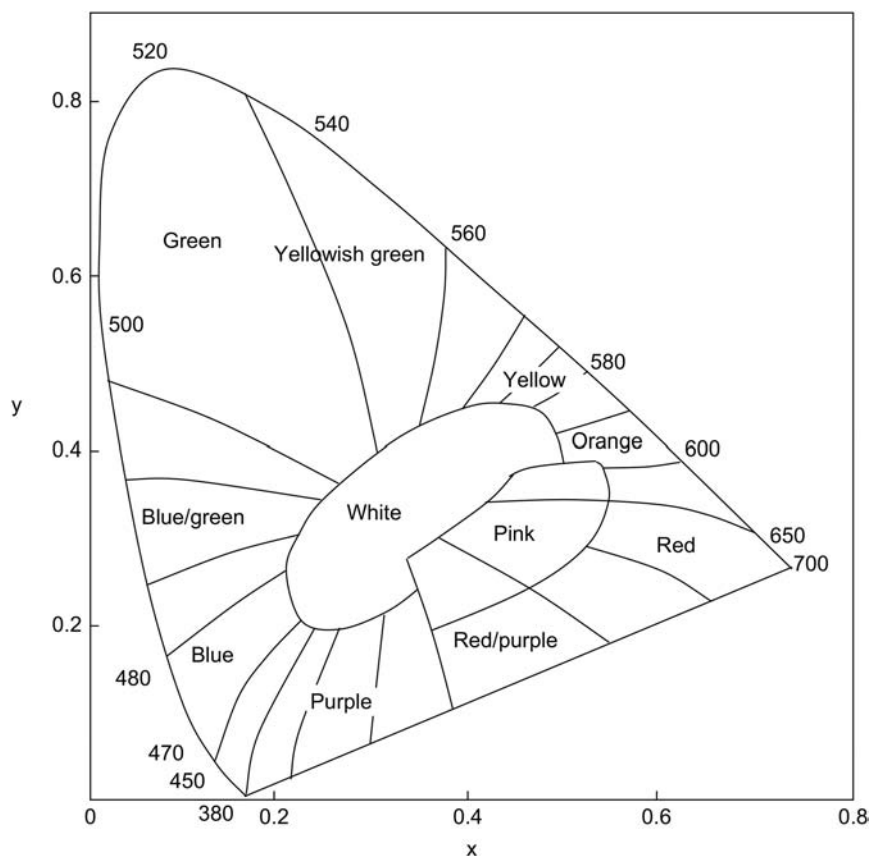


Figure 6.19 The CIE chromaticity diagram. The colour names shown on the diagram describe the colour perceived by a standard observer looking at a coloured surface illuminated by a standard illuminant (light source) with the theoretical spectral characteristics of ordinary daylight. A rather non-picturesque approach is usually adopted towards the naming of intermediate colours, largely dependent on the suffix “ish”. All shades of grey are merely more or less bright white.

of two real objects would differ in only one of these parameters; in most cases all three would differ. Users of modern computers with colour monitors and printers will have encountered some of the practical issues associated with these colour systems.

The Hunter L , a , b system is based on the concept of a colour space, with the colour defined by three coordinates. The vertical coordinate (L) runs from $L = 0$ (black) *via* grey to $L = 100$ (white). The horizontal coordinate (a) runs from $-a$ (green) to $+a$ (red). The other horizontal coordinate (b) runs from $-b$ (blue) to $+b$ (yellow). Purple and orange can be visualised as being located at $+a$, $-b$ and $+a$, $+b$, respectively (Figure 6.20b).

Armed with the theory, one can then approach the actual task of recording the colour of a foodstuff. One simple approach is by visual matching against a standard colour sample. Although apparently simple, this approach still requires a standardised light source. The outdoors sky, incandescent (tungsten) bulbs and fluorescent tubes may

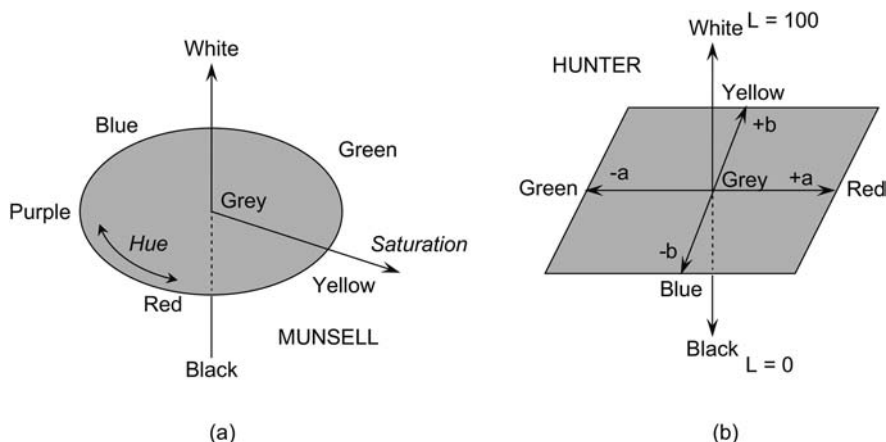


Figure 6.20 The (a) Munsell and (b) Hunter colour spaces. The mathematical relationships between these and the CIE system are well established and the parameters of a colour determined in one system can be mathematically related to those in the others.

all nominally give “daylight illumination”, but they differ considerably in their distribution of energy across the spectrum. Two samples may appear identical under a fluorescent lamp, but very different in a different type of illumination. This phenomenon is known as metamerism. In addition, visual assessment is affected by the colour sensitivity of the observer, including their age and whether they have “normal” colour vision. A further factor is the difficulty in describing small visual colour differences. The instrumental alternatives are colorimeters and spectrophotometers. A colorimeter uses filters to generate three light beams whose spectra match as closely as possible the CIE curves. Photocells measuring the quantity of light reflected (or transmitted) by the sample will then directly give the tristimulus values. Modern computerised instruments can give an instant conversion to the Hunter or Munsell value. More sophisticated spectrophotometers record the absorbance or transmittance of light at intervals, or even continuously, throughout the visible spectrum and then calculate the tristimulus values.

6.11.3 Colours from Microorganisms

This chapter has been largely concerned with the coloured substances naturally occurring in plant-derived foods and chemists’ efforts to supplement or replace them in processed food products. However, pigments synthesised by microorganisms are now beginning to appear in our diet and deserve some attention. There are three groups of microbial pigments to consider. The first are the pigments chemically and functionally identical, *i.e.* nature-identical, to some of the pigments of higher plants we are already familiar with, but synthesised by industrially cultivated microorganisms, *i.e.* products of biotechnology. The second, much smaller group contains pigments that are naturally synthesised by microorganisms during the manufacture of traditional

fermented food products. The third, also small, group contains pigments otherwise largely unknown in human diets that can be isolated from microorganisms harvested from natural environments.

- (i) Carotenoids synthesised by fermentation. A number of different carotenoids associated with human foods are synthesised by yeasts or filamentous fungi, including β -carotene, lycopene, astaxanthin, zeaxanthin and canthaxanthin. Two species of filamentous fungi, *Blakeslea trispora* and *Phycomyces blakesleanus*, members of the Class Zygomycetes (formerly the Phycomycetes), contain useful quantities of β -carotene. It is dissolved in the prominent oil globules that occur in the vegetative hyphae and can readily be extracted and purified. Although the yield of β -carotene from *B. trispora* (30 mg per g dry mass) is slightly lower than that from *P. blakesleanus*, the former is the preferred source because it is easier to grow in a bulk fermenter. The purified product is permitted for use as a food colour in Europe as E160a(ii), and in the USA and elsewhere.

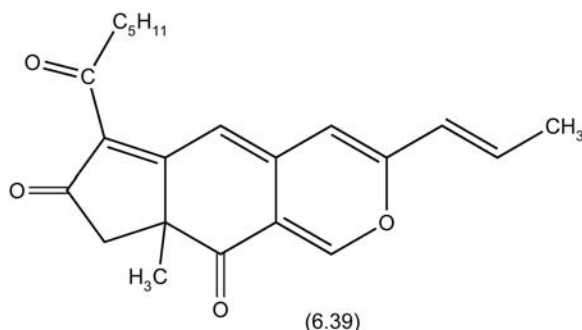
As lycopene is the immediate biosynthetic precursor of β -carotene, it has not proved difficult to obtain mutant strains of *B. trispora* that accumulate lycopene rather than β -carotene. Lycopene from this source is now commercially available and is accepted for use as a food colour in Europe (E160d) and the USA. From the point of view of food safety, it should be noted that the Zygomycete fungi are not normally associated with mycotoxin production (see Chapter 10, Section 10.3).

The possible synthesis of other carotenoids, including astaxanthin, zeaxanthin and canthaxanthin, by microorganisms, notably by species of yeasts, has been extensively studied in recent years, but at the time of writing had not come to commercial fruition.

- (ii) Microbial pigments in traditional fermented foods. Foodstuffs depending on microbial activity for their long-term stability and nutritional value have made important contributions to the diet of human populations for millennia. Cheese, wine and beer are the obvious examples in European and other Western cultures. In Asia, soya beans, rice and fish are all used as the basic raw materials of a great variety of food products stabilised by microbial fermentation. Of particular interest in the context of food colours is the “red mould rice” widely used in Indonesia, southern China and Japan.

Various species of the mould genera *Monascus*, notably *Monascus purpureus*, *Monascus ruber* and *Monascus anka*, which grow readily on starchy substrates such as steamed rice, are used. The cells of these moulds contain a variety of yellow, orange and purple pigments, which collectively impart a purplish-red colour to the rice. Rubropunctatine (6.39) is one of the purple pigments. The pigments tend to be unstable once extracted from the fungal cells and are most often used in the form of dried and pulverised rice grains on which the moulds have grown. To date, this material has not been approved for use as a food colour in Europe or the USA, largely due to the possible presence of other fungal metabolites. One group of these are the monacolins, hypercholesterolaemic agents (*i.e.* substances which lower the cholesterol content of

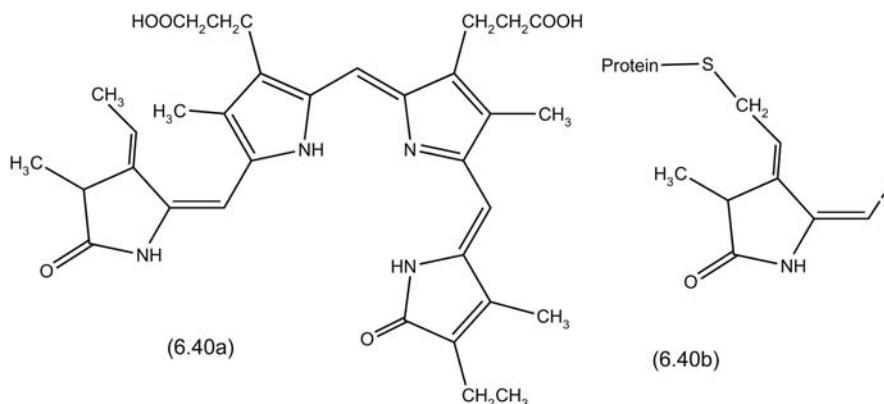
the blood). Although these might be considered beneficial, the wide variations in the amounts found in powdered pigment preparations actually present a serious potential hazard to consumers. Another issue is the occasional appearance of the mycotoxin citrinin. Despite these problem components, red mould rice, often known as “anka”, remains a popular and legal food colour in many Far Eastern countries.



- (iii) Microbial pigments without a history of use in human food. Until recently, there has been little incentive for food scientists to look beyond traditional human foods for potential new food colours. This situation began to change with the growing antagonism of consumers around the world towards synthetic food colours that developed after 2007. As far as red, yellow, orange, green and brown colours are concerned, the subsequent switch to natural colours has been managed with considerable success. However, a good natural blue colour has proved elusive, especially for food products with pH values of about 6 or higher. In reality, this problem was largely restricted to colouring food decorations and confectionery. None of the food raw materials humans consume, and whose colour food processors need to mimic, are naturally bright blue.

Confectionery manufacturers are now using the blue pigment known as spirulina obtained from the filamentous cyanobacterium (blue-green alga) *Arthrospira platensis*.^{xvi} This grows naturally in subtropical lakes in Africa, Asia and South America that are rich in carbonate and bicarbonate (pH 8–11) and is also well suited to commercial cultivation using sunlight or artificial light. The cells of these microorganisms contain substantial quantities of the blue protein pigment *C*-phycocyanin as a major component of their photosynthetic machinery. The chromophore of the protein (6.40a) is almost identical in structure to bilirubin, the breakdown product in mammals of the haem component of haemoglobin. Formula (6.40b) shows the point of its attachment to a sulfhydryl group of the *C*-phycocyanin protein. Techniques, as yet not disclosed in detail, have been developed for extracting the protein from the dried biomass of *A. platensis* in a form suitable for use as a food colour.

^{xvi} *Arthrospira platensis* and the closely related *A. maxima* were originally assigned by taxonomists to the genus *Spirulina* and the name “spirulina” has continued to be applied to the blue colour used by food manufacturers. Although often referred to as blue-green algae, *Arthrospira* spp. are, in fact, bacteria, specifically cyanobacteria, members of the phylum Cyanobacteria and the class Cyanophyceae.



The blue protein pigment is known commercially as spirulina, but there are few published details of the processes for its extraction and purification from algal cells. The regulatory status of spirulina is not currently finalised in Europe. If it is to be approved as a food additive (complete with an E number) alongside the other natural colours such as anthocyanins and carotenoids, *etc.*, then spirulina would have to undergo time-consuming and expensive toxicological scrutiny. The alternative is the rather dubious classification of “colouring food stuff”, the best-known example of this being saffron (see Section 6.2). At present in the UK, it is left to local authorities to challenge spirulina's unregulated use, but any prosecution would have to demonstrate that its use was injurious to health. Unsurprisingly, no prosecution had taken place by 2020. In the USA, spirulina extract is exempt from certification by the Food and Drug Administration for the coloration of confectionery and a variety of other food products. One reason for the fairly relaxed attitude adopted by the authorities to spirulina is the result of its long history of use as a dietary supplement in the form of tablets of compressed dried spirulina biomass.

Further Reading

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7 Flavours

However much nutritionists, both professional and amateur, may wish otherwise, it is the flavour and appearance of food, rather than its vitamins and fibre, that win compliments at the dinner table. This attitude on the part of diners is not as short-sighted as it might at first appear. The sense organs concerned in the detection of taste and odour, collectively referred to as flavour, have evolved to perform an essential function: to establish what is and what is not suitable as food. As a general rule, palatability can be equated with nutritional value and most of the exceptions are related to excess. For example, to the genuinely hungry sweetness is a clear indicator of the presence of energy-yielding sugars, whereas to the parent of a well-fed European child sweetness implies dental caries and obesity. Conversely, their bitterness and astringency will prevent us from eating many poisonous plants, but these flavours are valued elements in the flavour of beer and tea.

We usually regard taste as a property of liquids or solids, and gases in solution, which can be detected in the mouth, not only by receptor cells in the taste buds of the tongue, but also elsewhere in the oral cavity. Odour (or aroma, or smell) is similarly regarded as a property of volatile substances detected by the receptor cells of the olfactory systems of the nose. Very few flavours, and even fewer complete foodstuffs, allow a clear distinction to be made between odour and taste.

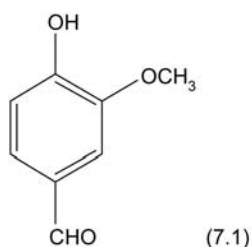
There are several distinct approaches to the study of flavour. Chemists are most interested in the range of substances that elicit a particular flavour, *i.e.* invoke a particular response from our sense organs – in particular, which features of the chemical structure or reactivity they have in common. This line of enquiry was hinted at by the Greek philosopher Theophrastus (*c.* 372–287 BC) who declared:

τὸν δὲ πικρὸν ἐκ μικρῶν καὶ περιφερῶν τὴν περιφέρειαν εἰ ληχότα καὶ καμπὰς ἔχουσαν. διὸ καὶ γλίσχρον καὶ κολλώδη. ἀλμυρον δὲ τὸν ἐκ μεγάλων καὶ οὐ περιφερῶν, ἀλλ' ἐπ' ἐνίων μὲν σκαληνῶ ...ⁱ

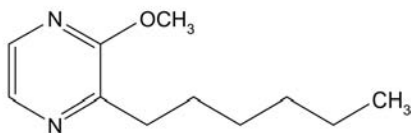
ⁱ“Bitter taste is therefore caused by small, smooth, rounded atoms, whose circumference is actually sinuous; therefore it is both sticky and viscous. Salt taste is caused by large rounded atoms, but in some cases jagged ones ...”

Understanding these features can inform efforts to develop artificial flavourings, which may have advantages over their natural counterparts – for example, in relation to the familiar problems associated with the excessive consumption of salt and sugar. Another obvious motive for such work is that it enhances the possibility of natural flavours being simulated for use in processed foods.

The research chemist faces a number of special difficulties in investigations of flavour. The first of these is that there is no physical or chemical probe available for the detection of the specific substances of interest. There is no flavour equivalent of the spectrophotometer, which quantifies the light-absorbing properties of the substances we perceive as colour. Instead, an analytical procedure may depend on the skill of a trained taste panel or the nose of an experimenter. Another complication is that the characteristic flavour of a food is rarely due to a single substance. When the mixture of substances involved in a particular flavour has apparently been elucidated and the components reassembled in the correct proportions, it is commonly found that the flavour is not the same as the original. This is usually because certain substances, present in exceedingly small proportions, without an obvious flavour of their own and readily overlooked by the analyst, can have a great influence on the overall flavour of the food. These difficulties are not likely to be underestimated if it is realised just how sensitive the nose can be. For example, vanillin (7.1), the essential element of vanilla flavour, can be detected by most individuals at a level of 0.1 ppm.



(7.1)



(7.2)

There are many other substances to which we are far more sensitive than this. For example, the odour threshold (*i.e.* the lowest concentration at which most humans can detect a substance) of 2-methoxy-3-hexylpyrazine (7.2) is reported to be 1×10^{-6} ppm, which is one part in 1 000 000 000 000. Interestingly, as the lengths of the pyrazine side-chains shorten, we become progressively less sensitive to the odour. The corresponding figures for the 2-methoxy-3-ethyl-, 2-methoxy-3-methyl- and 2-methoxypyrazines are 4×10^{-4} , 4×10^{-3} and 7×10^{-1} ppm, respectively. The earthy smell of geosmin (5.19), mentioned in the previous chapter in connection with beetroot, is reputed to be detectable at 0.5×10^{-6} ppm.

Gas chromatography has proved to be the most valuable analytical technique available to the flavour chemist. The most sophisticated modern instruments are capable of detecting as little as 10^{-14} g of a component in a mixture, and 10^{-10} g is within the capability of many commercially available gas chromatographs. The peaks on a gas chromatogram can be tentatively identified by comparing the elution characteristics with those of known substances. Firm identification requires the isolation of the substances

emerging from the chromatography column (for example, by condensation at very low temperatures) and their identity confirmed by techniques such as infrared spectrometry, nuclear magnetic resonance or mass spectrometry. Instruments are available in which the link between gas chromatography and mass spectrometry is handled automatically and the substance identified automatically by comparing its mass spectrum with a library of known spectra.

Since it is the flavour, more than anything else, which enables us to distinguish one foodstuff from another, it would be surprising if the total number of different flavours was much smaller than the total number of different foodstuffs. Moreover, the flavour compounds are much harder to classify according to their chemistry than by their contribution to the chemistry of particular foods. However, we can make a reasonable distinction between tastes and odours, and this forms the basis of the organisation of this chapter. As we shall see, the scale of these subdivisions tends to reflect the intensity of research and the volume of knowledge available, rather than the relative importance of one flavour compared with another, which is an impossible judgement.

7.1 Taste

The flavour sensations detected in the mouth, and particularly by the tongue, are usually described as tastes. Several different taste sensations can be identified. First, there is the classic quartet of saltiness, sweetness, bitterness and sourness mentioned in school biology textbooks and often associated with different parts of the tongue. Then we have three other tastes: astringency, pungency (*i.e.* hotness) and meatiness (now often referred to as umami). The concept of seven basic tastes is actually not new – the Greek philosopher Aristotle has been credited with proposing the same number. His list omits meatiness, but includes “sandiness”, although it is not clear what he meant by this word. The substances involved in all these sensations have a number of characteristics in common that distinguish them from those associated with odour. Taste substances are usually polar, water-soluble and non-volatile. Besides their necessary volatility, odour substances are generally far less polar and elicit a much broader range of flavour sensations.

7.1.1 Sweetness

Of the various tastes detected by the taste buds of the tongue, sweetness has received by far the most attention from research workers. We usually think of sweetness as being the special characteristic of sugars, and this is certainly a major reason why sucrose is included in so many food products, whether created in the home or the factory. What is frequently overlooked is that most sugars are much less sweet than sucrose, and many are not sweet at all. Furthermore, our pursuit of low-calorie diet foods has increased our awareness of the fact that sweetness is in no way the prerogative of sugars: “saccharin” has become a household word.

As we shall see with other flavours, the quantitative measurement of the sweetness of a substance is peculiarly problematical. There are no laboratory instruments to

perform the task, and no absolute or even arbitrary units of sweetness, except perhaps, “one lump or two?” in a teacup. Instead, we have to rely on the human tongue and the hope that, if we average the findings of a large number of tongues, we can obtain useful data. The data we obtain will still not be in absolute units, but expressed relative to an arbitrary standard, usually sucrose. Table 7.1 gives relative sweetness data for a number of sugars and other substances. It can be a revealing exercise for students to devise for themselves procedures for establishing the relative sweetness of, say, glucose relative to sucrose. The degree of variation in the results between individual tongues can then be considered and a “class average” obtained to compare with the data in Table 7.1. Any practical work in this field should be carried out in the workrooms of the home economists, not chemistry laboratories.

A great deal of work has been devoted to identifying the molecular features of sweet-tasting substances that are responsible for the sensation of sweetness. One approach has been to survey as many sweet-tasting substances as possible to identify a common structural element. A second approach has been to prepare large numbers of derivatives of sweet-tasting substances in which potentially important groups have been either blocked or modified. This second approach led Shallenberger's group to propose, in 1967, a general structure for what they termed the “saporous unit”. By analogy with the chromophores of coloured substances (see Chapter 6, Section 6.10), the term “glycophore” has largely replaced saporous unit. They suggested the AH,B system shown in Figure 7.1, where A and B represent electronegative atoms (usually oxygen) and AH and YH denote the hydrogen-bonding capability.

It has been shown that, for sweetness, the distance between the electronegative atom B and the hydrogen atom H bound to A must be close to 3 Å (*i.e.* 0.3 nm). Not only can it be assumed that the corresponding groups on the surface of a receptor protein (X and YH) of the taste bud epithelial cells are a similar distance apart, but also that, if AH and B were any closer, then they would form an intermolecular hydrogen bond of their own rather than with a receptor protein.

Studies of numerous sugar derivatives have established that in aldohexoses, such as glucose, the 3,4- α -glycol structure provides the principal AH,B system. The conformation of the sugar ring is of crucial importance to the degree of sweetness. For the α -glycol AH,B system to have the correct dimensions, the two hydroxyl groups must be

Table 7.1 Relative sweetness values reported for sugars and sugar alcohols. As discussed in the text, these values must be regarded typical rather than absolute.

Sugar	Relative sweetness		Sugar alcohol	Relative sweetness	
	By weight	By molarity		By weight	By molarity
Sucrose	1.00	1.00	Xylitol	1.00	0.44
D-Fructose	1.52	0.80	Maltitol	0.90	0.90
D-Glucose	0.76	0.40	Sorbitol	0.60	0.32
D-Galactose	0.50	0.26	Isomalt	0.55	0.55
Lactose	0.33	0.33	Mannitol	0.50	0.27
Maltose	0.33	0.33	Lactitol	0.37	0.37
Trehalose	0.25	0.25			
D-Ribose	0.15	0.06			

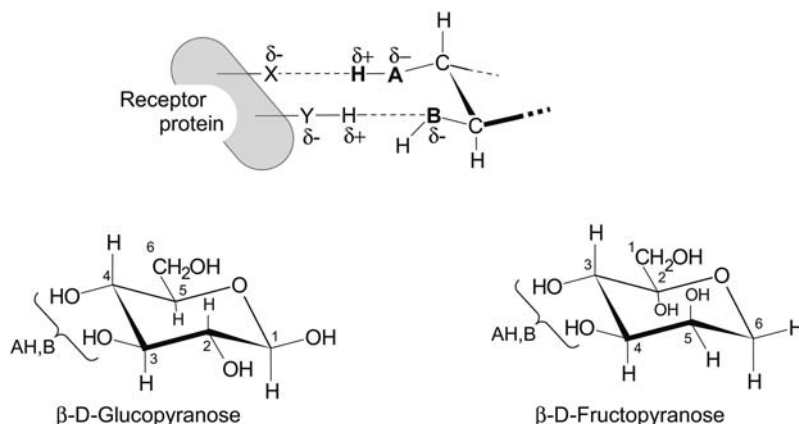


Figure 7.1 Shallenberger's "sorous unit". The recognised AH,B elements of glucose and fructose are also shown. Although not immediately obvious, this portrayal of β -D-fructopyranose is entirely compatible with that in Chapter 2, Section 2.4 (2.28); it is shown here from an unusual viewpoint.

in the *skewed* (or *gauche*) conformation. If the α -glycol is in the eclipsed conformation, then the two hydroxyl groups are sufficiently close for the formation of an intramolecular hydrogen bond, which excludes the possibility of interaction with a receptor protein. In the *anti* conformation, the distance between the hydroxyl groups is too great for the correct interaction with the receptor protein. Inspection of the conformation of $C1$ β -D-glucopyranose (see Chapter 2, Section 2.4) shows that all the hydroxyl groups attached directly to the ring (at C-1, C-2, C-3 and C-4) are potential members of sorous units. By contrast, $1C$ β -D-glucopyranose has all its α -glycol structures in the *anti* conformation, making the distances between the hydroxyl groups too large for binding to the receptor. If D-glucose could be persuaded to take up the $1C$ conformation (it can't), then we would predict that this form would not be sweet.

The presence of an AH,B system of the correct dimensions is clearly not the only factor. For example, the *axial* C-4 hydroxyl of D-galactose is able to form an intramolecular hydrogen bond with the ring oxygen, which explains why D-galactose is less sweet than D-glucose. The furanose form of β -D-fructose is believed to lack sweetness, which can be explained by the ease with which a hydrogen bond can form across the ring between the hydroxyl groups on C-2 and C-6. The observation that the disaccharide, trehalose, is not twice as sweet as D-glucose tells us that, although a sugar may have more than one sorous unit, only one of these at a time can actually bind with the receptor. It should not then be surprising that high molecular weight polymers of glucose, such as starch, are completely tasteless. The relative sweetness of a range of sugars is shown in Table 7.1.

There are clearly vast numbers of substances that contain an AH,B system of the correct dimensions, but which are actually tasteless, or even bitter. These include the L-amino acids, in which it is the ionised carboxyl and amino groups that could constitute a well-defined AH,B system. The fact that the corresponding D-amino acids are sweet led to the proposal of a third binding site, and with it the concept of a three-

dimensional receptor site. This third binding is assumed to involve a hydrophobic site on the sweet-tasting molecule, known as γ , and a hydrophobic region of the binding site on the receptor protein. Figure 7.2 shows the most probable dimensions of the receptor and shows how this principle might apply to leucine, only the D-isomer of which tastes sweet.

Of course, sugars are not the only substances with a sweet taste and we are becoming increasingly familiar with “non-sugar sweeteners”, which have the advantage of a sweet taste without the calorific value disadvantage of sugars. Some of these are chemical derivatives of sugars, such as xylitol and sorbitol, but others, such as saccharine and aspartame, are entirely the products of synthetic chemistry. A variety of other substances have been identified as the natural sources of the sweetness of various unusual, *i.e.* not widely consumed, food plants. Two sweet-tasting proteins have also been identified. The relative sweetness values of some of the important non-sugar sweeteners are shown in Figure 7.3. These values are inevitably rather variable, partly because they have to be measured subjectively by human subjects, but also because the sweetness of a substance in solution is not linearly related to its concentration. Thus, if one prepares solutions of different sweeteners that have equal apparent sweetness, diluting both solutions by the same factor does not necessarily result in two equally sweet solutions.

The AH,B and γ concept has been extended to include many of the non-sugar sweeteners shown in Figure 7.3. The differences in relative sweetness are ascribed to both closeness of fit in geometrical terms and also the strength of the hydrogen bonds and hydrophobic interactions at the γ site. Identifying the AH,B and γ sites within a molecule is not always certain. For example, many authorities still record the AH and B sites

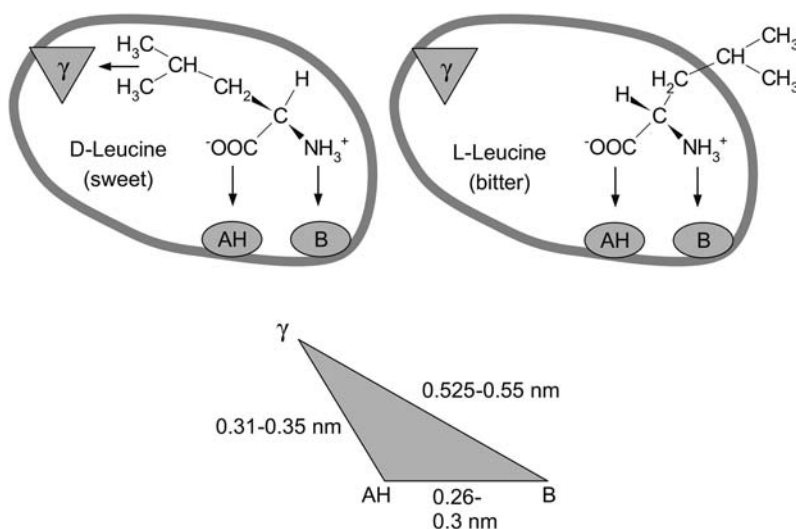


Figure 7.2 The third binding site (γ) proposed by Kier in 1972, as it affects the binding of D- and L-leucine. Opinions vary as to the exact dimensions of the triangle formed by AH,B and γ . It is assumed that other elements of the receptor site obstruct the L-leucine side-chain. Glycine, which has a weak sweet taste, has just a single hydrogen atom in place of the $(\text{CH}_3)_2\text{CHCH}_2$ group in leucine and can therefore gain access to the receptor site even though it does not bind to the γ site.

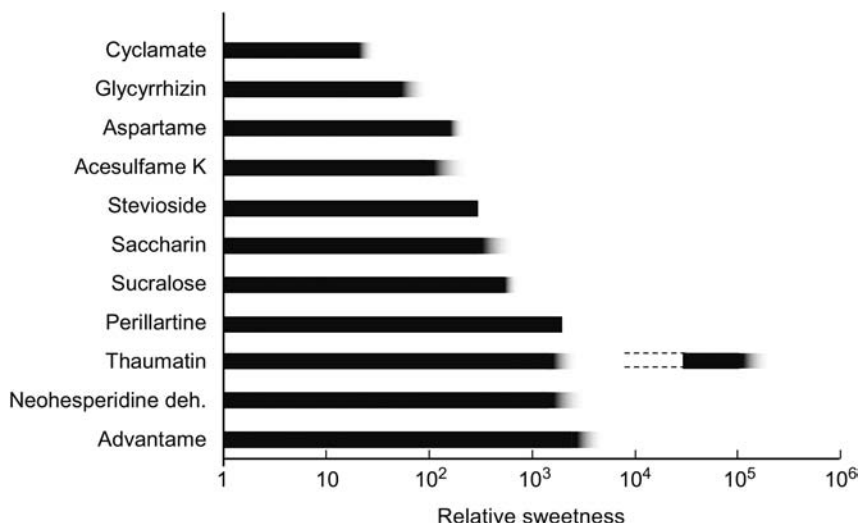


Figure 7.3 Relative sweetness of a range of non-sugar sweeteners. Sweetness is expressed on a logarithmic scale relative to sucrose (=1). Data are obtained by diluting a solution of the test substance until its sweetness is judged to be equal to that of a dilute sucrose solution and then relating its concentration to that of the sucrose standard. The blurring of most of the bars in this chart reflects the variations between the results from different laboratories. The apparent precision of the data for stevioside and perillartine is merely due to the shortage of published data. For the protein, thaumatin, the concentration ratios were also calculated on a molarity basis, giving the extremely high relative sweetness values plotted to the right.

of D-fructopyranose to be the C-2 and C-1 hydroxyl groups, although most favour C-3 and C-4, shown in Figure 7.1. The γ site appears to have much less significance in the sweetness of sugars than it does in the intensely sweet non-sugar sweeteners, some of whose chemical structures are shown in Figure 7.4.

For many years our understanding of the structural relationships between sweet-tasting substances and the receptor proteins that were assumed to occur in the membranes of taste bud cells did not advance beyond the AH,B concept discussed here. However, by the end of the twentieth century, new insights into mammalian molecular biology and genetics began to tell us much more. What has been learned applies to four of the basic tastes, *i.e.* sweetness, bitterness, sourness and meatiness (umami), and is outlined in the first of the Special Topics at the end of this chapter.

Sugar derivatives constitute an important class of sweeteners. Sugar alcohols, such as xylitol (2.18), sorbitol (2.19), mannitol (7.3), lactitol (7.4) and maltitol (7.5), are obtained from their parent mono- or disaccharides by hydrogenation. Xylitol was first produced commercially in Finland during the Second World War, when neither cane nor beet sugar was obtainable. The starting material, the hemicellulose xylan (see Chapter 3, Section 3.4), was originally obtained from birch wood, but is now obtained from cereal waste products such as corn cobs. After acid or enzymic hydrolysis, the resulting D-xylose is chemically hydrogenated to xylitol. In addition to some confectionery products, xylitol is frequently used to flavour toothpaste, mouthwash and pharmaceuticals.

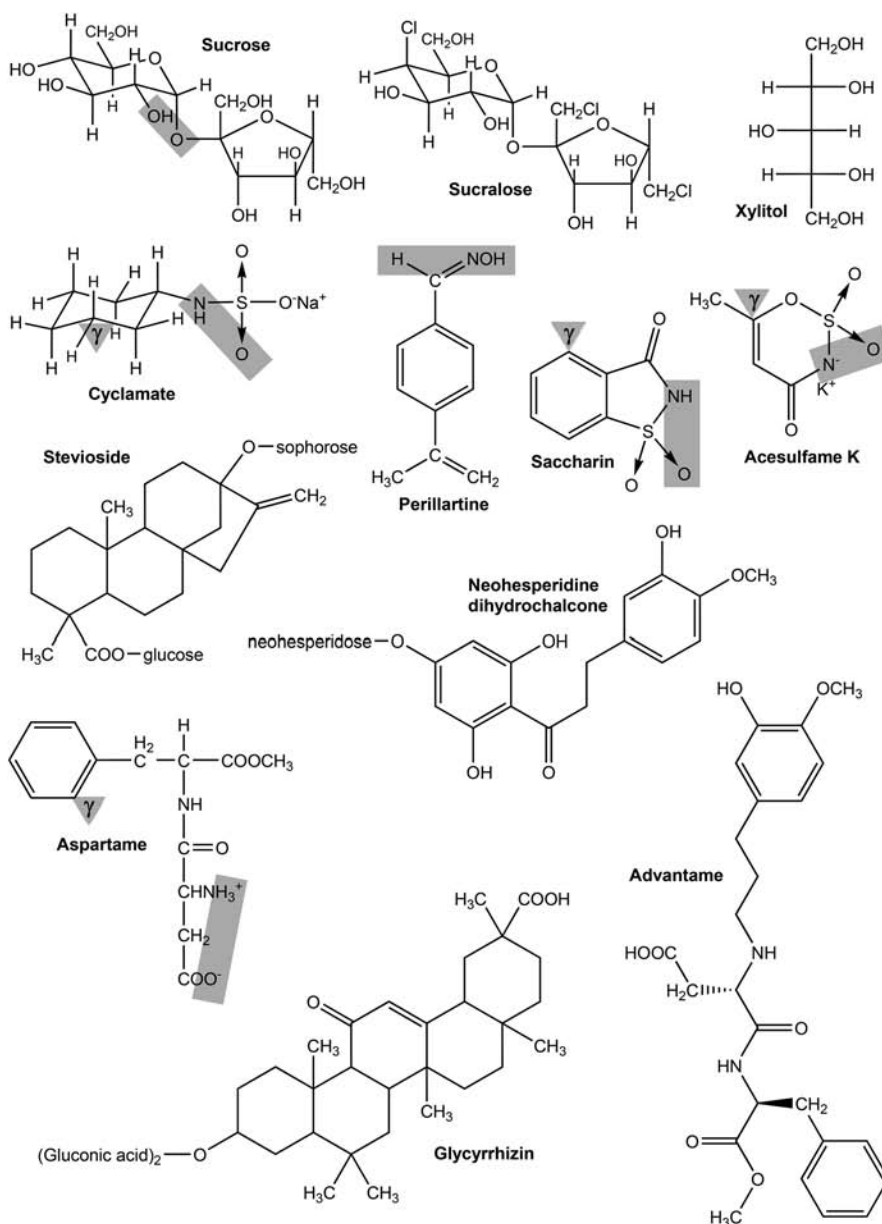
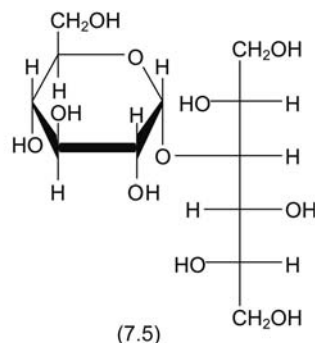
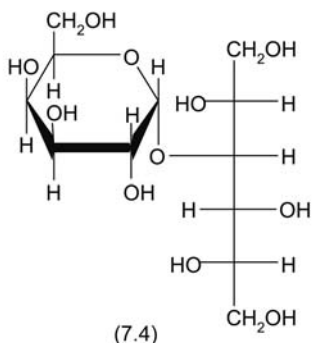
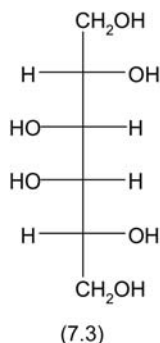
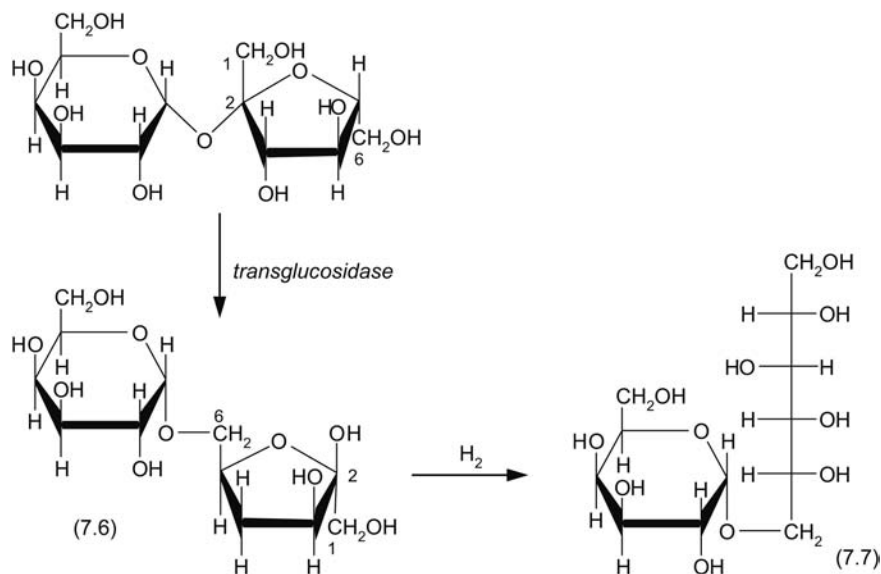


Figure 7.4 Chemical structures of non-sugar sweeteners, showing the AH,B and γ sites, where these have been assigned with reasonable confidence. The AH and B sites are shown by the grey bars, the γ sites by grey triangles. Neohesperidose is α -L-rhamnose-1,2- β -D-glucose; sophorose is 1,2- β -D-glucose-1,2- β -D-glucose.

It is particularly valuable for products aimed at consumers with diabetes because, unlike glucose, it does not require insulin for its uptake by muscle tissues, *etc*. A disadvantage is that it is only slowly taken up from small intestine and excessive consumption can lead to osmotic diarrhoea. Dogs mistakenly allowed treats containing xylitol absorb it extremely rapidly, with a concomitant rise in insulin levels and a potentially lethal fall in blood glucose levels (hypoglycaemia).



A related commercial commodity is isomalt. This is actually a mixture of 6- α -D-glucopyranosyl-(1,6)-D-sorbitol (7.7) and the corresponding mannitol derivative. It is manufactured in a novel process involving immobilised cells of the soil bacterium *Pro-taminobacter rubrum*. These cells carry an enzyme that is able to convert sucrose to its α -1,6 isomer isomaltulose (7.6), which is then chemically hydrogenated to give isomalt. Isomalt crystallises readily and is popular with commercial cake decorators and confectioners as a glazing agent and is a permitted food additive (E953).



Hydrogenated sugars are gaining importance as bulk, low-calorie sweeteners. In general, they are a little less sweet than sucrose. The term “bulk” refers to the fact that they can fill the other roles, besides sweetness, which sucrose fulfils in flour and sugar confectionery, but being largely unabsorbed by the small intestine they do not contribute to calorific intake. On the other hand, they do carry the water-binding properties of sugars into the large intestine and have the potential, if sufficient is consumed, to cause osmotic diarrhoea. (Isomalt shares the same positive and negative attributes.) They have often been used in the formulation of commercial confectionery products marketed as being suitable for people with diabetes, although medical authorities

disapprove of this approach to diabetes management. Various health issues, particularly obesity and diabetes, have been associated with high dietary sugar intakes by both medical professionals and the general public. Rather than simply reducing the overall sweetness of our diets by reducing our intake of sugar, we have, to some extent, turned to non-sugar sweeteners to maintain the taste of the manufactured foods that we have become accustomed to.

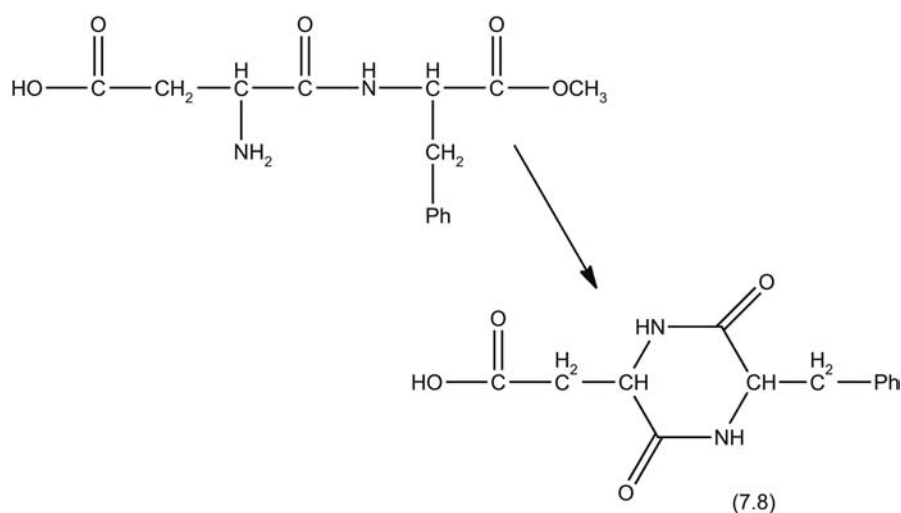
Non-sugar sweeteners have a much longer history than one might imagine. The attractive sweetness of saccharin was noticed within weeks of its first synthesis in 1878 and it was quickly exploited as a sweetening agent. By the turn of that century, about 200 tons were being produced annually by a German company and in 1901 the Monsanto Chemical Company was created in the USA to manufacture it. Shortages of sucrose during both world wars led to its widespread use in Europe, both in the home and in commercial products such as soft drinks. The advantages of saccharin include its stability to processing procedures and the essentially clean bill of health it has received from toxicologists. It is also inexpensive: in terms of sweetening power, only 2% of the cost of sucrose. To many consumers it offers the advantage of being a sweetener that contributes no “calories” either to the human diet or the diet of the bacteria that cause dental caries. Its main disadvantage is an unpleasant metallic aftertaste that often leads to it being blended with sucrose to reduce, rather than eliminate, the calorific value of a product.

More recently, several other non-sugar sweeteners have been introduced, some entirely the products of chemical synthesis, but others more natural in origin. Many of these are now permitted in many countries around the world, including those listed in Table 7.2. Two synthetic sweeteners, sodium cyclamate and acesulphame K (Figure 7.3), share some structural features with saccharin. Both have a superior taste profile to saccharin, lacking its metallic aftertaste, and retain all the other dietary advantages. However, cyclamate fell foul of a single carcinogenicity test in 1969 and was immediately banned in the USA, with many other countries following suit. Subsequent biological testing did not support this conclusion, however, and it has been reinstated in many countries around the world, although is still prohibited in the USA.

Like saccharin, the sweet taste of aspartame, L-aspartyl-L-phenylalanine methyl ester (see Figure 7.4), was discovered by chance. In the case of aspartame, it followed a lapse in laboratory hygiene on the part of a chemist working on a quite different problem. Aspartame has a clean, sucrose-like flavour, which has made it extremely popular with soft drink manufacturers. Its main drawback is its instability outside the pH range 3–6, especially at temperatures $>70^{\circ}\text{C}$. Its decomposition follows two distinct pathways: (i) simple hydrolysis to its components, aspartic acid, phenylalanine and methanol; and (ii) methanol loss, followed by cyclisation to a diketopiperazine (7.8). Aspartame’s instability at elevated temperatures makes it unsuitable for use in foods that are heated, either during commercial manufacture or preparation in the home. This rules out its use in baked or canned products and most confectionery. However, the other non-sugar intense sweeteners, although chemically stable, cannot make the essential contribution to the physical, *i.e.* textural, properties of cakes, confectionery, jam, *etc.*

Table 7.2 Non-nutritive sweeteners permitted for food use in Europe and elsewhere in 2022. The INS number for a particular food additive is identical to its E number, except in lacking the “E”. “Y” means that the sweetener is permitted.

	INS/E number	Europe (incl. UK)	USA	Canada	Australia and New Zealand
Sorbitol	420	Y	Y	Y	Y
Mannitol	421	Y		Y	Y
Acesulfame K	950	Y	Y	Y	Y
Aspartame	951	Y	Y	Y	Y
Cyclamate	952	Y			Y
Isomalt	953	Y	Y	Y	Y
Saccharin	954	Y	Y	Y	Y
Sucralose	955	Y	Y	Y	Y
Alitame	956				Y
Thaumatococin	957	Y	Y	Y	Y
Neohesperidin	959	Y	Y		
Steviol glycosides	960	Y			Y
Neotame	961	Y	Y	Y	Y
Maltitol	965	Y	Y	Y	Y
Lactitol	966	Y	Y	Y	Y
Xylitol	967	Y	Y	Y	Y
Erythritol	968	Y	Y	Y	Y
Advantame	969	Y	Y	Y	Y
Glycyrrhizinate		Y	Y		



Fortunately, the diketopiperazine has proved to present no toxicological problem, although aspartame itself has caused some concern. The initial attraction of aspartame was that its natural hydrolysis in the gastrointestinal tract would result in only products whose “naturalness” at reasonable levels could not be doubted. However, the possibility arose that the small proportion of people (about 1 in 10 000) who are affected by the inherited disease phenylketonuria might be at risk because their capacity to cope with even a small excess of phenylalanine is dangerously impaired. The quantities of phenylalanine derived from aspartame are tiny compared with the quantities liberated by the

ordinary digestion of dietary proteins and, in fact, the dietary regime for people with this disease is so exacting that avoiding food and drinks containing aspartame would be only a minor issue. The potential hazard to symptomless carriers of the defective gene has been shown to be insignificant.

Another potential problem for all consumers of aspartame is the methanol that is liberated when it is consumed. However, ordinary pectins, especially those in tomato juice, citrus juices and some vegetables, also contain methanol (see Chapter 3, Section 3.2). This is liberated during digestion in the gastrointestinal tract and passes into the bloodstream. The normal concentrations of methanol in distilled spirits, such as gin, brandy and whisky, are also significant, if low. However, even very high intakes of soft drinks (including “diet” drinks lacking any nutritive sugars) sweetened with aspartame do not appear to increase blood methanol concentrations to the levels associated with these supposedly “natural” sources. *Acceptable Daily Intake* (ADI) figures for the human consumption of aspartame, and other non-sugar sweeteners that have been established by the European Food Safety Authority (EFSA) and also by the US Food and Drug Administration (FDA) are shown in Table 7.3. Estimates of the mean intakes of aspartame vary widely, but are highest among children, where they may reach 40% of the ADI.

Sucralose is an intense sweetener, derived from sucrose, which is becoming increasingly popular in both soft drinks and confectionery, as well as for domestic use (see Table 7.2). As with many other intense sweeteners, it was discovered by accident. In 1976, chemists were exploring the possibility of using chlorinated sugar derivatives as intermediates in the synthesis of novel pesticides. A telephoned instruction to a research student to “test” what is now known as sucralose was misheard and instead it was “tasted”, revealing its intense sweetness. Although derived from sucrose by selective chlorination, in strictly chemical terms it is a galactose derivative in which the glucose ring has isomerised (see Figure 7.4). It has the great advantage of being completely stable under any conditions of temperature and pH likely to be encountered in culinary or food processing situations.

Advantame is the most recent intense sweetener to gain widespread acceptance (in 2013 in Europe, 2014 in the USA), reported to be at least 20 000 times the sweetness of sucrose. The industrial synthesis of advantame uses aspartame as one of its raw materials and, like aspartame, it releases phenylalanine when it is broken down

Table 7.3 Acceptable daily intakes for non-sugar sweeteners.

Sweetener	ADI (mg per kg body weight per day)	
	Europe (EFSA)	USA (FDA)
Advantame	5	32.8
Acesulfame K	9	15
Aspartame	40	50
Cyclamate	7	
Neotame	2	0.3
Neohesperidine DC	5	
Saccharin	5	15
Steviol glycosides	4	
Sucralose	15	5

in the gastrointestinal tract. However, because its relative sweetness is so high, the amount anyone is likely to consume is extremely small and the amount of free phenylalanine that it may contribute to the diet will not present any hazard to people with phenylketonuria.

A number of naturally occurring intense sweeteners have attracted attention. Although neohesperidine dihydrochalcone is not known to occur in nature, some of its close chemical relatives are the source of the strong sweet taste of a number of traditional food plants consumed around the world. It is obtained by the chemical conversion of naringin (7.10) (the source of the bitterness of grapefruit, *Citrus paradisi*) to neohesperidine, followed by hydrogenation to the chalcone. Neohesperidine can also be obtained directly from the peel of the bitter, or Seville, orange (*Citrus aurantium*), the fruit of choice for the manufacture of marmalade. As it is quite expensive, it is not usually used on its own in food, but has been found to enhance the potency of many other types of sweeteners. One unusual property is that it is able to mask the bitterness of some other substances, making it an attractive ingredient in some pharmaceutical products.

Glycyrrhizin (see Figure 7.4) is the natural sweet component of liquorice root (*Glycyrrhiza glabra*). Extracts containing glycyrrhizin are widely used in the Far East for flavouring and sweetening foods and drinks, medicines and tobacco. Liquorice-based confectionery is now less popular in the UK than it once was (*i.e.* in the authors' childhood). Glycyrrhizin is also known to have a range of less welcome biological activities. Although its anti-allergenic, anti-inflammatory and expectorant properties could be regarded as beneficial, its effects on kidney function and blood pressure certainly could not. For this reason, it is only permitted as a flavouring in confectionery and soft drinks where its total consumption is unlikely to be high, but not as a sweetening agent. The well-known laxative action of liquorice may or may not be attributable to glycyrrhizin, but this does ensure that its level of consumption is to some extent self-limiting.

Stevioside (see Figure 7.4) is the principal terpenoid glycoside extracted from the leaves of *Stevia rebaudiana*, a member of the sunflower family cultivated in some parts of the Far East and South America. Steviol glycosides are around 150–300 times sweeter than sugar and only a small amount is needed to match the sweetness of regular sugar. Extracts containing stevioside are approved for food use in some countries, but reservations about its safety (like glycyrrhizin, it has a wide range of biological activities) mean that it has only recently been widely permitted – for example, only since 2011 in the European Union. In the USA, it has been awarded Generally Recognised As Safe (GRAS) status.

Sweetness is not the prerogative of relatively small molecules. Thaumatin is the best known example of a sweet-tasting protein, with a molecular weight of 22 000. It is intensely sweet, especially if compared with sucrose on a molecule for molecule basis (see Figure 7.3). It is obtained from the fruit of a West African tree, katemfe (*Thaumatococcus daniellii*), and is marketed under the name Talin™. It is unusually resistant to thermal denaturation, making it suitable for cooked products, but is totally hydrolysed to amino acids by the human digestive system. It has been approved for use in a number of countries, including the USA and the EC, but is more likely to find application as a flavour enhancer than as a sweetener in its own right.

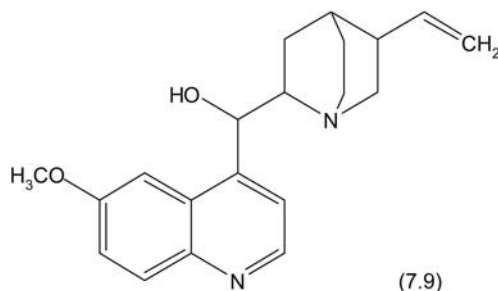
Monellin is another sweet-tasting protein that has been studied extensively. It is a remarkably small protein, consisting of two polypeptide chains with a total of only 94 amino acids. It occurs in the fruit of a West African shrub, *Dioscoreophyllum cumminsii*, that presumably acquired its common name, “serendipity berry”, from English-speaking colonialists. It has been chemically synthesised and is variously reported as being 800–4000 times sweeter than sucrose. Japan remains the only country where it is accepted as a permitted food additive.

Miraculin is the only other protein that has received much attention that *might* be described as sweet-tasting. It is also found in the fruit of a West African shrub, *Synsepalum dulcificum*, the miracle berry. The fruit, and purified miraculin, do not have a noticeably sweet taste. However, once the taste buds of the tongue have been exposed to miraculin, acidic foods and drinks lose their sour taste and are discerned to be sweet. This effect can last for up to an hour. At best, this effect can be regarded as a pleasant novelty, but it is difficult to envisage any useful application. Nevertheless, studies of the activity of miraculin at the molecular level are providing useful insights into the behaviour of the sweetness receptor proteins.

7.1.2 Bitterness

The apparently contrasting tastes of bitterness and sweetness have been linked together by the poetically and/or romantically inclined for centuries. However, this linkage at the emotional level is also supported at the chemical level. For the food scientist, the two are often regarded as opposite sides of the same coin. Several types of substance that taste bitter have, as we shall see, a close structural relationship to sweet-tasting substances. Inorganic salts are a notable exception; most of these have a very bitter taste and, as discussed in the next section, relatively few taste salty.

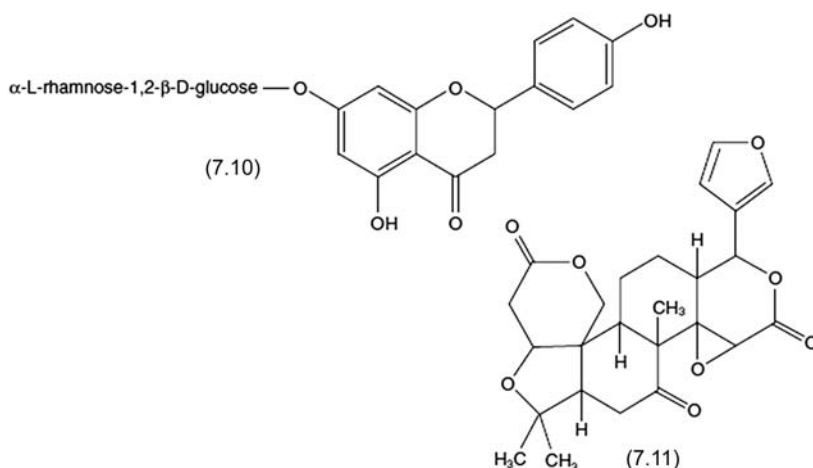
Bitterness is often a warning of potential toxicity. The microbial breakdown of food materials often results in the generation of bitter-tasting substances, and many plant toxins have a bitter taste. Although it may be unwise to generalise, it may be significant that the human taste threshold for naturally occurring bitter substances is typically very much lower than that for naturally occurring sweet-tasting substances. For instance, the threshold in the case of quinine (7.9) is only $25 \mu\text{mol dm}^{-3}$, compared with $10\,000 \mu\text{mol dm}^{-3}$ for sucrose. Bitterness is also a defining characteristic of the alkaloids, whose toxicity is discussed in Chapter 10.



The ability to perceive bitterness has almost certainly evolved to protect humans, and presumably other animals, from the dangers posed by the alkaloids present in many

plants. Alkaloids are organic compounds containing a basic nitrogen atom in a heterocyclic ring. Many alkaloids have profound pharmacological effects on animals, in addition to their extremely bitter taste: nicotine, atropine and emetine are all alkaloids. Thanks to its traditional medicinal use (as an antimalarial drug), quinine is one of the best-known alkaloids, now widely used to impart a mild bitter element to the taste of soft drinks, such as bitter lemon and tonic water.

Phenolic substances in the form of flavonoids are important sources of bitterness in fruit, particularly in citrus juices. The best known is naringin (7.10), a glycoside of the flavanone naringenin with the disaccharide neohesperidose, which occurs in grapefruit and Seville oranges. Its bitterness is such that it can be detected at a dilution of 1 in 50 000. Another bitter substance in citrus juices is limonin (7.11), sometimes formed from tasteless precursors during commercial juice extraction.



Bitterness is a sought-after characteristic in many types of beer, particularly those brewed in the UK. Bitterness is achieved by adding hops to the wort, the sugary extract from the malt, when it is boiled and then cooled prior to the actual fermentation. Hops are the dried flowers of the hop plant, *Humulus lupulus*, and they are rich both in the volatile compounds that give beer its characteristic odour, and in resins that include the bitter substances. The most important bittering agents in beer are the so-called α -acids. As shown in Figure 7.5, these isomerise during the “boil” to give forms that are much more soluble in water and much more bitter. The different varieties of hops contain different proportions of the three α -acids and the structurally related, but less important, β -acids.

Until recently, the bitter taste of many amino acids and oligopeptides was of only academic interest. However, the need for the more efficient use of the proteins in whey, waste blood, *etc.*, has focused attention on the properties of the peptides obtained by partial hydrolysis of these proteins by enzymes or acids. These protein hydrolysates are being developed as highly nutritious food additives, with many useful effects on food texture. Examination of the tastes of the amino acids in protein hydrolysates shows that bitterness is exclusively the property of the hydrophobic L-amino acids valine, leucine, isoleucine, phenylalanine, tyrosine and tryptophan. Whether a peptide is bitter or not depends on

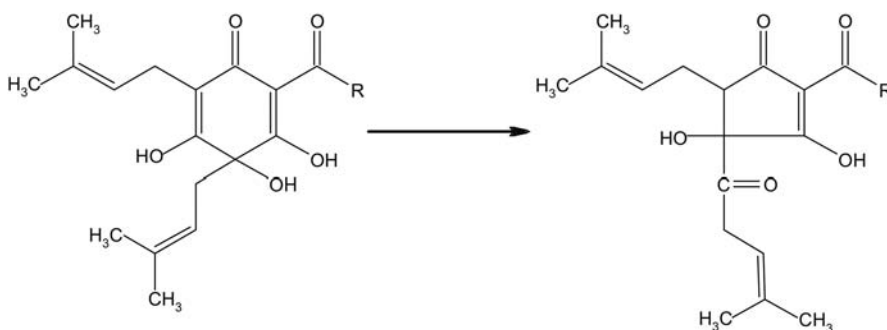


Figure 7.5 Isomerisation of the α -acids in hops. R is $(\text{CH}_3)_2\text{CHCH}_2-$ in humulone, $(\text{CH}_3)_2\text{CH}-$ in cohumulone and $\text{C}_2\text{H}_5(\text{CH}_3)\text{CH}-$ in adhumulone.

the average hydrophobicity of its amino acid residues. A great deal of work on the conformation of peptides in relation to their taste has demonstrated that the structural requirements for bitterness mirror those required for sweetness in sugars and other compounds. There is a similar requirement for a correctly spaced pair of hydrophilic groups (AH and B in sugars), one basic and one acidic, together with a hydrophobic group, in the correct spatial relationship to the hydrophilic groups. The ability to predict the probable taste of a peptide can be linked to our knowledge of amino acid sequences, to predict the appearance of bitter, and therefore unwelcome, peptides in the hydrolysates of particular proteins. For example, caseins and soya proteins are rich in hydrophobic amino acids and therefore tend to yield bitter peptides. One possible solution is to ensure that the hydrolysis is limited to producing fragments with molecular weights above about 6000, which are too large to interact with the taste receptors.

In terms of chemical structure, close relatives of many non-sugar sweeteners are found to taste bitter. One example is the isomer of aspartame in which the L-phenylalanine residue is replaced by D-phenylalanine. A comparison of the structures of three other intense sweeteners and their bitter-tasting analogues is shown in Figure 7.6. Since there is, at best, only a limited market for novel bittering agents, the most likely outcome of these observations is a greater insight into the structure and function of the sweet taste receptor proteins.

7.1.3 Saltiness

Saltiness is most readily detected on the sides and tip of the tongue and is elicited by many inorganic salts besides sodium chloride. However, the taste of salts other than sodium chloride has very little relevance to food. The contribution of sodium chloride to food flavour is often underestimated, as anyone who has experienced the awful taste of bread made without salt will testify. We frequently add salt to both meat and vegetables during cooking to enhance their flavour,ⁱⁱ but the sodium ions naturally present in many foods have a major role in their flavour. Besides this tendency to stimulate meaty

ⁱⁱGourmet chefs lay great stress on the “seasoning” of their creations, by which they appear to mean the addition of “just the right amount” of sodium chloride.

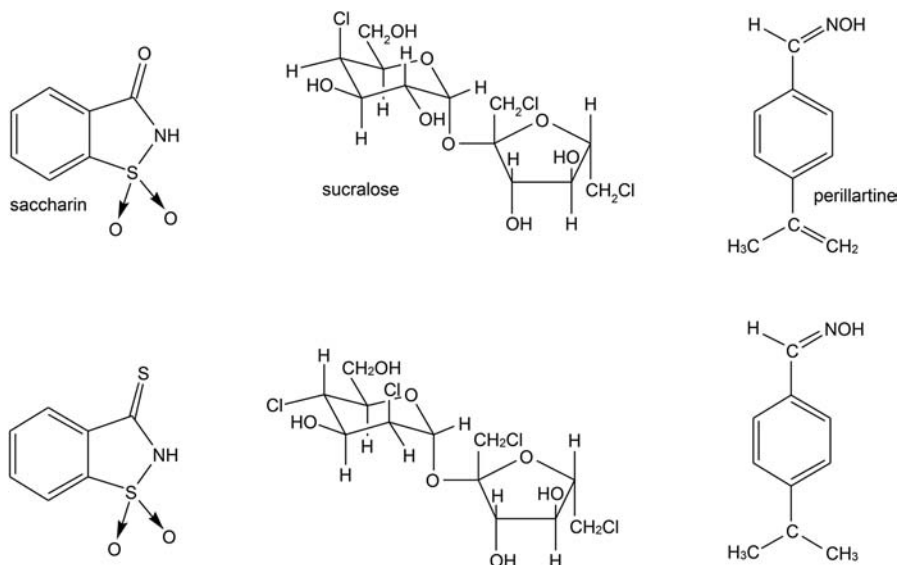


Figure 7.6 Chemical structures of three intense sweeteners compared with their bitter-tasting analogues (unnamed).

flavours, salt tends to decrease the sweetness of sugars, to give the richer, more rounded flavour required in many confectionery products.

As mentioned in the previous section, inorganic salts tend to taste either bitter or salty. Studies with the alkali metal halides have suggested that the structural criterion that distinguishes saltiness from bitterness is simply a matter of size. Where the sum of the ionic diameters is below that of potassium bromide (0.658 nm), a salt that tastes both salty and bitter, a salty taste predominates. Sodium chloride, at 0.556 nm, is an obvious example; potassium iodide, however, at 0.706 nm, merely tastes bitter. Magnesium salts, such as the chloride, MgCl_2 (0.850 nm), also have a bitter taste.

Our response to saltiness, like that to sourness, is very dependent on the concentration. From a biological point of view this is hardly surprising. Monitoring and controlling the concentration of sodium ions is an essential activity for cells and tissues throughout the body and the ability to assess salt concentration is in no way unique to the tongue. In as far as it is presently understood, the actual mechanism by which the cells of the tongue detect saltiness is dealt with in the first of the Special Topics at the end of this chapter.

In most foods, the sodium chloride that we encounter is already in solution, but this is not true of many snack foods. The salt, often mixed with other flavourings, is applied to the snack product – for example, potato crisps (chips in the USA) – as dry crystals. Nutritionists have recognised that the salt contained in snack foods makes a significant contribution to the sodium content of the diet and the consequential elevation of blood pressure. For consumers, a simple reduction of the salt content would have an unacceptable impact on their flavour, but an alternative strategy has been suggested. The impact of the salt taste lasts for only a few seconds in the mouth as saliva dissolves and then dilutes the salt. Reducing the size of the salt crystals, from about 200–500 μm

to 5–10 μm , allows a reduced amount of salt to deliver an equally strong burst of salt flavour because the smaller crystals dissolve so much quicker. Sadly, this approach will not succeed in any food system where the salt is in solution before it reaches the mouth.

Other aspects of the role of salt in food are dealt with elsewhere in this book: as an antimicrobial preservative in Chapter 9 and its contribution to elevated blood pressure in Chapter 11.

7.1.4 Sourness

Sourness is a property of solutions of high acidity, *i.e.* low pH. Although from time to time it has been suggested that it is the undissociated forms of ethanoic acid, citric acid, *etc.* that occur in fruit juices, vinegar and pickles, the current view remains that the taste-sensitive cells of the taste buds are responding to the concentration of hydrogen ions, H^+ .ⁱⁱⁱ The mechanism(s) by which hydrogen ions are tasted are not fully established, but, as with salt taste, are likely to reflect mechanisms elsewhere in the body where the pH of body fluids is monitored.

In most fruit and fruit juices, citric acid (7.12) and malic acid (7.13) account for almost all the acidity. Tartaric acid (7.14) is characteristic of grapes, blackberries are especially rich in isocitric acid (7.15) and rhubarb is, of course, well known for the presence of oxalic acid (7.16), which occurs together with substantial amounts of malic and citric acids. Some typical organic acid contents of citrus and grape juices are shown in Table 7.4

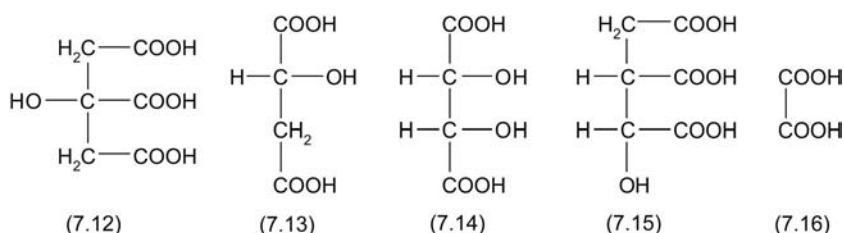


Table 7.4 Organic acid content of some fruit juices. The values here are typical, but wide variations occur between different varieties and are particularly influenced by the degree of ripeness. Grapes grown in relatively cold climates such as northern Europe, and the wines produced from them, have much higher acid content than those from warmer, sunnier climates.

	Acid content (mmol dm^{-3})		
	Malic	Citric	Tartaric
Orange	13	51	—
Grapefruit	42	100	—
Lemon	17	220	—
Grape	7	16	80

ⁱⁱⁱIn aqueous solutions, hydrogen ions are hydrated to form hydronium ions, H_3O^+ , so that, strictly speaking, it is these whose role in sour taste is under consideration here.

Ethanoic (acetic) acid, CH_3COOH , at levels around 5%, gives vinegar and the products derived from it, their sourness. However, in many pickled products, including pickled cabbage and cucumbers, it is lactic acid, derived from the sugar in the vegetables by bacterial fermentation, which is responsible for the low pH and sourness. One should not overlook the contribution that acetic acid makes to the odour of foods – for example, when fried fish and chips are dowsed in vinegar. Lactic acid, derived from lactose by fermentation, occurs in cheese at about 2%, contributing some of its sharpness. In the form of its sodium salt, lactic acid is also used to give the vinegar taste to “salt and vinegar” flavoured potato crisps.

7.1.5 Astringency

Astringency is a sensation clearly related to bitterness, but is registered throughout the oral cavity as well as on the tongue. We tend to register astringency in terms of “mouth feel” and, although it may therefore be incorrect to regard this sensation as a taste, we should not let semantics spoil our appetite. The reaction between polyphenolic substances and enzyme proteins was mentioned in Chapter 6 (Section 6.5) and it is believed that the sensation of astringency results from similar reactions involving proteins in the saliva, or possibly on the surface of the tongue, palate, *etc.*

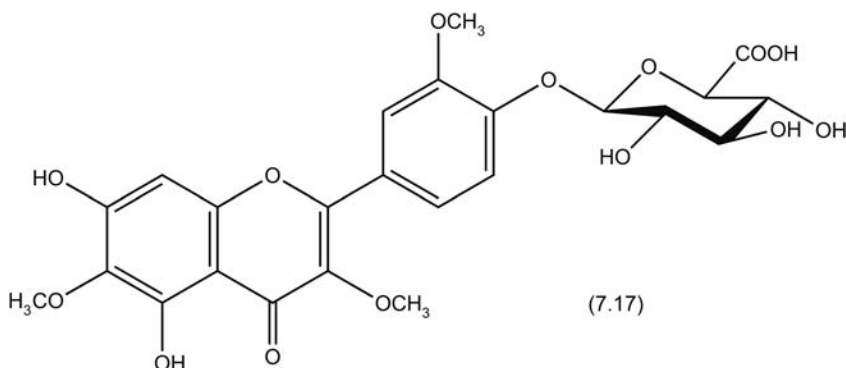
Although an undesirable characteristic in many food situations, a degree of astringency is usually regarded as a desirable characteristic of fruit, and also in cider. However, it is in red wine and tea that it is most important. In both these beverages astringency is associated with the high content of polyphenolic compounds, collectively described in this context as tannins, that are also involved in giving them their colour (as described in Chapter 6). In black tea, the polyphenolics are generally considered to contribute some of the astringency, but it is the gallyl groups of the epigallocatechin gallate (see Figure 6.12) that are primarily responsible. Epigallocatechin itself is merely bitter, with no discernible astringency, whereas the astringency of epicatechin gallate becomes apparent at concentrations >50 mg per 100 mL, a level comparable with that in normally brewed tea. There is some evidence that it is the interaction between the gallyl groups and caffeine (which alone is merely bitter) that produces astringency.

When we drink black tea, we usually feel the need to moderate its astringency. Two techniques are available, the choice being determined by custom and geography. In the USA, and in other countries where coffee rather than tea is the primary source of caffeine, lemon juice is often added to tea. This lowers the pH, repressing the ionisation of the gallate carboxyl groups and reducing the astringency. The alternative approach, deeply ingrained in the culture of the British Isles, is to add milk. The milk proteins successfully compete for the polyphenolics with the proteins lining the mouth. This technique is limited by the availability of pasteurised milk. The more aggressive heat treatments, such as UHT or sterilisation, generate flavour compounds (presumably from the Maillard reaction) and these clash with the delicate flavour of tea, with unpleasant results. Coffee, whose flavour is more dependent on Maillard reaction products, is less sensitive to the heat treatment of any milk that is added to it.

In wine, the situation is more complex because there are many more elements in the flavour, but all the evidence suggests that it is flavonoids similar to those found in

tea that are responsible for the astringency. In addition to their obvious anthocyanin content, which does contribute a little to the taste, red wines may contain up to 800 mg dm^{-3} of catechins (as in Figures 6.12 and 6.17), compared with no more than 50 mg dm^{-3} in white wine. The mellowing of the taste of red wine as it ages has been shown to be the result, in part, of the polymerisation of the proanthocyanidins (see Figure 6.17).

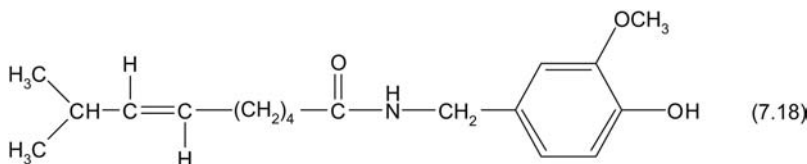
Astringency is a desirable property of some vegetables, notably spinach, *Spinacia oleracea*. A number of glycosides and glucuronides have been implicated in the astringent taste, including the example of a glucuronide shown here (7.17).



7.1.6 Pungency

Pungency, sometimes referred to as “hotness”, is another sensation experienced in the entire oral cavity and elsewhere, notably the anal mucosa. It is now common to link pungency with astringency, together with the cooling effect of certain compounds, under the general heading “chemesthesis”. This is analogous to “somesthesis”, the sensory system in the skin that responds to mechanical stimuli such as touch. The effect of chemesthetic stimulants may be better described as chemical irritation and has much in common with the perception of pain.

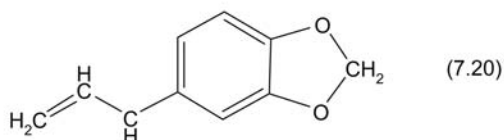
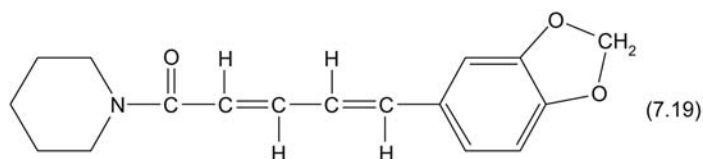
Pungency is the essential characteristic of a number of important spices, but it is also found in many vegetables in the Family Brassicaceae (previously known as the Cruciferae), which includes the genus *Brassica*. The genus *Capsicum* includes the red and green chillies, those from the larger *Capsicum annuum* being much less pungent than those from the ferocious little fruit of *Capsicum frutescens*. The active components in species of *Capsicum* are known as capsaicinoids. The most abundant and most pungent of these are capsaicin (7.18) and also dihydrocapsaicin.



Since 1912, when the scale was first devised, the relative pungencies of different varieties of chillies have been reported in Scoville heat units (SHU). The Scoville scale is entirely empirical, being based on the response of panels of testers. A known weight of

dried pepper is extracted with ethanol, which is then diluted with water and the average minimum concentration that is detectably hot is then used to position the pepper on the Scoville scale. The results are neither a precise nor accurate measure of the capsaicin concentration (1 SHU corresponds approximately to $18 \mu\text{g g}^{-1}$ of pure capsaicin), but provide a useful method for comparing the strengths of different peppers, as illustrated in Table 7.5. High-performance liquid chromatography has largely replaced taste panels for the actual determination of the relative strength of peppers, but the results are still referred back to Scoville units.

Pepper, both black and white, comes from *Piper nigrum*. The pungent component is piperine (7.19), amounting to about 5% of the peppercorn, together with traces of less pungent isomers that have one or both of the double bonds in the *cis* form. From time to time, concern has been expressed about the possible carcinogenicity of piperine. This is due to its close structural similarity to saffrole (7.20), a major component of sassafras oil, which is now recognised to be carcinogenic. In the past, sassafras oil was used in beverages such as “root beer”,^{iv} a non-alcoholic drink popular in the USA.



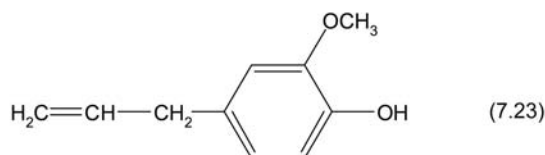
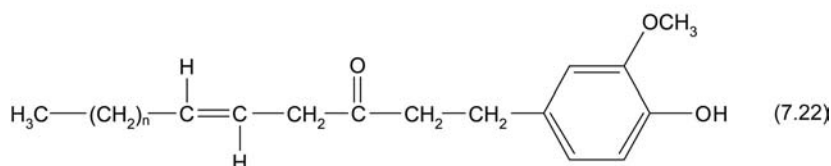
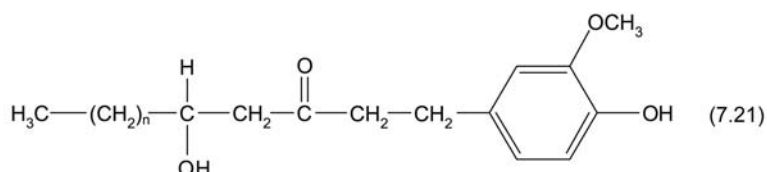
Gingerols (7.21) and shogaols (7.22), which show a striking structural similarity to capsaicin and piperine, are the active components of another pungent spice, ginger (*Zingiber officinale*). Gingerols and shogaols where $n = 4$ predominate, but higher homologues are present in small amounts. Ginger is sold in a number of different

Table 7.5 Relative strength of different species and varieties of *Capsicum*. Pure capsaicin and dihydrocapsaicin are rated at 16 000 000 and 9100 000 Scoville units, respectively.

Capsicum species	Popular name	Scoville units
<i>C. chinense</i> var. <i>Jacquin</i>	Habanero	100 000–350 000
<i>C. chinense</i>	Scotch bonnet	100 000–325 000
<i>C. baccatum</i> and <i>C. frutescens</i>	Cayenne pepper	30 000–50 000
<i>C. frutescens</i>	Tabasco pepper	30 000–50 000
<i>C. annuum</i>	Jalapeño	2500–5000
<i>C. annuum</i>	Pimento	100–500

^{iv}Traditionally made using the root bark of the sassafras tree, *Sassafras albidum*, or the vine of *Smilax ornata* (sarsaparilla) as the primary flavour. In the UK, the once popular “dandelion and burdock” soft drink was similarly manufactured using fermented dandelion (*Taraxacum officinale*) and burdock (*Arctium lappa*) roots. Modern versions of these drinks are flavoured artificially.

forms, *e.g.* green root ginger and dry powdered ginger, and these differ in the proportions of gingerols and shogaols that results from the readiness with which gingerols dehydrate to the corresponding shogaols. The vanillyl side-chain is also found in eugenol (7.23), the pungent component of cloves. In addition to its culinary applications, ginger has a long history of use as a medicine, especially in Asia and the Far East. Readers interested in this aspect of ginger will find the review by Baliga *et al.*^v a useful introduction.



The final group of pungent food components to be considered are the glucosinolates, found in plants belonging to the Family Brassicaceae, which includes the brassicas (*e.g.* cabbage, Brussels sprouts, broccoli) and many other plants renowned for their pungency when raw, such as horseradish, black and white mustards, and radish. Glucosinolates are usually found in these plants at levels up to about 2 mmol (or about 2 g) kg⁻¹ fresh, with the highest amounts in the most pungent species or varieties. The glucosinolates are not themselves pungent, but all the plants in which they occur also contain the enzyme myrosinase. This is present in the cytoplasm of the cells, but it only encounters its natural substrates, the glucosinolates, when the plant tissue is disrupted when we, or grazing animals, chew it. This also happens if the plant suffers insect or other damage in the field, or when it is chopped up in the kitchen.

As shown in Figure 7.7, myrosinase catalyses the hydrolysis of the unusual carbon-sulfur bond, liberating thiohydroxamate-*O*-sulfates. These compounds are unstable and, depending on the conditions, spontaneously form isothiocyanates or nitriles. The volatile isothiocyanates predominate and it is these that provide the pungent taste. Isothiocyanates are collectively referred to as mustard oils. The glucosinolates found

^vM. S. Baliga, *et al.*, Update on the chemopreventive effects of ginger and its phytochemicals, *Crit. Rev. Food Sci. Nutr.*, 2011, 51, 499.

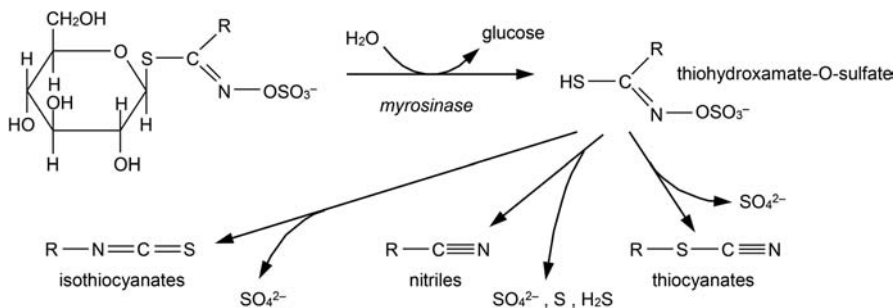
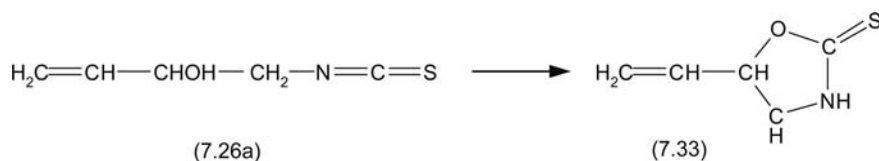


Figure 7.7 Breakdown of glucosinolates to form isothiocyanates and other products. Nitrile production is favoured by low pH, as encountered, for example, in the production of sauerkraut.

in various cultivated members of the Family Brassicaceae are shown in Figure 7.8. In the cases of sinigrin and glucotropaeolin, the thiocyanates are formed by the enzymic isomerisation of isothiocyanates.

Nitrile formation is a significant issue in the utilisation of rapeseed meal, the residue remaining after the extraction of rapeseed oil. Another problem with rapeseed meal is the tendency of isothiocyanates that have a hydroxyl group in the β -position, such as that derived from progoitrin (7.26a), to cyclise spontaneously to oxazolidine-thiones (7.33). These compounds, known as goitrins, interfere with iodine uptake by the thyroid gland, resulting in goitre, *i.e.* the massive enlargement of the thyroid gland and deficiency of the hormone thyroxine. For these reasons, plant-breeders have made great efforts to develop varieties of rape with very low levels of glucosinolates. The oil from these varieties, known as “canola oil”, is also low in erucic acid (see Chapter 4, Section 4.1). These efforts of plant-breeders are not without their downside because the breakdown products of glucosinolates are known to be important in defending plants against insect and fungal pathogens.



The suggestion that milk from cattle fed largely on rapeseed meal and other brassicas might be harmful need not be taken seriously. The only time goitrins have proved to be a problem for human consumers was during the winter of 1916–1917 in Germany. Food shortages during the First World War led to turnips (*Brassica rapa*) becoming a staple food, replacing both potatoes and cereals. Combined with the other effects of malnutrition, the incidence of goitre rose sharply.

The lower incidence of cancer in populations whose diet contains a high proportion of fruit and vegetables is now well recognised, and at least some of the beneficial effects, especially in relation to cancers of the colon, can be related to the consumption of glucosinolates from cruciferous vegetables. In experiments with laboratory animals, and also using tissue cultures of human cells, allyl isothiocyanate (7.34) (from sinigrin) and

Principal or characteristic glucosinolates

Cabbage	<i>Brassica oleracea</i>	sinigrin (7.24), glucobrassicin (7.25), progoitrin (7.26)
Brussels sprouts	<i>B. oleracea</i> var. <i>gemmifer</i>	glucobrassicin, neoglucobrassicin (7.27)
Broccoli	<i>B. oleracea</i> var. <i>italica</i>	glucobrassicin, glucoraphanin, (7.28)
Cauliflower	<i>B. botrytis</i> var. <i>cauliflora</i>	sinigrin, glucobrassicin, neoglucobrassicin
Black mustard	<i>B. nigrum</i>	sinigrin
White mustard	<i>B. alba</i>	sinalbin (7.29)
Rape	<i>B. napus</i>	progoitrin, glucobrassicin, neoglucobrassicin
Horse-radish	<i>Amoracia lapathifolia</i>	sinigrin
Radish	<i>Raphanus sativus</i>	glucoraphasatin (7.30)
Watercress	<i>Nasturtium officinalis</i>	gluconasturtiin (7.31)
Cress	<i>Lepidium sativum</i>	glucotropaeolin (7.32)

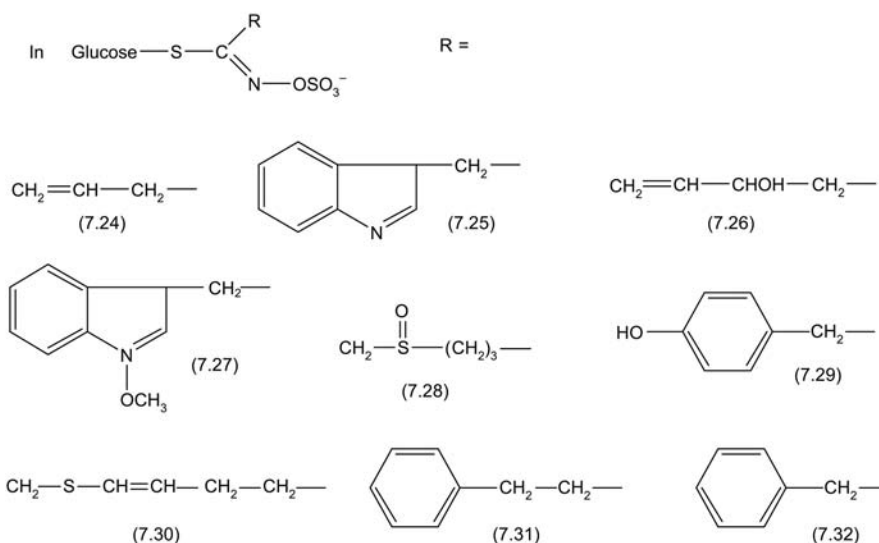
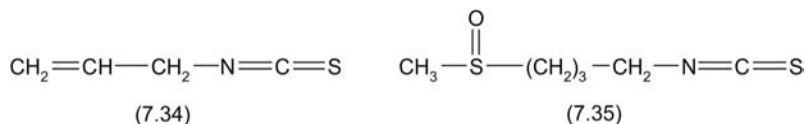


Figure 7.8 Glucosinolates found in members of the Brassicaceae family.

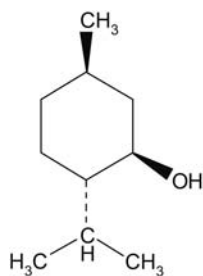
sulforophane (7.35) (from glucoraphanin) have been shown, at realistic concentrations, to kill cancerous cells. Sulforophane appears to increase the production of glutathione transferases, a group of enzymes involved in the neutralisation of certain carcinogens.



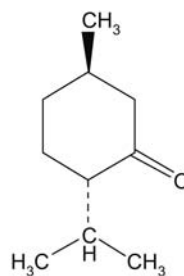
When vegetables belonging to the Family Brassicaceae are cooked, ideally by pouring boiling water over them, followed by a few minutes at 100 °C, the myrosinase is destroyed before tissue disruption brings the enzyme and substrate into contact and isothiocyanates are not formed. Instead, a wide range of other sulfur compounds arise and are responsible for giving over-boiled cabbage and other similar vegetables their characteristic, and unattractive, odour. The difference in flavour, after cooking, between fresh Brussels sprouts and those previously frozen, has been shown to reflect the

different rates of myrosinase destruction caused respectively by commercial blanching (always a precursor to commercial freezing) and domestic cooking.

The sensation of cooling is a minor taste response mentioned here simply because, like pungency, it is another manifestation of chemesthesis and is the natural opposite of heat. Cooling sensations are associated with some confectionery and also products such as toothpaste and mouthwash. Just as the burning taste of pungent substances is believed to be registered by similar nerve endings to those responding to high temperatures, the cooling taste is assumed to invoke a response in the nerves that register low temperatures. The best-known cooling substance is (–)-menthol (7.36). It is the dominant component of peppermint oil, extracted from *Mentha piperita* and *Mentha arvensis*, used in confectionery, the other major component being menthone (7.37). Menthone and the optical isomers of (–)-menthol all share the characteristic minty odour, but it is only (–)-menthol itself that produces a cooling sensation in the mouth. Some other substances, notably xylitol (1.18), can also produce a cooling effect, but this is usually attributed to their large negative heat of solution. When the solid xylitol included in chewing gum dissolves in saliva, its temperature briefly falls.



(7.36)



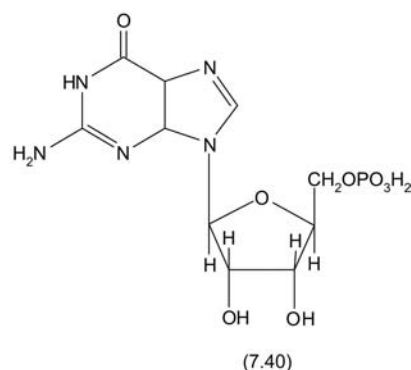
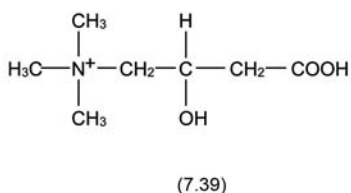
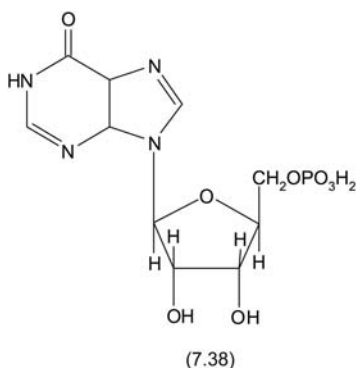
(7.37)

7.1.7 Meatiness

Of all the taste classes, the most recent to attract the attention of scientists, as well as food manufacturers, chefs and consumers, is meatiness. Although the flavour of meat is obviously an amalgam of our responses to both volatile and non-volatile compounds, a meaty taste has come to be associated with two particular compounds, inosine monophosphate (IMP, 7.38) and monosodium glutamate (MSG, 7.39). Both substances were first identified as active components in food ingredients used to enhance the flavour of traditional Japanese dishes. The Japanese word うま味 (pronounced *umami*) is applied to the particular taste of these two compounds and others related to them. The literal translation of *umami* is “delicious taste”, but it also carries the implication of meatiness, savoury or broth-like. Food writers, television chefs, *etc.* have familiarised the general public with the concept and the word *umami* has found a place in the English language in its own right.

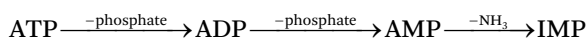
MSG was identified in 1908 as the source of the taste of the seaweed *konbu*, used to make a broth popular in Japan. Its commercial extraction and its use as a food

seasoning followed almost immediately, but it is now manufactured by a bacterial fermentation process. A few years later, IMP was isolated from a dried fish preparation (bonito flakes, made from skipjack tuna), also widely used in Japanese cooking. The fermented fish sauces used in Ancient Rome (known at the time as *garum* or *liquamen*), and still popular in the Far East, are essentially umami seasonings, rich in both MSG and ribonucleotides. Fish muscle was at one time the source for commercial preparations of all the 5'-nucleotides, but enzymic hydrolysates of RNA are now the usual source.



Alone, neither MSG nor IMP has a particularly strong taste. They need to be at a concentration above about 300 mg dm^{-3} before they begin to make much impact. However, when present together they show a remarkable synergy. A mixture of equal proportions of IMP and MSG tastes about 20 times stronger than a similar weight of one alone. Although the mechanism of this synergism is not understood, its importance to food manufacturers is obvious. The addition of only a small amount of one of these substances (usually MSG) to a food product that naturally contains even modest amounts of the other can have a dramatic effect on the flavour. Thus MSG is often described as a flavour enhancer rather than a flavouring in dried soups and similar products, at levels giving about 1 g dm^{-3} in the final dish. Although they are much more expensive, preparations of IMP, often blended with guanosine monophosphate (7.40), are now used by some food manufacturers.

Glutamic acid in the form of MSG is naturally present at low levels in the muscle tissue of live animals, but the higher concentrations in meat are mainly derived from the protein breakdown that occurs during the *post mortem* ageing process. IMP is a breakdown product of the adenosine monophosphate, AMP, which accumulates as adenosine triphosphate (ATP) is utilised in the *post mortem* metabolism of muscle:



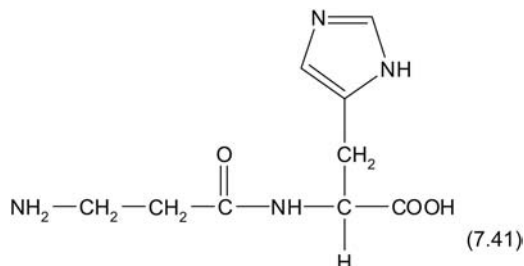
Some data for the levels of umami substances are given in Table 7.6. It is not difficult to correlate these data with one's personal impression of the flavour of many of these foods. The use of tomatoes to bring out the flavour of meat, and parmesan as a garnish

Table 7.6 Typical levels of umami substances found in a representative range of foods. The gaps in the table indicate an absence of data rather than a zero concentration. The foods listed here include several that are important, and characteristic, of the Japanese diet and the subject of much more research in Japanese laboratories than characteristically European foods. Much of this data is from K. Nimomiya, Umami: a universal taste, *Food Rev. Int.*, 2002, **18**, 23; the remainder is from the Umami Information Centre, <https://www.umamiinfo.com/> (last accessed in 2020).

	MSG	IMP	GMP
	mg per 100 g		
Kombu (an edible kelp seaweed, pheophyceae, brown algae)	2240		
Nori (an edible seaweed <i>Pyropia</i> sp. – Rhodophyceae, red algae)	550–1350	1–40	3–80
Bonito flakes (katsuo-bushi, a type of dried tuna)	30–40	470–700	
Fresh tuna		188	
Sardines	280	193	
Prawns	43	92	30
Cod	9	44	
Salmon	20		
Pork	9	260	2
Chicken	22	283	5
Parmesan cheese	1200		
Emmental cheese	308		
Shiitake mushroom (dried, <i>Lentinus edodes</i>)	1060		150
Fish sauce (nam pra)	950		
Tomato juice	260		
Human milk	22		
Cow's milk	2		

for minestrone soup, are readily supported by these data. The difference in MSG levels between human and cow's milk is also interesting, and one could speculate that it reflects the different dietary priorities of the herbivorous cow and the omnivorous human. By the time it is eaten, any protein-rich food will have degraded sufficiently to have accumulated detectable levels of umami substances, so that our taste for them provides a good test for the likely presence of protein. Results obtained by the pet food industry suggest that domestic cats, being obligate carnivores and using high-protein foods to supply their nutritional needs, are much more sensitive to umami substances than are humans.

Although IMP and MSG provide the basic meatiness of meat and meat products, the subtle differences in flavour between different meats depends on variations in their proportions of the two and also involve trace amounts of a number of other flavour compounds. For example, the IMP contents of beef and pork are roughly similar, but beef contains significantly more free amino acids than pork, including twice as much MSG. Lamb is particularly rich in MSG, but contains much lower levels of dipeptides, such as carnosine (7.41), which also contribute to the meat flavour. It has been suggested that the difference in flavour between veal and beef is the result of increasing levels of free amino acids as the animal matures. Any *post mortem* breakdown will also contribute free amino acids, including glutamate, to the taste of roasted meat.

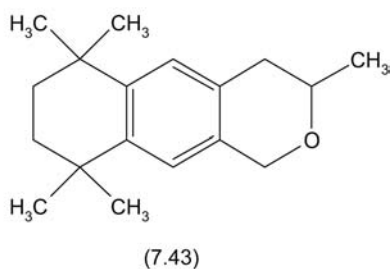
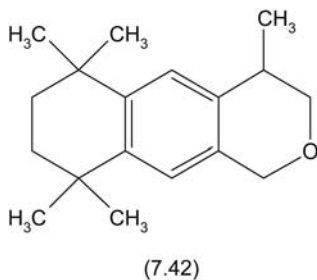


Although there are some classic dishes that contain raw meat (*e.g.* steak tartare), cooking is obviously the origin of the innumerable volatile compounds that are important in the flavour of meat. These are discussed in the second half of this chapter.

7.2 Odour

Although our sense of taste provides a broad outline of the nature of a potential food-stuff, it is odour that fills in the detail. Counting the number of different substances we are able to detect is impossible, as is any attempt to list all the different odours available. It is also impossible to draw a line between food odours and the host of other odours, more often referred to as smells, that we encounter every day and that have nothing to do with food. Yet another complication is that natural odours, as we perceive them, are hardly ever generated by single pure substances – for example, a typical fruit may have as many as 200 different volatile components. All of these will contribute to the overall odour, but may, in total, comprise only a few parts per million of the fruit.

Attempts have been made to classify odours and to relate the different classes to elements of chemical structure. Seven primary odours were described by Amoore in 1972: camphoraceous, ethereal, musky, floral, minty, pungent and putrid. However, if these correspond to seven types of receptors in the nose, many substances must be able to interact with more than one receptor. Since most of the odours we experience in the real world outside the laboratory are caused by mixtures of a great many different substances, with several types of receptors responding simultaneously, the brain is left to make sense of an amalgam of nerve impulses, in much the same way as it does with colour vision. By analogy with what is now well understood in relation to taste, our olfactory organs must be equipped with cell membranes carrying receptor proteins able to discriminate between molecules with quite similar structures, including optical isomers. For example, of the two isochroman derivatives illustrated here, one (7.42) has an intense musk odour, whereas the other (7.43) is odourless.



The organisation of the following sections is therefore based on a few broad groups of foodstuffs and makes no claim to be comprehensive.

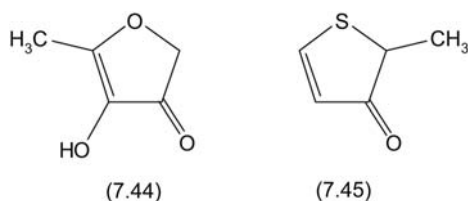
7.2.1 Meat

The first half of this chapter concluded with an account of the taste of meat and it seems reasonable to return to this food to emphasise the dependence of flavour on both taste and odour. There are three major groups of compounds that contribute to the flavour of meat and one of these, the umami substances and dipeptides, was discussed in the earlier section. The other two groups are:

- heterocyclic compounds derived from amino acids, nucleotides, sugars and thiamin *via* the Maillard reaction (see Chapter 2) during cooking; and
- unsaturated fatty acid breakdown products (see Chapter 4), also formed during cooking.

The Maillard reaction can yield a great diversity of carbonyl compounds from the residual sugars (derived from glycogen and nucleotides) in meat, including 2-oxopropanol (2.31), 2,3-butanedione (2.37), hydroxypropanone (2.38) and hydroxymethylfurfural (Figure 2.13). The Strecker degradation (Figure 2.16) of these with most amino acids yields the corresponding aldehydes, but, as shown in Figure 7.9, cysteine gives rise to both hydrogen sulfide and ammonia, which can contribute to the formation of a variety of heterocyclic compounds, such as thiazoles and oxazoles, that are important in the flavour of roasted meat. The reaction of hydrogen sulfide with aldehydes can give rise to ring structures containing multiple sulfur atoms that are believed to be particularly important in the flavour of boiled (as opposed to roasted) meat. Several other sulfur compounds that contribute to the flavour of cooked meat have been traced back to the thermal decomposition the vitamin thiamin.

The thermal decomposition of ribonucleotides (*e.g.* IMP, (7.38)) has been shown to lead to compounds such as methyl furanolone (7.44), which not only have a pronounced meaty odour themselves, but can also be the precursors of sulfur-containing compounds (the sulfur is derived from the breakdown of cysteine and methionine) – for example, methylthiophenone (7.45), which also have a strong meaty odour.



Fatty acid breakdown, along the lines of the autoxidation reactions described in Chapter 4, is also an important source of the characteristic odours of meat products. The low content of polyunsaturated fatty acids in the depot fat and intramuscular fat of beef, lamb and pork suggests that the polar membrane lipids of muscle cells are the

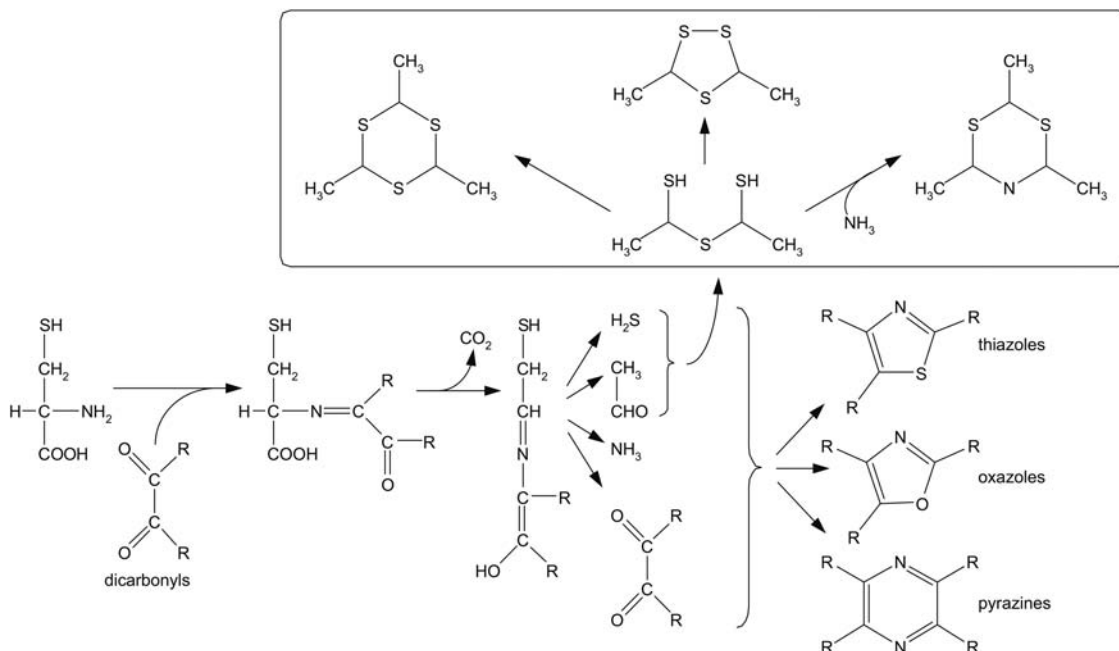
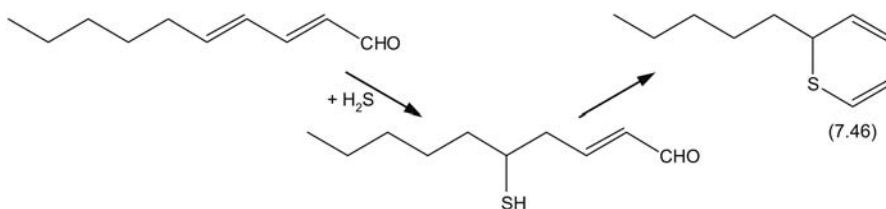


Figure 7.9 Formation of heterocyclic meat flavour compounds following the Strecker degradation of cysteine. For simplicity, the complex sequence of condensation reactions postulated to lead to the ring compounds is not shown. R represents any of the terminal fragments of sugar molecules that can arise in the Maillard reaction. It is likely that aldehydes and dicarbonyls from the degradation of other amino acids will join in the sequence and provide an even greater diversity of end-products. The boxed reactions show the formation, from acetaldehyde and hydrogen sulfide, of the cyclic sulfides identified in the flavour of boiled meat.

most likely source of the autoxidation products significant to flavour. Arachidonic acid has been shown to break down on heating to give four aldehydes, which together provide the distinctive odour of cooked chicken:



Aldehyde fatty acid fragments such as these can react with hydrogen sulfide or ammonia to provide yet another source of odorous heterocyclic compounds (7.46).



7.2.2 Fruit

The flavour of fruit is definitely a blend of taste and odour. The taste – an aggregate of sweetness due to sugars, the sourness of organic acids and sometimes the astringency of polyphenolic compounds – provides the basic identity that confirms that one is consuming fruit. Even a simple solution of sucrose and citric acid tastes “fruity” to most people. However, to distinguish between different fruits we rely heavily on the distinctive odour of the fruit’s volatile components. If one is suffering from a cold, which effectively, but temporarily, eliminates the sense of smell, it is extremely difficult to distinguish between raspberry and strawberry flavours.

A typical fruit may contain as many as 200 different volatile components, but even in total these may comprise only a few parts per million of the total fruit. It is generally observed that citrus fruit odours are dominated by terpenoids (see Chapter 6, Section 6.2), whereas they are much less in evidence in other fruit.^{vi} Long lists of compounds have been assembled for many fruits, which show that acids, alcohols (and the esters resulting from their combination), aldehydes and ketones predominate. Considering apples as a typical example, we find:

- at least 20 aliphatic acids, ranging from formic to *n*-decanoic;
- at least 27 aliphatic alcohols, covering a corresponding range of chemical structures;
- over 70 esters, from the combination of the predominant alcohols and acids;
- 26 aldehydes and ketones, also with structures corresponding to the acids and alcohols; and
- smaller numbers of miscellaneous ethers, acetals, terpenoids and other hydrocarbons.

This adds up to a total of 131 separate compounds. However, it is important to remember that by no means all of these substances make a significant contribution: volatility does not equate to aroma. Another point is that a very high proportion of these compounds are common to many different fruits. For example, of the 17 esters identified in banana volatiles, only five are not also found in apples. The particularly common occurrence of aldehydes, alcohols and acids that have linear or branched (*e.g.* isopropionyl) chains of up to seven carbon atoms, often with an occasional double bond, suggests some common origins. In fact, there are two sources, both represented in many fruits. During ripening, cell breakdown is accompanied by oxidation of the unsaturated fatty acids in membrane lipids, catalysed by the enzyme lipoxygenase. This converts fatty acids with a *cis,cis*-methylene-interrupted diene system (as in linoleic acid) into hydroperoxides. These break down either spontaneously or under the influence of other enzymes to give aldehydes, as shown in Figure 7.10, which are important elements of vegetable flavour. The pattern of reaction products is very similar to that found in the non-enzymic autoxidation of fatty acids. The other sources of aldehydes are amino acids. Enzymic transamination and decarboxylation of free amino acids often accompany ripening – for example, leucine gives rise to 3-methylbutanal (7.47):

^{vi}The term “berry-type” aroma is sometimes used to cover all non-citrus fruit, a satisfactory term when applied to raspberries and similar fruit, but somewhat stretched to cover apples or bananas.

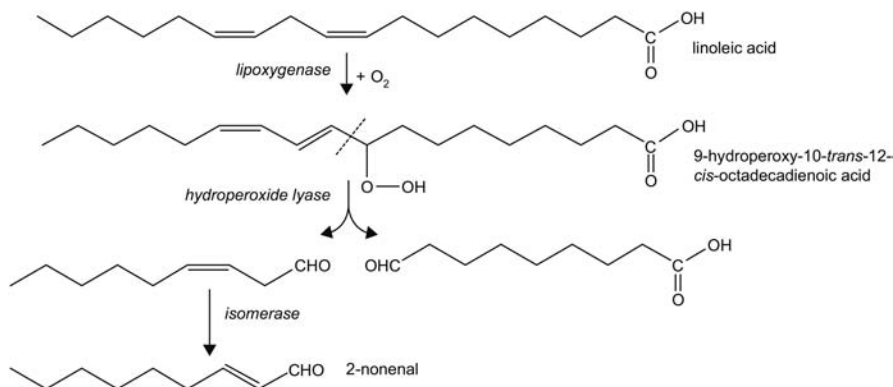
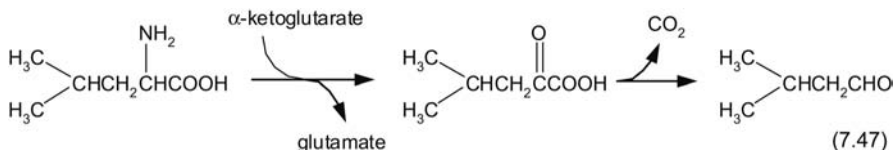


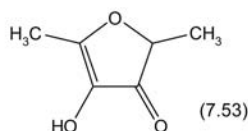
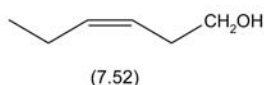
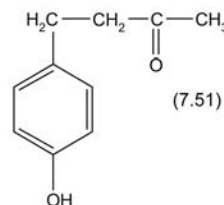
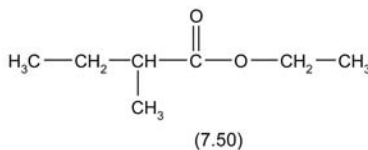
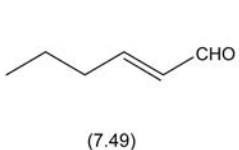
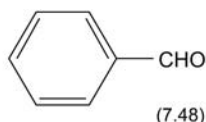
Figure 7.10 Formation of *trans*-2-nonenal following the breakdown of linoleic acid initiated by lipoxygenase action. Lipoxygenases in some plant species react at the other end of the conjugated diene system, giving, for example, 13-hydroperoxy-9-*cis*-11-*trans*-octadecadienoic acid, which inevitably leads to a very different collection of end-products.



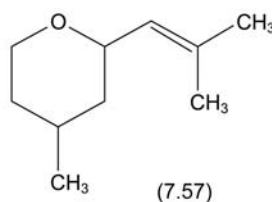
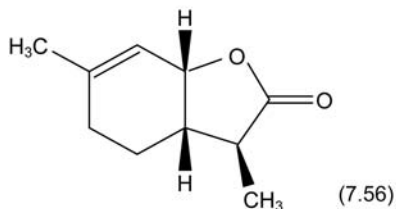
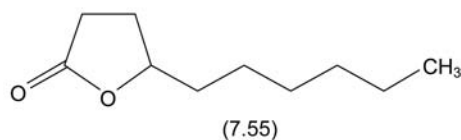
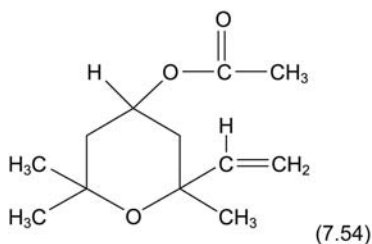
Aldehydes are not only important in fruit and vegetables. During alcoholic fermentation, yeast enzymes catalyse similar reactions with the amino acids from the malt or other raw materials. These aldehydes, and the alcohols derived from them, are important as flavour compounds in many alcoholic drinks, particularly in distilled spirits (where they are described as fusel oils) and where they have a special responsibility for the headaches associated with hangovers. Other enzyme systems in plant tissues are responsible for the reduction of aldehydes to alcohols, or their oxidation to carboxylic acids, and the combination of these to form esters.

In many cases, the distinctive character of a particular fruit flavour is dependent on one or two fairly unique “character impact” substances. For example, iso-pentyl acetate is recognised as the crucial element in banana flavour, although eugenol (7.23) and some of its derivatives contribute to the mellow, full-bodied aroma of the ripe fruit. Benzaldehyde (7.48) is the character impact substance in cherries and almonds. It is difficult to establish to what extent hydrocyanic acid (HCN), which is also present at levels of a few ppm in cherries, contributes to their aroma. Hexanal ($CH_3(CH_2)_4CHO$) and 2-hexenal (7.49) provide what are described as green or unripe odours in a number of fruits and vegetables. In at least one variety of apple (Golden Delicious), they have been shown to balance the ripe note in the aroma given specifically by ethyl 2-methyl butyrate (6.50). The olfactory thresholds of these three substances are very low (0.005, 0.017 and 0.0001 ppm, respectively). The distinctive character of raspberry aroma is due mostly to 4-(*p*-hydroxyphenyl)-2-butanone (7.51), often referred to as “raspberry ketone”, but the fresh grassy aroma characteristic of the fresh fruit, notably lacking in many raspberry-flavoured products, is principally due to *cis*-3-hexenol (7.52) supplemented by α - and

β -ionone (see Chapter 6, Section 6.2). Furaneol, 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (7.53), is a major constituent of strawberry flavour.

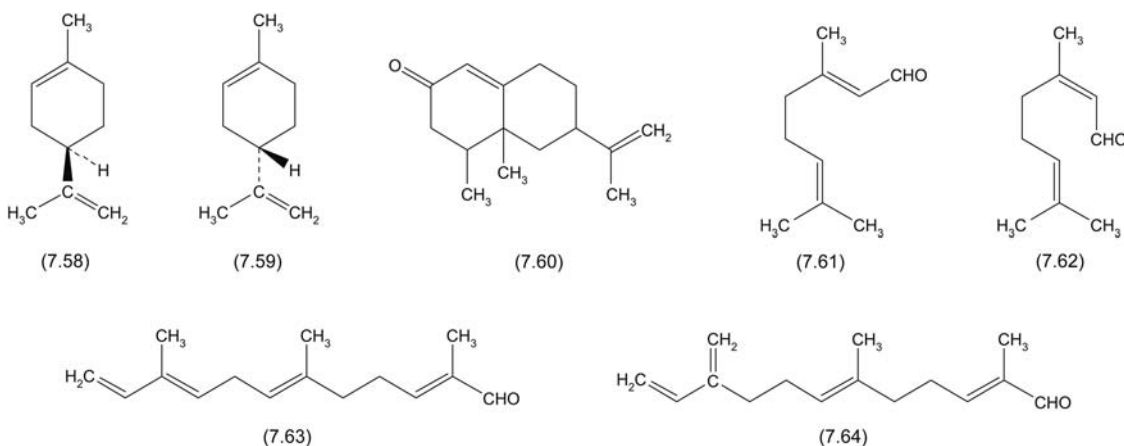


Wine presents special challenges to the flavour chemist. Most varieties of red wine, *e.g.* Merlot, Grenache, *etc.*, share many of the same flavour compounds, merely in different proportions. Wine experts are well known for describing the aroma of a wine in terms of other fruit. For example, the presence of 2,6,6'-trimethyl-2-vinyl-4-acetoxytetrahydropyran (7.54) in a red wine is said to impart a blackcurrant aroma. Similarly, the peach and pineapple aromas of several red wines have been attributed to γ -decalactone (7.55) and "wine lactone" (3a,4,5,7a-tetrahydro-3,6-dimethyl benzofuran-2(3*H*)-one; (7.56)), respectively. *cis*-Rose oxide (4-methyl-2-(2-methyl-1-propenyl)tetrahydrofuran; (7.57)) has been identified as the source of the lychee (*Litchi chinensis*) aroma characteristic of Gewürztraminer, a white wine.



The character impact substances in many fruits, especially citrus fruits, are terpenoids. Terpenoids can often be isolated from plant materials by steam distillation. The oily

fraction of the distillate is known as the “essential oil”, and these are often used as flavourings in drinks, such as gin and vodka. The essential oils of citrus fruits (from the entire fruit, including the peel) consist mainly of terpenes, of which the monoterpene (+)-limonene (7.58) is usually at least 80%. The optical isomer of (+)-limonene, (–)-limonene (7.59), is reported to smell of lemons rather than oranges. However, (+)-limonene is not nearly as important a flavour component as the oxygenated terpenoids, which occur in much smaller amounts in the oil. For example, fresh grapefruit juice contains about 16 ppm of (+)-limonene carried over from the peel during juice preparation, but the characteristic aromatic flavour of grapefruit is due to the presence of much smaller amounts of (+)-nootkatone (7.60). The characteristic bitter taste of grapefruit juice was mentioned earlier in this chapter. The character impact substance in lemons is citral, which is more correctly described as a mixture of the isomers geranial (7.61) and neral (7.62). Most varieties of oranges do not appear to have a clearly identified character impact substance, but the mandarin orange, *Citrus reticulata*, is characterised by α -sinensal (7.63). The various varieties of the sweet or Valencia orange, *Citrus sinensis*, contain β -sinensal (7.64).



7.2.3 Vegetables

Compared with fruit, vegetables tend to have milder and more subtle flavours in which aroma is much less significant than taste. The aromas of fruit have evolved to entice insects, birds and other consumers into assisting with seed distribution and related activities. This hardly applies to the leaves of a cabbage or the tubers of a potato. The list of vegetables whose flavour is sufficiently desirable to prompt food manufacturers to seek out artificial alternatives to their flavours is very short – mushrooms and onions – and it certainly does not include either broccoli or beans. The character impact substance of the common mushroom, *Agaricus bisporus*, is 1-octen-3-ol, $\text{CH}_3(\text{CH}_2)_4\text{CHOHCH}=\text{CH}_2$.

Truffles should also be mentioned at this point. They are the famously expensive subterranean fruiting bodies, resembling mushrooms, of a genus of symbiotic ascomycete fungi, *Tuber* spp. They grow symbiotically in association with the roots various species of trees and are harvested with aid of dogs trained to detect their characteristic odour. This odour is attributed a large number of different volatiles, including sulfur

compounds and the products of fatty acid breakdown, that defy systematic, or brief, consideration. Truffle oil is used to impart the flavour and aroma of truffles to a variety of dishes. The finest, and most expensive, truffle oils are made by infusing olive oil with actual truffles. However, most of the truffle oil on sale consists of olive oil flavoured with synthetic 2,4-dithiapentane, $\text{CH}_3\text{-S-CH}_2\text{-S-CH}_3$, a major contributor to the natural odour of truffles.

Many vegetables owe their flavour to the activity of the enzyme *lipoxygenase*. The action of this enzyme is shown in Figure 7.10. 2-*trans*-Nonenal, the end-product shown in Figure 7.10, is an important contributor to the aroma of cucumbers (*Cucumis sativus*), although 2-*trans*-6-*cis*-nonadienal is recognised as the character impact substance. Lipoxygenase activity is not always a good thing, however. High levels of the enzyme occur in peas and beans and, if they are physically damaged during harvesting, the enzyme is able to react with unsaturated fatty acids released from the cell membranes, giving rise to off-flavours, notably “delay off-flavour” in peas. The delay in this case is the unintended few hours between the peas being mechanically removed from the pod by the vining machine and the blanching process. Blanching, usually comprising a few seconds in a blast of steam, is designed to inactivate enzymes such as lipoxygenase.

Onion (*Allium cepa*) and garlic (*Allium sativum*) are by far the most studied vegetables in terms of flavour. All members of this genus contain *S*-alkyl cysteine sulfoxides (see Figure 7.11). The 1-propenyl derivative is particularly associated with onions and the allyl derivative with garlic. Just as in the formation of isothiocyanates in cruciferous plants, the compounds that occur in the raw, undamaged plant tissues are odourless. It is only when the garlic is crushed, or the onion is sliced, that the enzyme alliinase gains access to its substrates and the flavour becomes obvious. The fate of the unstable sulfenic acid produced by the action of the alliinase depends on the species. In the case of onions, a second enzyme, known as LF synthase (LF = lachrymatory factor), gives rise to thiopropionaldehyde-*S*-oxide (also known as propanethial-*S*-oxide). This substance is the source of the legendary lachrymatory effect of onions. In garlic, which lacks LF

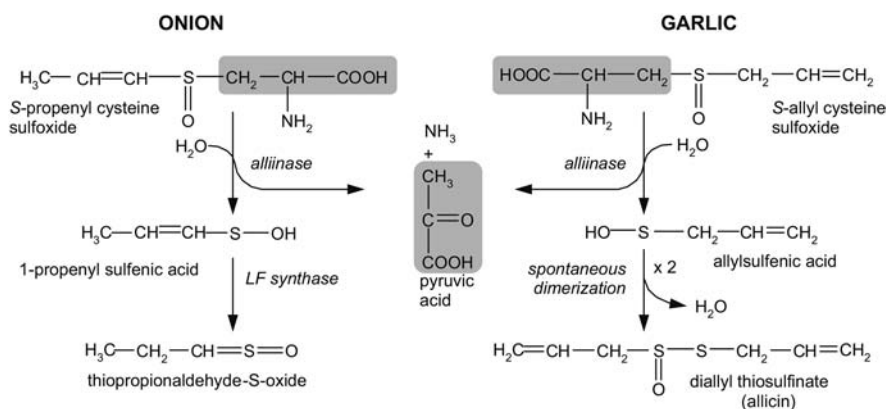


Figure 7.11 Breakdown of *S*-alkyl cysteine sulfoxides in onion and garlic. The pyruvate moiety is shaded.

synthase, the reactivity of the sulfenic acid of the allyl sulfenic group leads to dimerisation to allicin, well known for its antibacterial properties.

The immediate biosynthetic precursors of *S*-alkyl cysteine sulfoxides are the γ -glutamyl derivatives (7.65) and considerable amounts of these compounds are present in the fresh plant material. They are not susceptible to the action of alliinase, but these, and any traces of the amino acid cysteine, are able to participate in the great variety of exchange, dimerisation and condensation reactions that occur when onions and garlic are cooked. Figure 7.12 illustrates some of the potential reactions that can lead to the formation of sulfonates and sulfides that contribute to the flavour of cooked onion or garlic. Other, non-sulfur, flavour compounds, such as 2-methylbut-2-enal ($\text{CH}_3\text{CH}=\text{C}(\text{CH}_3)\text{CHO}$) and 2-methylpent-2-enal ($\text{CH}_3\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)\text{CHO}$), are formed in similar reactions.

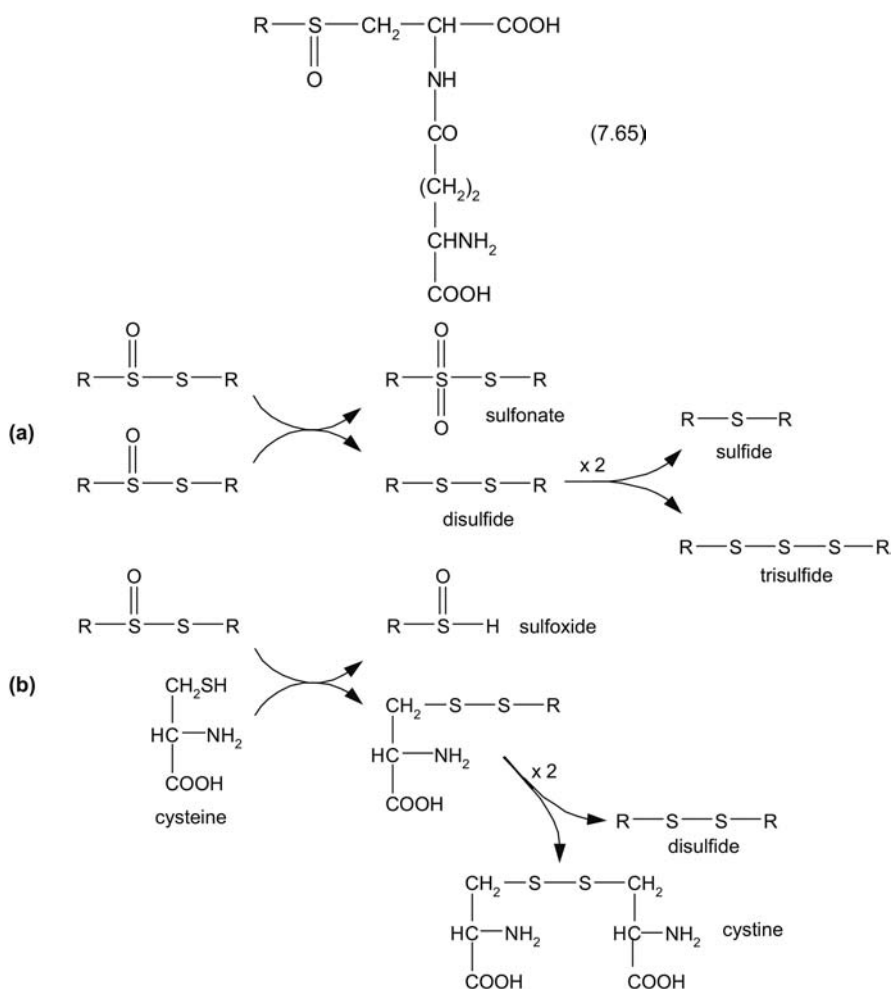
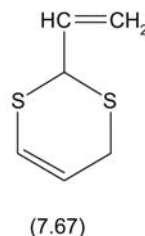
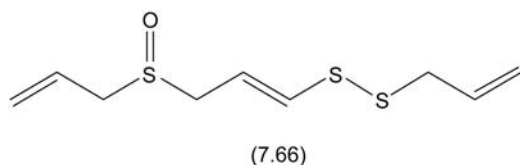


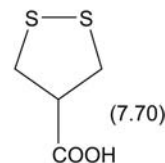
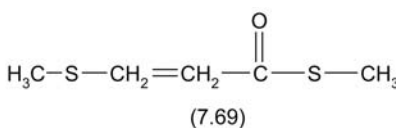
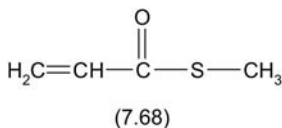
Figure 7.12 Exchange reactions taking place when onions and garlic are cooked involving (a) two alkyl thiosulfinate molecules and (b) an alkyl sulfinate with cysteine, both leading to the formation of sulfonates and volatile odorous sulfides. R may represent an allyl ($\text{CH}_2=\text{CHCH}_2-$), 1-propenyl ($\text{CH}_3\text{CH}=\text{CH}-$) or methyl (CH_3-) group.

Di- and trisulfides tend to be most significant when cooking takes place in water. However, in fried dishes, including stir-fries, other compounds are more significant, including (*E*)-ajoene^{vii} (7.66) and 2-vinyl-[4*H*]-1,2-dithiin (7.67).



A brief mention should also be made of the medicinal properties of garlic. In spite of its long history of use in traditional medicine, results from modern clinical trials with human subjects have been rather variable. Most studies show reductions in blood cholesterol levels of between 10 and 15% when 0.5–1.0 g of garlic powder (equivalent to 1.5–3.0 g fresh garlic) is consumed daily for two or three months. Allicin has recognised antibacterial properties and it is also a potent antithrombotic agent, inhibiting the aggregation of platelets. However, it remains questionable whether the practical health benefits of these effects can compensate for the social impact of this level of garlic consumption. The odour-free capsules of garlic oil available as “alternative” medicines are suspected to contain only small amounts of these biologically active substances.

Asparagus is another vegetable in which sulfur compounds make an important contribution to flavour. However, the flavour of the vegetable itself is of less scientific interest than the unpleasant smell it imparts to the urine of consumers. The most likely compounds in urine causing the “rotten cabbage” smell are methyl mercaptan (methanethiol, CH₃SH), *S*-methylthioacrylate (7.68) and *S*-methyl-3-(methylthio)thiopropionate (7.69). These are assumed to be derived from asparagusic acid (7.70), which, unlike most of the other sulfur compounds in asparagus, has not been encountered in any other vegetable. It has been suggested that only about 40% of asparagus eaters can detect this smell in their urine, but whether this is due to deficiencies in the sense of smell of the majority or their sulfur metabolism has not been established.



7.2.4 Herbs and Spices

These important sources of food flavour are usually considered together, not least because the distinction between them is not always clear. Both terms describe plant materials used specifically as flavourings rather than for any nutrients they may

^{vii}The (*E*) identifies the configuration of the central double bond as the *trans* form. (*Z*)-Ajoene, with the double bond *cis*, also occurs. Ajo is the Spanish word for garlic.

contain. The culinary use of the word “herb” tends to follow from its botanical meaning, *i.e.* a plant with no woody tissues, as in “herbaceous border”, and is applied to leafy flavouring materials such as mint, basil, rosemary and sage. By contrast, “spices” are usually derived from non-leafy plant tissues, such as seeds (nutmeg), flower buds (cloves), roots (ginger) or tree bark (cinnamon). The principal compounds responsible for the flavour of a selection of herbs and spices are listed in Figure 7.13. Three important spices, ginger and black and white pepper, were considered in detail earlier in this chapter.

Principal aroma compounds

Basil	<i>Ocimum basilicum</i>	methylchavicol (7.71)
Rosemary	<i>Rosmarinus officinalis</i>	verbenone (7.72), 1,8 cineole (7.73)
Sage	<i>Salvia officinalis</i>	α -thujone (7.74), camphor (7.75)
Oregano	<i>Origanum</i> spp.	carvacrol (7.76), thymol (7.77)
Peppermint	<i>Mentha piperita</i>	(-)-menthol (7.78), menthone (7.79), menthofuran (7.80)
Spearmint	<i>M. spicata</i>	(-)-carvone (7.81)
Caraway	<i>Carum carvi</i>	(+)-carvone (7.82)
Nutmeg	<i>Myristica fragrans</i>	(+)-sabinine (7.83)
Cardamom	<i>Elettaria cardomomum</i>	α -terpinyl acetate (7.84)

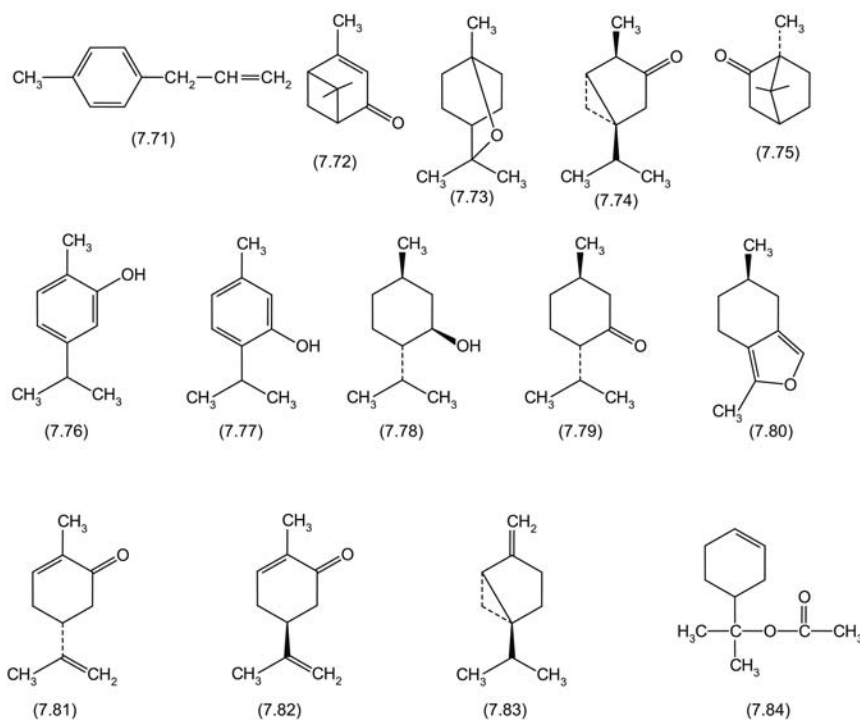


Figure 7.13 Principal aroma compounds in a selection of herbs and spices. The compounds shown are not necessarily the most abundant, but are the ones believed to impart the characteristic flavour notes. The carvones ((7.81) and (7.82)) demonstrate the common observation that optical isomers often differ markedly in aroma. (The $-\text{CH}_3$ groups carried by the bridging carbon atoms in (7.72) and (7.75) have been omitted for clarity.)

7.2.5 Synthetic Flavourings

A principal objective for the flavour chemist is the provision of synthetic flavourings, particularly for use by food manufacturers. An important trend in recent years has been the replacement of simple esters, with their sweet and unsubtle odour (*e.g.* the amyl acetate in pear drops), with lighter and fresher (and often much more expensive) flavourings based on our knowledge of the natural flavour components. For commercial reasons, very little information about the detailed composition of their products is ever released by flavour manufacturers, but the patent literature can give some insights. Table 7.7 provides an example of a formulation for use in confectionery and similar products. Mixtures such as that described usually provide only the starting point for the formulation of a commercial flavouring. This flavouring is typical in that alongside numerous chemically defined substances, the formulation includes small amounts of essential oils, in this case both lemon and orange oils, so that presentation of a complete full list of all the individual substances present would be too long to be meaningful.

Flavour manufacturers are able to customise their products for individual food manufacturers, including retail chains offering own-label products, by making small changes to the proportions of the ingredients and by including, or omitting, particular components. It is also important to remember that most of these compounds will have been identified as components of the original fruit of which the flavour is being mimicked. Before arriving at the final formulation, the flavourist will be guided initially by the identity and proportions of the volatiles in the original fruit, as determined by gas chromatography linked with mass spectrometry. However, the final composition will owe as much to the highly skilled nose of the flavourist as it does to science.

In recent decades, a demand has developed in the food industry for flavourings beyond traditional fruit flavours. For example, the very high price of cocoa has stimulated demand for good synthetic chocolate flavourings. The natural flavour of chocolate has been extensively investigated, but not exhaustively. Two groups of compounds have been implicated, sulfides (*e.g.* dimethyl sulfide and related sulfur compounds) and pyrazines (see Figure 7.9). No fewer than 57 different pyrazines have been identified in cocoa volatiles. A patented formulation for a chocolate flavouring containing a representative pyrazine and sulfide is shown in Table 7.8. It is, of course, not known whether this particular formulation has ever been used.

Table 7.7 A synthetic banana flavouring. The concentrations in the final product of even the most abundant of these components will only be of the order of parts per million.

Major components	Minor components	Trace components
Amyl acetate	Amyl valerate	Acetaldehyde
Amyl butyrate	Benzyl propionate	Butyl acetate
Ethyl butyrate	Cyclohexyl propionate	2-Hexenal
Isoamyl acetate	Ethyl caproate	Isoamyl alcohol
Isoamyl butyrate	Geranyl propionate	Lemon oil
Linalool	Heliotropin	α -Ionone
	Vanillin	Methyl heptanone
		Orange oil

Table 7.8 A synthetic chocolate flavouring (US Patent No. 3619210). The proportions of carriers, solvents, *etc.* have been omitted.

	Parts
Dimethyl sulfide	1
2,6-Dimethylpyrazine	3324
Ethyl vanillin	143
Isovaleraldehyde	100

Snack foods are now marketed in a remarkable range of synthetic savoury flavours: “smoky bacon” and “barbecue beef” are typical examples. The synthesis of flavours such as these by replicating the range of volatiles found in the original foods presents a number of problems. First, the flavour compounds are not usually present in the original raw materials – they arise during cooking, mostly *via* the Maillard reaction or the thermal degradation or oxidation of unsaturated fatty acids. Second, products of the Maillard reaction often have highly complex molecular structures that place them beyond the scope of commercial synthetic organic chemistry. A third problem is that, in isolation, such compounds would be unlikely to gain the necessary safety clearance from the regulatory authorities. The solution has been the development of what are known as “process flavours” (or “thermal process flavourings” in European Union regulations).

Process flavours are produced by heating precisely specified mixtures of proteins, amino acids, sugars and fats together under closely controlled conditions. The raw materials must be toxicologically acceptable as food ingredients and the heating conditions (time, temperature and pH) must resemble those encountered in normal cooking. Comparison of the composition of a food product’s raw materials with the range of flavour volatiles formed during cooking will often enable the chemist to predict the mixture of amino acids, *etc.* that is most likely to produce a good process flavour. Another type of process flavour that also has a role in many snack products is “liquid smoke”, considered in Chapter 9.

7.3 Special Topics

7.3.1 Taste Receptors and Cells

The correlation of a sweet taste with the particular molecular features of sugars that led to the AH,B concept was discussed earlier this chapter. Unfortunately, the relative simplicity of this essentially chemical approach did not lend itself to the other basic tastes – bitterness, meatiness (umami), saltiness and sourness – until advances in biology, particularly genetics and molecular biology, could be brought to bear on the issue. What follows is an essentially biological/biochemical approach to this topic, which should throw more light on the subject of taste as a whole.

The several thousand taste buds of the human tongue are located on raised protrusions known as papillae. Each taste bud consists of 30–100 elongated taste receptor cells (TRCs) that have fine projections (microvilli) extending to the surface of the tongue

and maintain contact with the saliva and its solutes. Each TRC is responsive to one of the five basic tastes and each type of TRC is innervated by individual nerve fibres. The membrane proteins active in taste, the taste receptor proteins, are located in the cell membranes of the TRCs.

In 1999–2001, three genes involved in the detection of sweet and umami compounds, T1R1, T1R2 and T1R3, were identified in rodents and humans. The DNA sequence of these genes showed them to code for proteins that belonged to a class known to include receptors in a variety of physiological processes, the G protein coupled receptors (GPCRs).^{viii} These proteins were synthesised to different degrees in the various types of taste bud cells. The T1R2 and T1R3 proteins were shown to form a dimer that functioned as a receptor for sweet taste and the T1R1–T1R3 dimer as an umami receptor.^{ix} The T1R2–T1R3 dimer has at least three binding sites (analogous to the *active* sites of enzymes), which are found in the region, or domain, that projects outwards from the surface of the cell membrane. On the basis of its shape, this is known as a Venus fly trap domain. One of these sites binds small, sweet-tasting molecules such as sugars (and hosts the AH,B structure) and most non-sugar sweeteners; another binds sweet-tasting macromolecules, such as the proteins thaumatin and monellin. A third site is believed to bind neohesperidine dihydrochalcone and also, fortuitously, some artificial sweeteners. Models of the three-dimensional structure of the receptor molecule, computed from the amino acid sequence, show that these sites actually overlap.

Whichever site is occupied, the result is a small change in the overall shape of the receptor dimer, the bulk of which is embedded in the cell membrane. This change (which biochemists will recognise as a similar phenomenon to the changes in regulatory enzymes when ligands bind at allosteric sites) is ultimately relayed to the part of the protein dimer that projects into the cytoplasm of the taste bud cell and initiates the cascade of biochemical events that leads ultimately to the generation of a nerve impulse.

The T1R1–T1R3 dimers of human origin have been shown to be receptors for L-glutamate (*i.e.* MSG), although not any other amino acids, a selectivity clearly characteristic of umami. The response to L-glutamate is enhanced by the presence of the 5'-ribonucleotides IMP and GMP, confirming the role of the T1R1–T1R3 dimer in the umami response and providing a simple explanation for the synergism between MSG and the ribonucleotides. It is curious that the T1R3 monomer is common to both umami and sweetness receptors. The mechanism of the effect of IMP and GMP on the response to L-glutamate is believed to involve the binding the ribonucleotide molecule to the receptor protein at a distinct, *allosteric*, site, resulting in a conformational change in the receptor protein that makes its L-glutamate binding site more receptive.

The taste receptors for sweetness and umami have relatively simple tasks in identifying two small subsets of similar nutrient molecules. The task for bitterness receptors

^{viii}GPCRs carry tightly bound guanosine phosphates (hence the “G”) and initiate the cascade of biochemical events that leads ultimately to the generation of a nerve impulse.

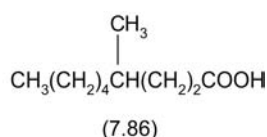
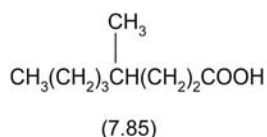
^{ix}Domestic cats, and other members of the Family Felidae, are indifferent to sweetness and lack the *T1R2* gene. Being obligate carnivores, the ability to detect sweet carbohydrates would not give them any biological advantage.

is much more difficult because the diversity of potentially toxic molecules is so great. The only simplifying factor is that the potential responses are consolidated into one, bitterness, to show that all of these substances are to be avoided. About 30 different bitterness receptor proteins (GPCRs), otherwise known as T2Rs, are involved and most, if not all, are found in the same TRCs. This means that most bitterness TRCs behave as universal bitterness detectors and do not discriminate between one type of bitter substance or another.

Although only a limited number of species have been examined in detail, it has been shown that the inability of birds to taste the pungency of capsaicin is caused by their lack of appropriate receptor proteins. The evolutionary advantage to the *Capsicum* genus of having birds as potential seed distributors is clear; the advantage to birds in having peppers as a potential food source is less obvious.

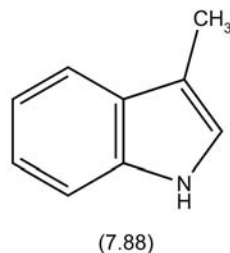
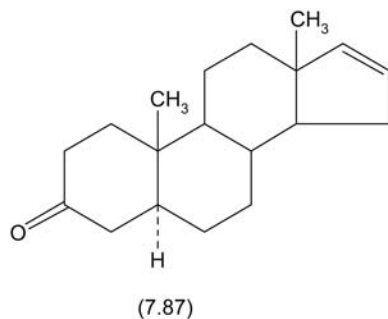
7.3.2 Off-flavours in Meat

Mutton, the meat from mature sheep, is often regarded as having an unattractive odour compared with lamb. This has been attributed to the accumulation of traces of free branched-chain fatty acids in the depot fat triglycerides, the most important being 4-methyloctanoic (7.85) and 4-methylnonanoic acids (7.86).



“Boar taint” is a problem encountered with pig meat. Male animals tend to be leaner than females and, in theory, this should make them preferable for meat production, but this unfortunately presents a difficulty for pig farmers. As the uncastrated male pig reaches about 90 kg live weight,^x it accumulates 5-androst-16-ene-3-one (7.87) in its body fat. This by-product of normal steroid metabolism is the cause of boar taint. Being volatile, the steroid is driven off when the meat is cooked and its odour may cause offence. Interestingly, not everyone is able to smell it. More than 90% of women, but only about 55% of men, are able to detect it at typical cooking concentrations and, of those that can, far more women than men find the smell unpleasant. One can only speculate about the implications for human behaviour or physiology of these sex differences. The odour is described as having much in common with both urine and sweat and it is therefore hardly surprising that the meat industry would like to find a method of discouraging boars from synthesising it. Another substance with a very unpleasant smell, skatole (3-methylindole, (7.88)), has also been implicated in boar taint. It is synthesised by the intestinal flora of pigs (including females and uncastrated males) and migrates to the fatty tissue of the animal. Fortunately, its development can be reduced by manipulation of the pig’s diet.

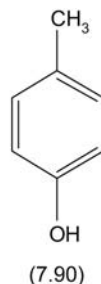
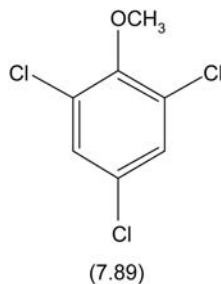
^x90 kg is the typical weight for a bacon pig; younger animals of around 65 kg are used for pork.



7.3.3 Taints

Undesirable flavours that are found in foods as a result of contamination are usually known as “taints”. An enormous diversity of substances can give rise to taints in food, and from a similar diversity of sources, although there is only space for a few examples here. The most important sources are storage areas, packaging materials and the cleaning agents used on processing plant. In many cases it can be extremely difficult to trace the source of the contamination. For example, a case was reported^{xi} in which a packaged foodstuff had an off-odour caused by trichloroanisole (7.89). This has a musty, mouldy odour detectable at three parts in 10.¹⁶ Some polyethylene packaging was found to be the source of the trichloroanisole and it was eventually shown that a single polyethylene bag, containing polyethylene granules used in the manufacture of the packaging material, had been stored on a wooden pallet. The wood had been previously treated with chlorinated phenolic compounds as preservatives. Some microorganisms are known to be capable of converting chlorinated phenolics into the corresponding anisoles.

Although this may be an extreme example, phenolic disinfectants are often the origin of such problems. Chlorophenol wood preservatives may find their way into the fibre-board used for food packaging. Disinfectants applied to buildings, or to bedding for livestock or poultry, can ultimately reach milk and meat at low, but detectable, levels. *p*-Cresol (7.90), a common component of disinfectants, is detectable down to two parts in 10.⁷ Trichloroanisole contamination of the cork can also give rise to “cork taint” in wine. This problem is rapidly disappearing as even fairly expensive wine is now sold in bottles sealed with screw caps.



^{xi}J. Ewender, *et al.*, in *Food and Packaging Materials – Chemical Interactions*, ed. P. Ackerman, M. Jägerstad and T. Olsen, Royal Society of Chemistry, Cambridge, 1995, p. 33.

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8 Vitamins

The vitamins are an untidy collection of complex organic nutrients that occur in the biological materials we consume as food (and in others we do not). In terms of chemical structure, they have nothing in common with one another and their biological functions similarly offer little help with their definition or classification. What does draw them together is that:

- they are essential components of the biochemical or physiological systems of animal life, and frequently plant and microbial life as well;
- as animals evolved, they lost their ability to synthesise these substances for themselves in adequate amounts;
- they tend to occur only in tiny amounts in biological materials; and
- a specific deficiency syndrome results from a deficiency in the tissues, whether by absence from the diet or failure to absorb them.

This specification rules out:

- trace metals and other minerals, since these are not organic;
- essential fatty acidsⁱ and indispensable amino acids,ⁱⁱ which are required in larger amounts;
- hormones, which are synthesised by the body as required and cannot be supplied in the diet; and
- substances which might well be beneficial in the treatment of some illness or condition, but whose absence does not invoke a disorder or disease in an otherwise healthy person.

Animals are distinguished from most other forms of life by their dependence on other organisms for food. Either directly or indirectly, plants are the fundamental source of basic nutrients and it is hardly surprising that animals have come to rely on plants for

ⁱSee Chapter 4.

ⁱⁱSee Chapter 5.

the supply of other substances as well. The risk of vitamin deficiency occurring in an otherwise adequate diet would have been slight at the time this dependence evolved, and was a small price to pay for no longer having to maintain the machinery for synthesising such a diverse range of complex substances. Several of the diseases now known to be caused by vitamin deficiency have been recognised since classical times. For example, accurate descriptions of scurvy, a disease caused by deficiency of ascorbic acid, including its association with seamen living on preserved food, go back to 1150 BC (the Ebers papyrus) and Hippocrates (c. 420 BC). Similarly, beriberi, caused by a deficiency of thiamin, was described in Chinese herbals dating back to around 2600 BC. Although the linkage to diet was noted, the idea that these conditions were caused by the *absence* of something from the diet was not widely accepted until the twentieth century. For example, pellagra, caused by a deficiency of niacin, was correctly associated with maize, but was assumed to be caused by the *presence* of a toxin in spoilt maize.

Even after the association of a disease with a dietary component had been confirmed, progress in the isolation and identification of the substance could only be made if a suitable animal model was available. The discovery of thiamin is a classic illustration of this. In 1886, Christian Eijkman was sent to Indonesia by the Dutch authorities to seek out the cause of beriberi, a disease whose symptoms include inflammation, diarrhoea, dementia and sores in the mouth. Medical opinion at the time was that it was a bacterial infection. However, Eijkman was unable to find a causative microorganism, but he did notice a paralytic illness resembling beriberi in the chickens kept by the laboratory staff to supply eggs. No connection with their nutrition or the human disease was made until the disease suddenly disappeared. This was quickly associated with a change in the chickens' diet: polished rice (*i.e.* rice milled to remove the husk, bran and germ) caused the disease, whereas brown unmilled rice prevented it. Eijkman guessed that the cause of the chickens' symptoms was very close to that of beriberi in humans and, from that moment, Eijkman had his experimental model. He quickly confirmed the link when he found that the incidence of beriberi among Javanese prisoners fed a diet of polished rice was 2.8%, but only 0.09% in those on a diet including brown rice. In 1894, a Japanese naval surgeon named Takaki had also concluded that beriberi was diet-related, based on his observation that adding milk and meat to the naval diet reduced the incidence of the disease. However, at the time he believed the problem to be a manifestation of protein deficiency.

Aqueous or alcoholic extracts of rice husks showed a similar effect, confirming the idea that the beriberi treatment might be related to a chemical substance. Subsequently, in 1912, Casimir Funk came close to isolating the anti-beriberi factor from rice husks, again using chickens to test the extracts. Suspecting that the factor was, in chemical terms, an amine, he invented the term *vitamine*, from "vital amine". He later observed:

I must admit that when I chose the word "vitamine" I was well aware that these substances might later prove to not all be of an amine nature. However it was necessary for me to use a name that would sound well and serve as a 'catch word'.

When it became apparent that other factors were not amines, the final "e" was dropped. Studies in the years up to 1914 on laboratory rats fed highly purified diets

of proteins, fats, starch and minerals demonstrated that “growth factors” were missing from such diets. McCullom and Davis suggested that there were two of these, one associated with milk fat and egg yolk, described as “fat-soluble A”, and another associated with wheat, milk and egg yolk, described as “water-soluble B”. The first of these became identified with the prevention of night-blindness. However, in the 1920s, a second fat-soluble factor was isolated and shown to prevent the bone disease rickets. By this time the, as yet unidentified, factor in fruit and vegetables that prevented scurvy was becoming known as vitamin C. This meant that with the letters A, B and C already spoken for, the anti-rickets (antirachitic) factor was named vitamin D.

Over the years water-soluble B has been resolved into a host of different vitamins, often referred to collectively as the “vitamin B complex”, although they are not physiologically bound to one another as might be implied by the word “complex”. Through the 1920s and 1930s, plant and animal foodstuffs yielded an increasing number of water- and fat-soluble factors that could be linked to the growth of laboratory animals or to their deficiency diseases. For a time, this led to a proliferation of apparent “vitamins” that, with suffixes, used up much of the alphabet. Once chemistry had advanced sufficiently to provide concise chemical definitions and structural formulae, many of these disappeared. Some turned out to be identical to vitamins already identified, or to be mixtures of other known vitamins. Occasionally, they were even found to lack the activity initially attributed to them. Vitamin D₁, considered briefly in Section 8.10, is a good example of a now forgotten vitamin. Others were substances that were interesting, but did not meet the criteria set out at the start of this chapter. Scientists now try to use specific names wherever possible. Where the members of a group of closely related substances, *e.g.* the tocopherols, have differing degrees of vitamin activity, it is better to refer to them collectively, as in this example, as ‘vitamin E’, rather than implying that all tocopherols are equally potent as vitamins.

One should not underestimate the difficulties that the isolation and identification of vitamins presented to the original researchers. For example, the first isolation of pantothenic acid required a quarter of a ton of sheep’s livers as the starting material. Although the chemical structures of most vitamins had been established by the end of the 1930s, that of cobalamin (vitamin B₁₂) was only elucidated in 1955, by Dorothy Hodgkin, at the University of Oxford.

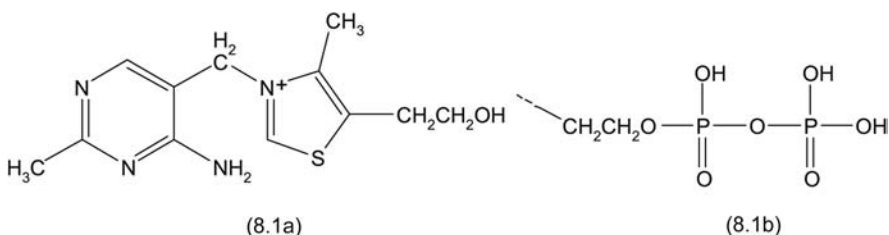
This brief detour into history may seem irrelevant to the modern food chemist, but it is important for us to appreciate how vitamin terminology evolved so that we can recognise when it is being misused. Even today it is common, especially among toiletry products and at the less responsible fringes of the food supplements industry, for obsolete terms to be resurrected and applied misleadingly.

The first tasks for chemists were the isolation of vitamins in a pure state and the determination of their chemical structures. Once these were known, chemists were then called upon to provide sensitive and accurate methods for the determination of vitamin levels in food materials. Initially, the only measurements available were biological assays, based on the relief of deficiency diseases in laboratory animals, but their high cost and the length of time they required made them unsuitable for routine measurements. Almost all vitamin analysis today is carried out using chromatographic methods, particularly high-performance liquid chromatography (HPLC). A second

objective for vitamin chemists was to devise commercially viable chemical syntheses for vitamins to make them available for clinical purposes and as dietary supplements. Commercially viable chemical routes are now available for the synthesis of most vitamins, but, in some cases, notably riboflavin and cobalamin, bacterial fermentation is a more economic method. Although the tocopherols can be chemically synthesised, they are more usually extracted from vegetable oils.

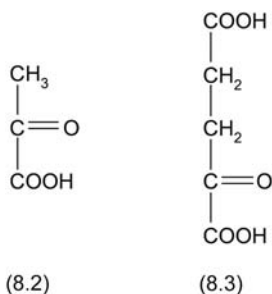
8.1 Thiamin (Vitamin B₁, Aneurine)

Thiamin occurs in foodstuffs either in its free state (8.1a) or as its pyrophosphate ester (8.1b) complexed with protein. No distinction is made between the two forms by the usual analytical techniques or in tables of food composition. Although small amounts occur in a wide range of foodstuffs, only a few can be regarded as useful sources. In general, thiamin is present in the greatest amounts (0.1–1.0 mg per 100 g) in carbohydrate-rich foodstuffs or foods associated with a high level of carbohydrate metabolism in the original living material.ⁱⁱⁱ Examples are legume seeds (*e.g.* raw peas, 0.74 mg per 100 g) and the embryo component of cereal grains (*e.g.* wheat germ, 1.80 mg per 100 g), which have high rates of carbohydrate metabolism during germination of the seed. Although both the muscle tissue and the liver of living animals contain very little carbohydrate, carbohydrate is the chief source of energy for these tissues. The reason why pork contains about ten times the amount of thiamin (about 1 mg per 100 g) than beef, lamb, poultry or fish is yet to be established.



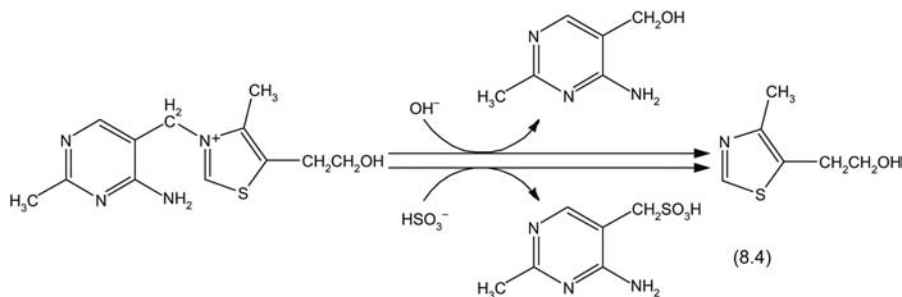
The association of thiamin with carbohydrates arises from its role in metabolism. Thiamin pyrophosphate is the prosthetic group of a number of important enzymes catalysing the oxidative decarboxylation of α -keto acids, including pyruvic acid (8.2) and α -ketoglutaric acid (oxoglutaric acid, 8.3), which occur as intermediates in the Krebs cycle, the key metabolic pathway of respiration. Details of the reactions involving coenzymes are not usually the province of food chemists, but the examples described in the first of the Special Topics at the end of this chapter do go some way towards explaining why the chemical structures of vitamins are so often complex. Thiamin, possibly in the form of the triphosphate, is believed to have a separate role in nerve function, but the details are yet to be established.

ⁱⁱⁱAll the data in this chapter on the vitamin content of particular foodstuffs is derived from McCance and Widdowson's Composition of Foods Integrated Dataset 2019, <https://www.gov.uk/government/publications/composition-of-foods-integrated-dataset-cofid> (last accessed in 2020).



Thiamin, like the other B vitamins, is usually determined by HPLC. The traditional chemical procedure, known as the thiochrome method, is rarely used today.

Thiamin is one of the most labile of vitamins. Only under mildly acid conditions, *i.e.* <pH 5, will it withstand heating. The reason for this is the readiness with which nucleophilic displacement reactions occur at the atom joining the two ring systems. Anions such as OH^- and, more significantly, sulfite, HSO_3^- , split the molecule into two fragments (8.4). This means that the sulfite added to fruits and vegetables to prevent browning will cause total destruction of the thiamin. Fortunately, these foods are not important sources of dietary thiamin. Heat treatments such as canning cause losses of up to 20% of the thiamin, especially when the pH is >6, but it is only in the baking of bread, where up to 30% may be lost, that these losses become really important.



The greatest losses of thiamin, in domestic cooking as well as in commercial food processing, occur simply as a result of its water solubility, rather than due to chemical attack. It is impossible to generalise about the extent of such losses, depending as they do on the amount of chopping and the soaking and cooking times *etc.*, but they can be minimised by making as much use as possible of meat drippings and vegetable cooking water – perhaps gravy^{iv} is nutritious after all.

Polishing rice, *i.e.* the mechanical process of removing the outer layers of the grain and the germ, enhances its storage life by denying invading moulds some of their nutrients. Although this practice is a major cause of the thiamin deficiency common in those parts of the world where rice is a staple, the difficulty of storing rice in warm, humid climates makes it essential. One way in which its effects can be minimised is by parboiling, *i.e.* partial boiling. The rice is steeped in water, steamed and dried. Ostensibly this is carried out

^{iv}Readers unfamiliar with British cuisine may not realise that roast meat is traditionally served with a sauce known as “gravy”, ideally made from the salted water in which vegetables have been cooked together with any meat residues in the roasting pan, thickened with flour and darkened with caramel (gravy browning).

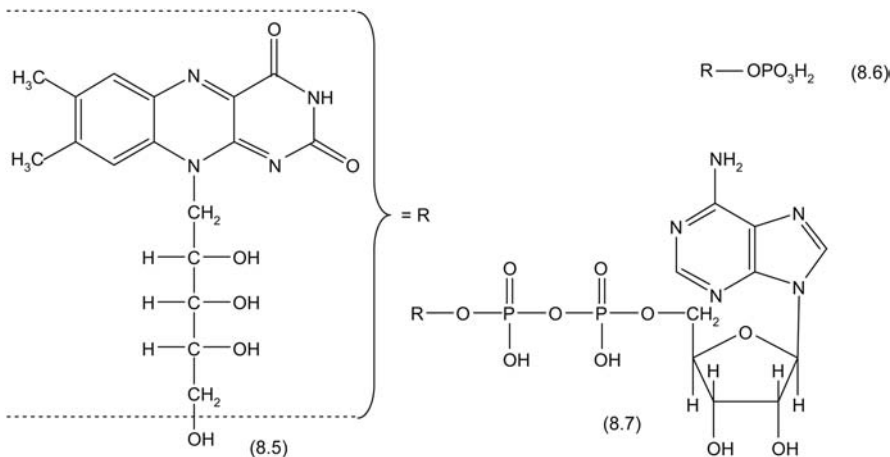
to make polishing easier, but, in fact, it allows the thiamin to diffuse from the germ into the endosperm, so that it is not lost when the outer layers are removed. Unfortunately, this does not have a beneficial effect on the availability of niacin. This vitamin's poor solubility means that it does not diffuse into the endosperm when the rice is steeped.

The question of how much of any particular vitamin is required to maintain health in various circumstances is more appropriate to textbooks of nutrition, but thiamin is an unusual case. Its involvement in carbohydrate metabolism has already been referred to and it now is recognised that the minimum intake of thiamin required by an individual will reflect the energy content of their diet. Authorities recommend a minimum thiamin intake of $96 \mu\text{g MJ}^{-1}$ (0.4 mg kcal^{-1}).

In some parts of the world, such as rural Thailand, where raw or fermented (but uncooked) fish is an important constituent of the diet, an enzyme found in shellfish and the viscera of freshwater fish has been implicated in thiamin deficiency. The enzyme, thiaminase I, breaks the link between the nitrogen atom of the thiazole ring and the $-\text{CH}_2$ group. Fortunately, the activity of the enzyme is destroyed by heating. Of probably greater nutritional significance are several thermostable anti-thiamin factors found in tea, coffee and many fruits and vegetables. They are all polyphenolics, notably caffeic acid (3,4-dihydroxycinnamic acid, 6.13), chlorogenic acid (6.24) and quercetin (6.35). They are recognised as leading to symptoms of thiamin deficiency, especially in communities where the habitual chewing of fermented tea leaves or betel nuts (seeds of areca palm, *Areca catechu*) is common. The high tannin content of black tea (see Chapter 6, Section 6.5.1) and dark roasted coffee has also been implicated in thiamin deficiency.

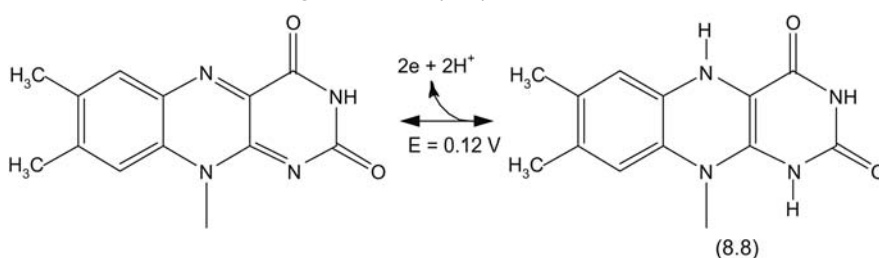
8.2 Riboflavin (Vitamin B₂)

The structure of this vitamin is usually presented as riboflavin itself (8.5), the isoalloxazine nucleus with a ribitol side-chain attached, but in most biological materials it occurs predominantly as two nucleotides: flavin mononucleotide (FMN, 8.6) and flavin adenine dinucleotide (FAD, 8.7). These occur both as prosthetic groups (see Chapter 12, Section 12.1.1) in the respiratory enzymes known as flavoproteins and also in the free state, when they are referred to as coenzymes.



The distribution of riboflavin in foodstuffs is similar to that of thiamin, in that it is present in at least small amounts in almost all biological tissues. It is particularly abundant in meat (0.2 mg per 100 g) and especially in liver (3.0 mg per 100 g). In contrast with thiamin, cereals are not a particularly rich source, but milk (0.15 mg per 100 g) and cheese (0.5 mg per 100 g) are valuable sources. Dried brewer's yeast and yeast extracts contain large amounts of riboflavin and many other vitamins, but it is interesting that the thiamin does not leak out of the yeast during fermentation, whereas riboflavin does (giving a concentration in beer of up to 40 µg per 100 g).

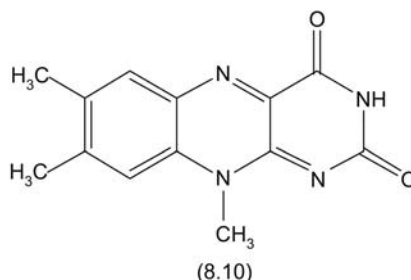
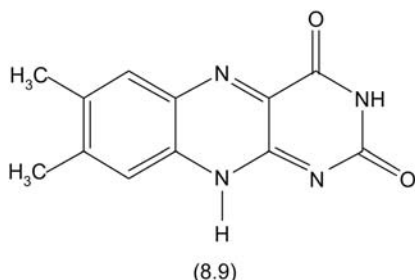
The flavoprotein enzymes of which riboflavin forms part of the prosthetic group are all involved in oxidation/reduction reactions. It is the isoalloxazine nucleus that receives electrons from the substrates which are being oxidised and donates electrons to the substrates which are being reduced (8.9):



Most flavoprotein enzymes are involved in the complex respiratory processes that occur in the mitochondria of living cells, but some take part in other aspects of metabolism. The enzyme glucose oxidase, mentioned in Chapter 2, is one example. This enzyme transfers two hydrogen atoms from C-1 of the glucose to a molecule of oxygen *via* the FAD prosthetic group, resulting in the formation of hydrogen peroxide.

Riboflavin is one of the most stable vitamins. The alkaline pH conditions in which it is unstable are rarely encountered in foodstuffs. The most important feature of its stability is its sensitivity to light. Although this is not significant in opaque foods such as meat, the effect can be dramatic in milk. It has been reported that as much as 50% of the riboflavin contained in the traditional glass bottle of milk is destroyed by two hours of exposure to bright sunlight. Although the widespread replacement of glass with polyethylene terephthalate (PET) can reduce riboflavin losses, only fully opaque cartons made from coated board solve this problem completely. The bright lights of supermarket displays may be just as damaging as sunlight. The principal product of this irradiation is known as lumichrome (8.9) and some lumiflavin (8.10) is also produced at neutral and alkaline pH values. The breakdown of riboflavin has wider significance than the simple loss of vitamin activity. Both the breakdown products are much stronger oxidising agents than riboflavin itself and catalyse the massive destruction of ascorbic acid (vitamin C). Even a small decrease in the riboflavin content of milk from this cause can lead to a near-total elimination of the ascorbic acid content. It is fortunate that milk is not an important source of vitamin C in most diets. The other important side-effect of riboflavin breakdown is that during the course of the reaction, which involves interactions with oxygen, highly reactive states of the oxygen molecule are produced, in particular singlet oxygen. Although this has only a transient existence,

it is able to initiate the autoxidation of unsaturated fatty acids in the milk fat, as discussed in Chapter 4. The result is an unpleasant off-flavour.



As vegetables are not an important source of riboflavin, losses by leaching during blanching or boiling in water are not significant. Other cooking operations, including roasting meat and baking cereals, also have negligible effects.

Riboflavin is not readily determined in the laboratory unless HPLC is available. The most commonly applied chemical technique requires an initial treatment with dilute hydrochloric acid at high temperatures to release the riboflavin from the proteins to which it is normally bound. After clean-up procedures, the extract is examined fluorimetrically. The absorbance by riboflavin at 440 nm is accompanied by an emission at 525 nm.

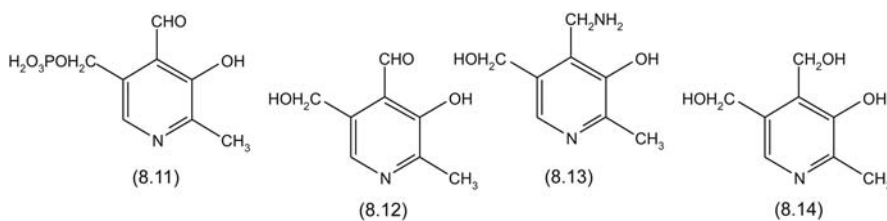
Before HPLC became widely available, laboratories that routinely determined the levels of many different vitamins in food often used microbiological assays. These depend on the existence of special strains of bacteria that have lost the ability to synthesise one particular vitamin for themselves. These special strains are obtained by subjecting more normal (*i.e.* wild-type) members of the species to irradiation or mutagenic chemicals and isolating mutants with the required characteristics. Specially prepared growth media are used, providing all the nutrients required by the test strain, except the vitamin being determined. The amount of bacterial growth in a culture using this medium depends on how much of the vitamin is present, either from a food sample or from a standard solution used for comparison. Although difficult to carry out, the sensitivity of microbiological assays, in experienced hands, is impressive. The assay for riboflavin using a strain of *Lactobacillus casei* covers the range 0–200 ng per sample, and this is one of the least sensitive vitamin assays. The vitamin B₁₂ assay using *Lactobacillus leichmannii* has an assay range of 0–0.2 ng per sample.

Riboflavin has been manufactured commercially since the 1930s. In 1980, the original, entirely chemical, synthesis was superseded by a process in which D-ribose synthesised by bacterial fermentation was the starting point for an elaborate series of chemical manipulations. This process has now, in turn, been superseded by an entirely biotechnological processes using mutant strains of either *Bacillus subtilis* or *Ashbya gossypii* (a filamentous fungus related to yeast).

Although a great deal is known about the biological functions of riboflavin, the symptoms of deficiency are not especially distinctive, being similar to those that are also associated with deficiencies of other B vitamins, notably pyridoxine. Although a degree of riboflavin deficiency is widespread among human populations, it is not believed to be fatal. One reason for this is that the riboflavin released from existing flavoproteins during the normal processes of protein turnover and cellular renewal is very efficiently reused in the synthesis of new flavoprotein molecules.

8.3 Vitamin B₆ (Pyridoxine)

The generic term “vitamin B₆” describes a group of substances including pyridoxal phosphate (8.11), the prosthetic group of a number of enzymes. The other members of the group are pyridoxal (8.12), pyridoxamine (8.13), pyridoxine (8.14) and the 5'-phosphates of pyridoxamine and pyridoxine. In the past, the term “pyridoxine” has often been used to cover all six, with the term “pyridoxol” used specifically for the alcohol (8.14). Pyridoxal phosphate is the form of the vitamin active in tissues, again as the prosthetic group of a number of enzymes. However, when the vitamin is identified in foodstuffs, it is in one of three forms, all having lost the phosphate group: pyridoxine, pyridoxal or pyridoxamine. In foodstuffs of plant origin, the first two of these usually predominate, whereas in animal materials the last two predominate. At least two-thirds of the vitamin in living tissues is probably present as enzyme prosthetic groups and, as such, tightly bound to protein. In muscle tissue, one particular enzyme, phosphorylase,^v makes up the major share of the total. Milk is one exception, with only about 10% being protein-bound.



Vitamin B₆ is widely distributed among foodstuffs, being found in at least small amounts (around 10 µg per 100 g) in almost all biological materials in which it has been sought. Meat and other animal tissues, and egg yolk, are particularly rich sources (about 500 µg per 100 g); milk and cheese contain about 50 µg per 100 g. The germ of wheat grains is another particularly rich source (about 500 µg per 100 g), accounting for >90% of the total in the complete grain. In consequence, a high proportion (75–90%) of the vitamin is lost in the manufacture of white flour. The wide distribution of this vitamin, however, means that any diet poor enough to be deficient in it will almost certainly also be deficient in most of the other B vitamins as well, and a specific clinical deficiency is rarely encountered in humans.

Compared with other vitamins active as enzyme prosthetic groups, pyridoxal phosphate is fairly versatile in terms of the types of reactions in which it is involved. Almost all the enzyme-catalysed reactions in which it participates have amino acids as substrates, with phosphorylase being a notable exception. Details of a transamination reaction, one of the best-known reactions involving pyridoxal phosphate, are given in the first of the Special Topics at the end of this chapter.

The question of the stability of vitamin B₆ during food processing is complicated by the fact that its different forms differ in stability, and that they also tend to interconvert during some processing operations. Pyridoxol is very stable to heat within the pH range usually encountered in foodstuffs. The other two forms are slightly less stable, but, as with the other water-soluble vitamins, leaching is the major cause of losses during

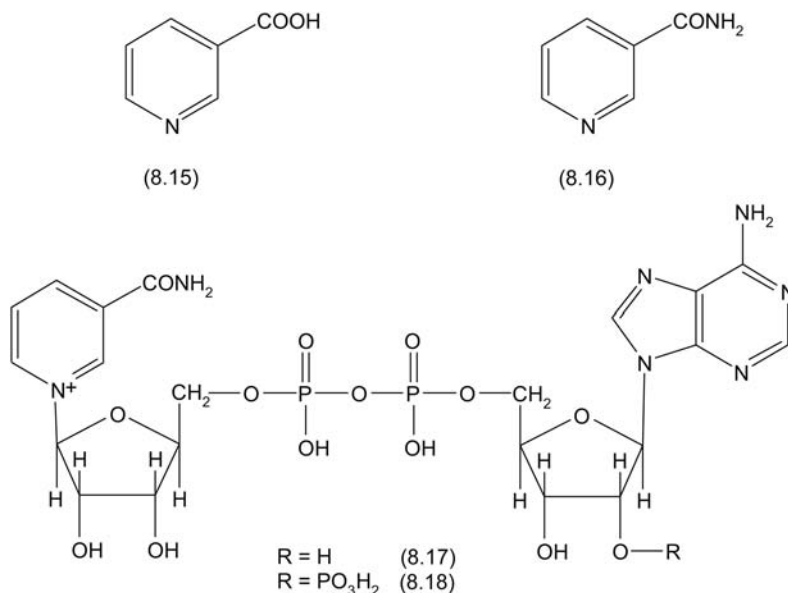
^vPhosphorylase catalyses the breakdown of glycogen, the energy reserve glucose polymer in animal tissues: glucose_(n) + phosphate → glucose_(n-1) + glucose-1-phosphate.

cooking and processing. Although there is little loss of vitamin activity during ordinary milk processing (pasteurisation or UHT treatment), the more drastic heating involved in the production of evaporated or condensed milk causes extensive conversion of pyridoxal, the predominant form in raw milk, to pyridoxamine. A similar change occurs during the boiling of ham. When milk is dried, extensive losses of pyridoxal can occur due to interactions with the sulfhydryl groups of proteins. Interaction with the free amino groups of proteins leads to the formation of pyridoxamine, which does not cause a loss of vitamin activity.

The analysis of pyridoxine levels in foods is not practical using chemical techniques and HPLC or microbiological methods must therefore be used. A special source of difficulty with the microbiological assay of this vitamin is that microorganisms differ in their response to the different forms of the vitamin, although apparently there are no differences in the responses of animals, including humans, to the different forms.

8.4 Niacin (Nicotinic Acid, Nicotinamide, Vitamin B₃)

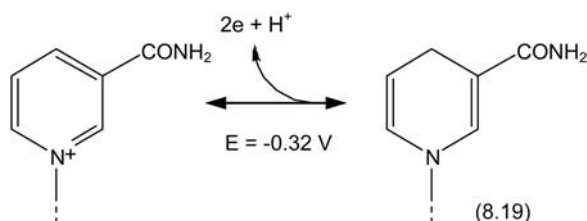
Niacin is a collective name for nicotinic acid (8.15) and its amide, nicotinamide (8.16).^{vi} Niacin is included in the vitamin B group (as are all the water-soluble vitamins except ascorbic acid), but its previous designation as vitamin B₃ is now obsolete. In living systems the pyridine ring occurs as a component of nicotinamide adenine dinucleotide (NAD, 8.17) and its phosphate derivative (NADP, 8.18).^{vii} NAD occurs as a prosthetic group in a few enzymes, *e.g.* glyceraldehyde-3-phosphate dehydrogenase, but most of the NAD and NADP in living cells functions as electron carriers in respiratory systems.



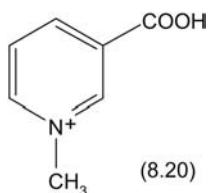
^{vi}In the USA, “niacin” specifically refers to nicotinic acid rather than to both forms of the vitamin. The obsolete term “vitamin B₃”, is usually only encountered in the marketing of vitamin supplements.

^{vii}In biochemistry textbooks it is usual to include the positive charge (see 8.18–8.20) in the abbreviated names of the oxidised forms: NAD⁺ and NADP⁺. In older literature, NAD and NADP are referred to as the di- and triphosphopyridine nucleotides, DPN and TPN, respectively.

For example, in most of the Krebs cycle reactions (the key metabolic pathway of respiration), the electrons removed from the organic acid substrates are transferred initially to NAD to form NADH (8.19) and are then used to reduce oxygen to water in the oxidative phosphorylation system of the mitochondria. The route from NAD involves first flavoproteins and then porphyrin-ring-containing proteins known as cytochromes. A striking feature of the reduction of NAD is the very high absorbance of NADH at 340 nm (molar extinction coefficient, $\epsilon = 6.22 \times 10^6$), a wavelength at which the oxidised form has almost no absorbance at all.



In general, the distribution of niacin in foodstuffs is similar to that of the other vitamins already discussed in this chapter. Although present in small amounts in all biological materials, it is meat (with 5–15 mg per 100 g) that provides the richest source of niacin in all but vegetarian or vegan diets. Dairy products, such as milk, cheese and eggs, are surprisingly poor sources, with no more than 0.1 mg per 100 g. Fruit and vegetables, particularly legume seeds, are useful sources (0.7–2.0 mg per 100 g). Green coffee beans contain trigonelline (*N*-methylnicotinic acid, 8.20), which is converted to nicotinic acid during the roasting process. The resulting levels of nicotinic acid range from 80 mg kg⁻¹ when medium roasted and up to 500 mg kg⁻¹ in dark roasted beans.



Many cereals contain apparently high levels of niacin (*e.g.* wholewheat flour, 5 mg per 100 g, and brown rice, 4.7 mg per 100 g). However, as with pyridoxine, milling can have drastic effects because a large proportion of the niacin is located in the germ; white bread and wholemeal bread contain 1.7 and 4.1 mg per 100 g, respectively.

To compound this problem, much of the niacin in cereals is tightly bound to other macromolecules in the grain. A small protein has been implicated in wheat, rice and maize and a polysaccharide, probably a hemicellulose, is also involved in both wheat and maize. The normal digestive processes in the small intestine do not liberate the vitamin and it remains unabsorbed. This problem is especially important for consumers of maize.^{viii} Before the middle of the eighteenth century, there was no record of pellagra, the disease caused by a deficiency of niacin, but after this time it became increasingly prevalent in southern Europe. People affected by pellagra show a diverse range of symptoms, including a characteristic form of dermatitis with degrees of dementia, diarrhoea and other symptoms.

^{viii}“Corn” in North America, Australia and New Zealand.

The increase is now clearly seen to be associated with the replacement of rye with maize as a dietary staple, but the connection was not made at the time. Pellagra was endemic in the southern USA, but the significance of its absence in population of the Yucatan peninsula, Mexico, where maize was first cultivated, was not recognised. Even when the link to maize was identified, it was assumed that a microbial toxin present in spoilt maize was to blame. It is now recognised that the reason for the apparent immunity to pellagra in Mexico is that the maize flour used for making tortillas, the dietary equivalent of bread, was traditionally ground from maize kernels that had first been treated with lime water. The purpose of the alkali was to weaken the husk of the maize kernel to make milling easier, but we now know that, under alkaline conditions, heating liberates the bound, unavailable niacin. Consumers of “corn chips” and other Westernised versions of tortillas will be reassured that alkali treatment of maize is still a key step in their manufacture.

The human body itself has a limited, but significant, ability to synthesise the vitamin itself. *Via* a rather tortuous metabolic pathway, the amino acid tryptophan (see Figure 5.2) can be converted to nicotinic acid. The effectiveness of this route is inevitably low as a result of the competing demands of protein synthesis for tryptophan. Experimental evidence suggests that about 60 mg of dietary tryptophan is required to replace 1 mg of dietary nicotinic acid. This explains the beneficial effect of milk and eggs in cases of pellagra, even though both are poor sources of the actual vitamin. It also illustrates the care needed in the interpretation of food composition tables when diets are being assessed. Niacin is stable under most conditions of cooking and processing, leaching being the only significant cause of losses. The availability of niacin may actually increase during the baking of cereals, especially in products rendered slightly alkaline by the use of baking powder.

8.5 Cobalamin (Cyanocobalamin, Vitamin B₁₂)

The chemical structure of cobalamin is by far the most complex of all the vitamins. As shown in Figure 8.1, its most prominent feature is a corrin ring system. This resembles the porphyrin ring systems found in chlorophyll (see Chapter 6) and haem proteins, *etc.* (see Chapter 5). However, it differs from these in two respects: in cobalamin, the coordinated metal atom is cobalt, rather than magnesium or iron, and the ring substituents are mostly amides. The fifth coordination position is occupied by a nucleotide-like structure based on 5,6-dimethylbenzimidazole, which is also attached to one of the amide groups in the corrin ring. The sixth coordination position (X in Figure 8.1), on the face of the corrin ring opposite the fifth position, can be occupied by a number of different groups. As normally isolated in the laboratory, the vitamin contains a CN group in the sixth position, hence the name cyanocobalamin. This form does not actually occur in nature, but is much more stable than the other forms and also readily absorbed by the body when supplied as a nutritional supplement or pharmaceutical preparation. In its functional state, in which it is the prosthetic group of a number of enzymes, it may have either a methyl group or a 5'-deoxyadenosyl residue in the sixth position.

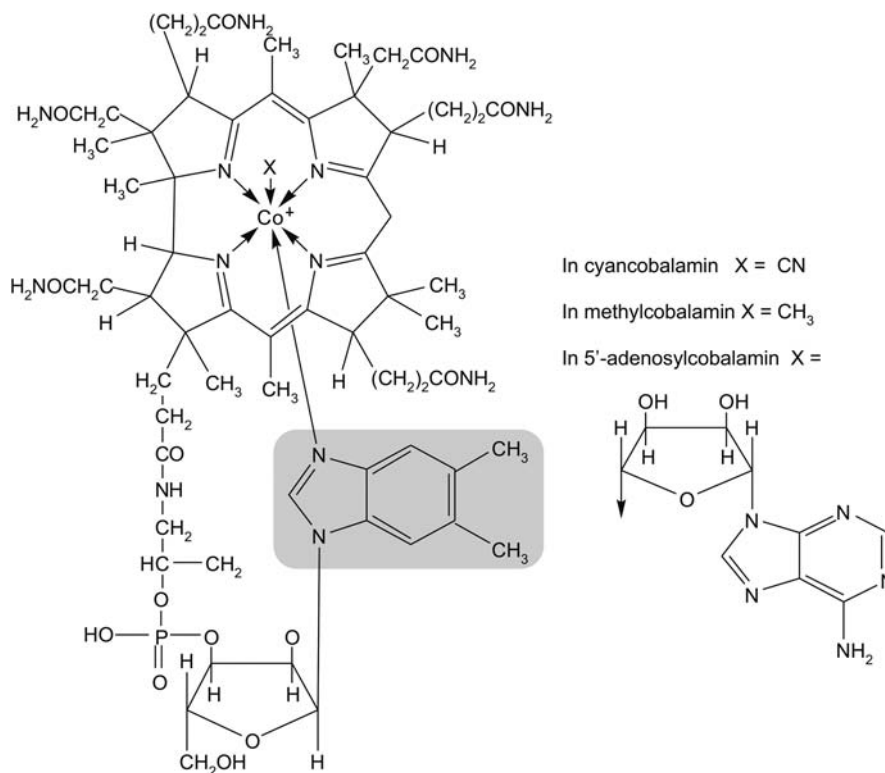


Figure 8.1 Structure of cobalamin and its derivatives. In the absence of these or other ligands (*i.e.* X) a water molecule occupies the sixth coordination position. The shaded region is the 5,6-dimethylbenzimidazole moiety, which is replaced by an adenine moiety in pseudovitamin B₁₂, a variant of cobalamin that actually lacks vitamin activity in humans but is the dominant variant found in “health food” products based on algae such as *Spirulina*.

The distribution of cobalamin in foodstuffs is most unusual. It is widely accepted that bacteria are the only organisms capable of synthesising it and it is therefore never found in higher plants. Possible exceptions to this are discussed in the following text. When trace amounts are detected in vegetables, the source is almost always surface contamination with faecal material that has been used as fertiliser. The bacterial flora of the colon collectively secretes a total of about 5 µg daily, but because there is no absorption from the colon, this is wastefully excreted. The daily requirement is about 1 µg. The vitamin occurs in useful quantities in most foodstuffs of animal origin (eggs 0.7 µg per 100 g, milk 0.3 µg per 100 g, meat (muscle tissue) 1–2 µg per 100 g, liver 40 µg per 100 g, kidney 20 µg per 100 g), but in all these cases the vitamin got there either from microorganisms or animal tissues consumed in the diet, or from synthesis by intestinal microorganisms high enough in the alimentary tract for absorption to occur.

As a result of the low requirement for this vitamin and its abundance in many foodstuffs, dietary deficiency is extremely unlikely, although vegans are advised to take

a B₁₂ supplement. It may take as long as 10 years after ceasing consumption of the vitamin for an otherwise healthy individual to show deficiency symptoms because the vitamin is lost from the body at an extremely slow rate. In contrast with the other water-soluble vitamins, the body is able to conserve vitamin B₁₂ by storing it in the liver.

The classic disease of apparent cobalamin deficiency is pernicious anaemia. This is almost always caused by an inability to absorb the vitamin rather than lack of it in the diet. People with pernicious anaemia are unable to synthesise a carbohydrate-rich protein known as “intrinsic factor”. This is usually secreted by the gastric mucosa and forms a complex with the vitamin. Only in this complexed form is the vitamin readily absorbed from the small intestine. In the 1920s, pernicious anaemia was successfully treated by patients consuming large daily amounts (100–200 g) of calves’ liver (apparently raw), which would provide enough of the vitamin to overcome its inefficient absorption.

In mammals, the catalytic activities of most cobalamin enzymes involve one-carbon units such as methyl groups, an area of intermediary metabolism in which coenzymes based on folic acid, another B vitamin, are also involved. The details are really outside the scope of food scientists, but will be touched on here to show how a deficiency of cobalamin leads to anaemia.

The synthesis of DNA is obviously dependent on a supply of the purines and pyrimidines (adenine, guanine, cytosine and thymine) contained in their component nucleotides. The synthesis of purines and pyrimidines depends on the supply of a particular form of tetrahydrofolate, the biochemically active form of folic acid. The enzyme reaction that maintains the supply of tetrahydrofolate has methyl cobalamin as a coenzyme. A deficiency of cobalamin thus inhibits DNA synthesis and the rapidly dividing cells of the bone marrow, which generate red blood cells (erythrocytes), are among the first to die. The resulting shortage of erythrocytes is known as megaloblastic anaemia. Not surprisingly, a deficiency of folic acid also causes anaemia, but pernicious anaemia caused by cobalamin deficiency is specifically accompanied by neuropathy, or damage to the nerve cells, which starts in the peripheral nervous system, but eventually reaches the spinal cord.

Despite the complexity of its structure, cobalamin is fairly stable to food processing and cooking conditions, and the losses that do occur are not sufficient to cause concern to nutritionists. As with all the water-soluble vitamins, leaching is the major cause of loss.

The microbiological assay mentioned earlier in this chapter (see Section 8.2) is the only practical method of determining the vitamin in foodstuffs. However, although suitable for measurements in food materials of animal origins, it has been found to be of limited value for materials of microbial origin. Potential sources of vitamin B₁₂ that meet the needs of vegan consumers have been sought and various species of cyanobacteria (*i.e.* blue-green algae) and unicellular algae have appeared to be suitable on the basis of the microbiological assay using *L. leichmannii*. In particular, tablets of compressed, dried cells of the cyanobacterium *Spirulina platensis* have been marketed for several years as an alternative source of the vitamin. Unfortunately, these microorganisms rarely contain the form of cobalamin active in humans, but instead contain a

variant known as “pseudovitamin B₁₂”. This supports the growth of *L. leichmannii* in the microbiological assay, but does not bind to the intrinsic factor that facilitates absorption of the vitamin into the bloodstream. Pseudovitamin B₁₂ differs from the active vitamin as shown in Figure 8.1.^{ix}

The complete chemical synthesis of vitamin B₁₂ was only achieved in 1973, with a sequence of about 70 different reactions, far too many to make commercial manufacture by this route feasible. Large numbers of bacterial species have been shown to produce the vitamin, but specialised strains of *Pseudomonas denitrificans* or *Propionibacterium freudenreichii* subsp. *shermanii* are now used almost exclusively for the commercial manufacture of vitamin B₁₂ supplements. *P. freudenreichii* has the special advantage of not synthesising any toxins during its culture.

8.6 Folic Acid (Folacin, Vitamin B₉)

Folic acid is the name given to a group of widely distributed compounds whose vitamin activity is related to that of cobalamin. The structures of folic acid and its relatives are shown in Figure 8.2.

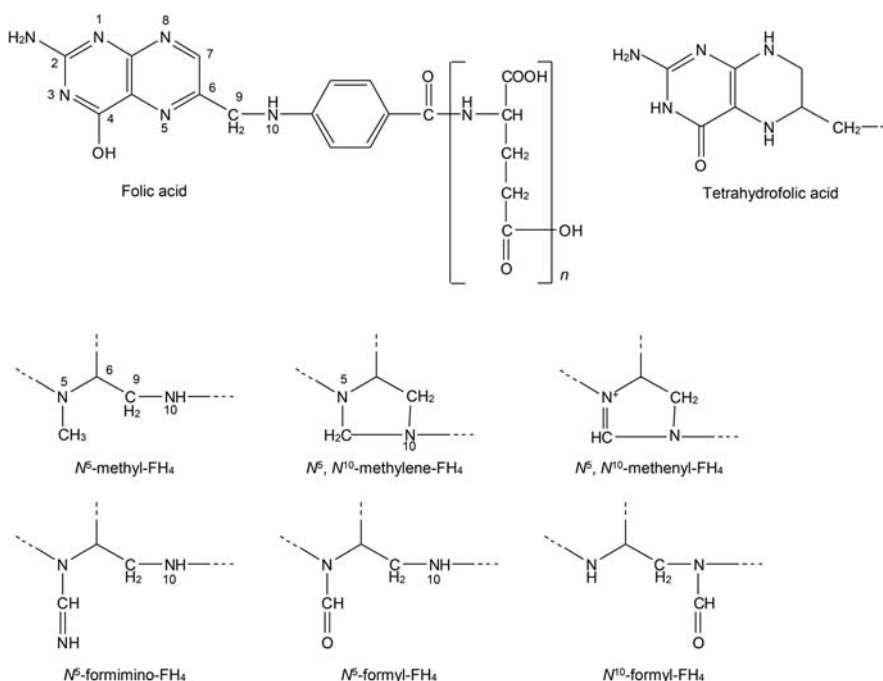


Figure 8.2 Structures of folic acid, tetrahydrofolic acid and the one-carbon units carried by tetrahydrofolate ($n = 3$ to 7).

^{ix}In 2020, reports appeared that suggested that a water plant common in Asia, duckweed (or water lentil), *Wolffia globosa*, is a potential plant source of vitamin B₁₂. Although duckweed has potential as source of supplementary proteins for human consumption, there is concern that it may prove to be another source, like *Spirulina*, of a pseudovitamin.

The form of the vitamin active as a coenzyme is tetrahydrofolate, which has the pteridine nucleus in its reduced state and several glutamate residues carried on the *p*-aminobenzoate moiety. The term “folic acid conjugate” is commonly used to refer to forms of the vitamin with three or more glutamate residues. Accurate determination of the folic acid content of foodstuffs is difficult, even using microbiological assays, because it is necessary to remove all but one of the glutamyl residues before consistent results are obtained. This can now be achieved by enzymic methods, but it has meant that earlier estimates of the folic acid content of foodstuffs could be orders of magnitude too low. In animal tissues, in which the pentaglutamyl conjugate predominates, liver is a particularly good source (300 µg per 100 g); muscle tissues contain much less (3–8 µg per 100 g). Leafy green vegetables, in which the heptaglutamyl conjugate predominates, have around 20–80 µg per 100 g) and probably constitute the most important source in modern diets.

As a coenzyme, tetrahydrofolate (FH₄; see Figure 8.2) is concerned solely with the metabolism of one-carbon units, which are bound to either N-5 or N-10. While bound to the coenzyme, the one-carbon unit may be oxidised, reduced or undergo other changes, giving the variety of forms shown in Figure 8.2. These are intimately involved in the metabolism of many amino acids, purines and pyrimidines (components of nucleotides and nucleic acids) and, indirectly, haem. In animals, the metabolism of methyl groups, which are mainly supplied in the diet by choline (see Figure 4.21), is particularly complex. Reactions requiring both folic acid and cobalamin are involved and it is therefore not surprising that there is some overlap in the deficiency symptoms of these two vitamins.

There is growing support for the view that folic acid deficiency is widespread, even in the comparatively well-fed populations of Europe and North America. A deficiency in folic acid in the very early stages of pregnancy (the periconceptual period) can lead to neural tube defects, such as spina bifida, in the fetus. An average diet may not supply sufficient folic acid and vitamin supplements are now recommended in the UK and elsewhere to ensure that all young women consume 0.4 mg daily to ensure adequate folate reserves in advance of pregnancy. An intake ten times this figure is recommended if a neural tube defect has affected a previous pregnancy. The importance of supplementation is increased by the observation that the bioavailability of naturally occurring folates is actually lower than that from folate supplements. There is also considerable support for the view that improving the levels of folates in the diet can reduce the incidence of strokes and other cardiovascular diseases.

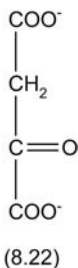
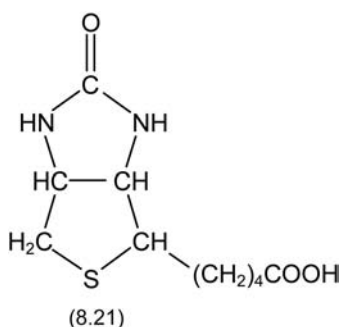
Routine dietary fortification with folic acid was not recommended in the past. This is because extra folic acid can alleviate, and therefore mask, the anaemia caused by cobalamin deficiency. However, folic acid has no effect on the neurological damage that can also result from cobalamin deficiency and, by the time this damage is detected, it cannot be reversed. In spite of this reservation, in 1998 the authorities in the USA made folic acid fortification mandatory in specified grain products, including bread-making flour and corn meal, at a level of 140 mg per 100 g. Since adopting this policy, rates of neural tube defects in the USA have almost halved. Various countries, including Australia, New Zealand and Canada, have introduced similar fortification. It has been estimated that about 13 million people in the UK have a low folate intake, with 700–900 pregnancies affected by neural tube defects each year.

In 2022, the UK authorities began consultations on the mandatory fortification of flour with 250 μg of folic acid per 100 g flour. Although this could undoubtedly improve the folic acid status of pregnant women, and other population groups, there is concern that it may increase the number of people consuming excessive levels of folic acid from inappropriate supplements and other voluntarily fortified foods. Long-term intake levels $>1 \text{ mg day}^{-1}$ (for adults, less for children) are not recommended. Elevated serum levels of homocysteine ($^-\text{OOC}-\text{CHNH}_3^+-\text{CH}_2-\text{CH}_2-\text{SH}$), an intermediate in the metabolism of methionine, have been linked to increased risks of heart disease. There are some indications that low doses of folic acid ($200 \mu\text{g day}^{-1}$) may be effective in lowering serum levels of homocysteine, the normal conversion of which to the amino acid methionine requires methyl tetrahydrofolate.

Folic acid and its derivatives are considered to be reasonably stable under the conditions prevailing in most cooking and food processing operations. Stability to heat and acidity depend both on the number of glutamyl residues and the nature of any attached one-carbon units. Oxidation can be a problem, but ascorbic acid provides a valuable protective effect. As with other water-soluble vitamins, most cooking and processing losses are the result of leaching.

8.7 Biotin (Vitamin B₇) and Pantothenic Acid (Vitamin B₅)

These two vitamins have little in common in terms of structure or function. What brings them together is the total absence of reports of deficiency symptoms in humans or animals, other than under the artificial conditions of the laboratory. Biotin (8.22) occurs as the prosthetic group of many enzymes that catalyse carboxylation reactions, such as the conversion of pyruvate (8.2) to oxaloacetate (8.23).



Most foodstuffs contain at least a few micrograms of these vitamins per 100 g (assayed microbiologically) and there appears to be a more than adequate amount present in any

otherwise satisfactory diet. The only known deficiency of biotin occurs when extremely large quantities of raw egg are eaten. Egg white contains a protein, avidin, which complexes biotin, but which is denatured when eggs are cooked. Biotin is apparently stable under cooking and processing procedures.

As with biotin, much more is known about the biochemical function of pantothenic acid (Figure 8.3) than about its status as a food component. It occurs in two forms in living systems: as part of the prosthetic group of the acyl carrier protein component of the fatty acid synthetase complex and as part of coenzyme A, the carrier of acetyl (ethanoyl) and other acyl groups in most metabolic systems. In both of these roles, the acyl group is attached by a thioester link (see Figure 8.3) to the sulfhydryl group, which replaces the carboxyl group of the free vitamin. Pantothenic acid is extremely widely distributed, usually at about 0.2–0.5 mg per 100 g, which is generally assumed to provide a more than adequate dietary intake. Liver contains especially high levels, about 5–10 mg per 100 g. Fairly high losses of pantothenic acid have been reported to occur during food processing, mostly due to leaching, but some decomposition is also known to occur during heating under alkaline or acid conditions.

The term “provitamin” is used to describe substances that are direct precursors of vitamins and their presence in the diet can be a substitute for the vitamin itself. The most obvious example is β -carotene, whose relationship to retinol, vitamin A, was described in Chapter 6 and will be given more attention later in this chapter. However, the term “provitamin B₅” has been adopted for panthenol (8.23) and its ethyl ester. These are sometimes encountered as ingredients of cosmetics and shampoos and their description as provitamins seems to be connected more with marketing than nutrition. Although panthenol can be absorbed by the skin (at least by the skin of laboratory rats)

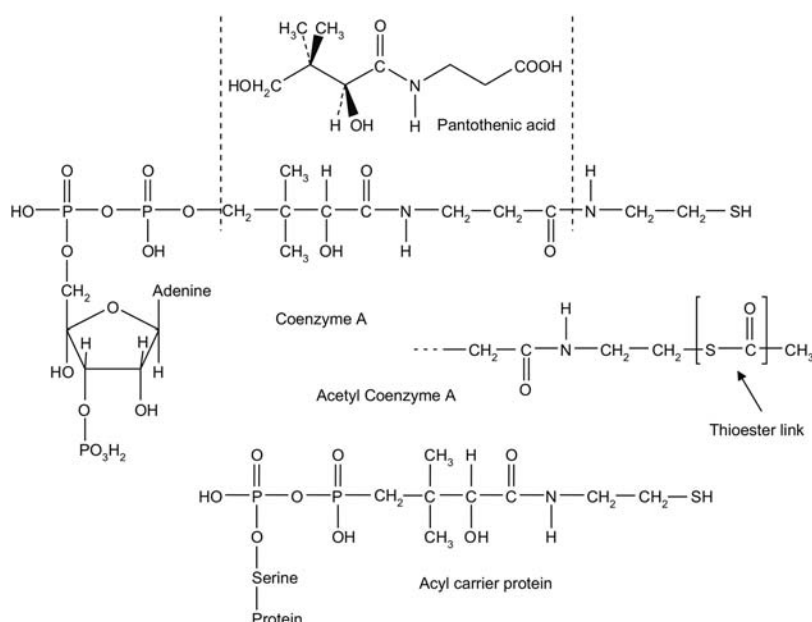
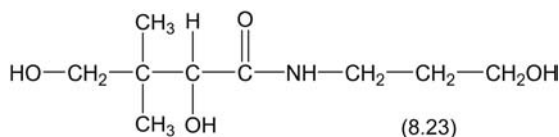


Figure 8.3 Pantothenic acid and its relationships with coenzyme A and acyl carrier protein.

and converted to pantothenic acid, which then appears in urine, the relevance of this to the nutrition of the skin or the appearance of one's hair is doubtful.



8.8 Ascorbic Acid (Vitamin C)

Ascorbic acid is a rather curious vitamin. It occurs widely in plant tissues and it is also synthesised by almost all mammals, and is not therefore a vitamin for them, the exceptions being the primates, guinea pigs and some species of fruit-eating bats. The only discernible common feature of this otherwise disparate collection of mammals is a liking for fruit, a rich source of the vitamin. The structures of L-ascorbate ($\text{p}K_{\text{a}}$ 4.04 at 25 °C) and some related substances are shown in Figure 8.4. The oxidised form, dehydro-L-ascorbic acid (DHAA) (an unfortunate name, since the molecule has no ionisable groups), is distributed with the reduced form in small, rarely accurately determined, proportions. DHAA has virtually the same vitamin activity as L-ascorbate. The mirror image isomer, most commonly referred to as erythorbic acid, has no vitamin activity, but does behave similarly to ascorbate in many food oxidation/reduction systems and it therefore finds some similar applications as a food additive.

Much more is known about the distribution of ascorbic acid in foodstuffs than any other vitamin. This is not because ascorbic acid is inherently more interesting or more important, but simply because it is much easier to determine than the other vitamins, so that when a research worker or student decides to do an experimental project “on vitamins”, then this is the one that tends to be chosen. The richest sources are fruits, but as the data in Table 8.1 show, wide variations do occur. It has to be remembered that a foodstuff that is eaten in large quantities will be an important dietary source even if it contains only modest levels of the vitamin. The very high levels to be found in parsley are nutritionally insignificant when compared with the quantity in the new potatoes it is used to garnish. Cereals contain only trace amounts, and of the animal tissue foods

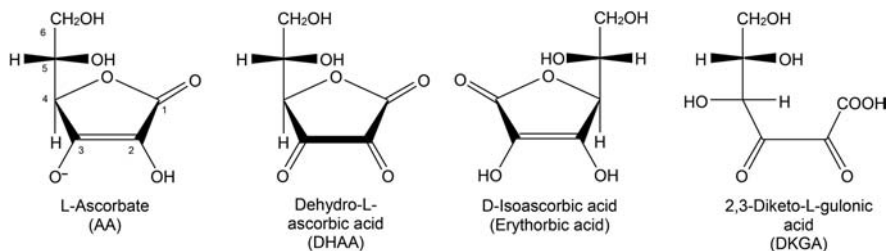


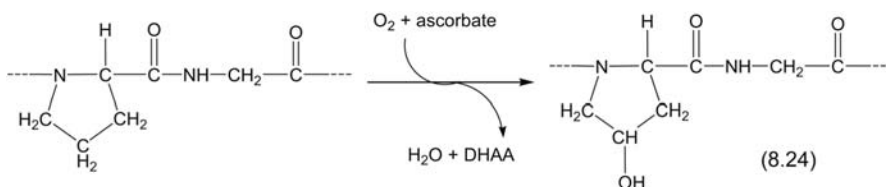
Figure 8.4 Structures of ascorbic acid and related substances.

Table 8.1 Typical ascorbic acid contents of some fruit and vegetables. Values for particular samples may vary considerably from these figures, which refer to content of the edible part, when fresh.

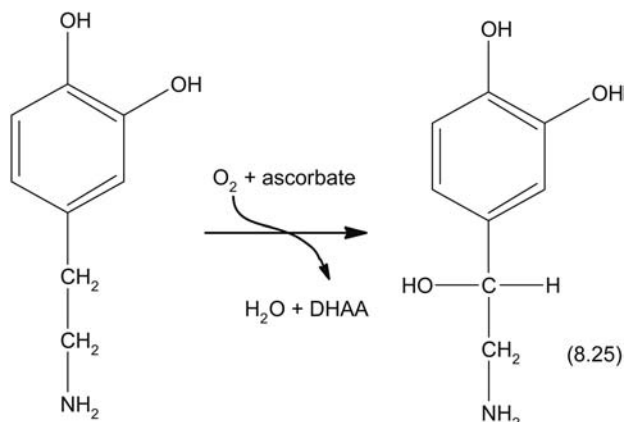
Ascorbic acid (mg per 100 g)		Ascorbic acid (mg per 100 g)	
Plums	3	Carrots	6
Apples	6	Old potatoes	8
Peaches	7	Peas	25
Bananas	11	New potatoes	30
Pineapple	25	Cabbage	40
Tomatoes	25	Cauliflower	60
Citrus fruit	50	Broccoli	110
Strawberries	60	Horseradish	120
Blackcurrants	200	Sweet red peppers	140
Rose hips	1000	Parsley	150

only liver (30 mg per 100 g) is a valuable source. Human milk provides enough ascorbic acid (4–6 mg per 100 mL) to prevent scurvy in breastfed infants. Untreated cow's milk contains about 2 mg per 100 mL, but pasteurisation or other heat treatments considerably reduce this figure.

The role of ascorbic acid in mammalian physiology and biochemistry is far from fully understood, but is very different from its role in plants. In plants, its function is as an antioxidant (see Chapter 4, Section 4.3.3), in particular helping to protect the photosynthetic machinery of the chloroplasts from the effects of singlet oxygen and other highly reactive oxygen species (see Chapter 4, Section 4.6.2). Glutathione is also involved in this activity (see Chapter 5, Section 5.5.5). In animals, it is involved in hydroxylation reactions. It has been implicated in a number of reactions, particularly in the formation of collagen, the connective tissue protein. After incorporation into the collagen polypeptide chain, proline (see Figure 5.2) is hydroxylated to hydroxyproline in a complex reaction for which ascorbic acid is essential (8.24).



Many of the symptoms of scurvy, the best-known ascorbic acid deficiency disease, are to be expected from a failure of normal connective tissue formation. For example, wounds fail to heal, the gums bleed, internal bleeding occurs and the joints become painful. In advanced cases of the disease, the bones become extremely painful and fracture easily. Behavioural disturbances, including personality changes and listlessness, are also encountered and are possibly linked to reduced activity of the enzyme dopamine hydroxylase, which catalyses the conversion of 3,4-dihydroxyphenylethylamine (dopamine, a derivative of tyrosine) to noradrenaline (8.25).



There is also evidence that the uptake of non-haem iron (see Chapter 11, Section 11.2.1) from the duodenum is enhanced by the presence of high levels of ascorbic acid. Ascorbic acid reduces iron from the ferric (Fe^{III}) state, in which it normally occurs in food, to its ferrous (Fe^{II}) state. Compared with ferrous iron, very little ferric iron is absorbed because it forms the insoluble hydroxide. Unfortunately, the amounts of additional ascorbic acid required to double the iron uptake from a typical meal are fairly high, about 100 mg. Ascorbic acid also has a role in the transport of iron in the blood and its mobilisation from reserves.

The diversity of the roles of ascorbic acid in human physiology and biochemistry probably accounts for its adoption as a wonder cure-all for a number of conditions and diseases, particularly the common cold. The actual evidence for the value of extra-high doses (up to 5 g per day, more than 100 times the recommended dietary intake) is extremely flimsy, something that cannot be said for the profits to be made by selling ascorbic acid as a supplement.

There are many different routes available for the chemical synthesis of ascorbic acid, but the most usual commercial route is shown in Figure 8.5, known as the Reichstein process. This is an interesting combination of traditional synthetic chemistry with biotechnology that was first introduced in the 1930s and continues to be used today.

Almost all of the numerous methods devised for the determination of ascorbic acid depend on measurement of its reducing properties by titration with an oxidising agent, the most popular being 2,6-dichlorophenolindophenol (DCPIP, 8.26). DCPIP is blue in its oxidised form, but colourless when reduced. Highly coloured plant extracts, such as blackcurrant juice, obviously present problems because the end-point of the titration is obscured. There are numerous, more or less satisfactory, techniques for circumventing the problem. One example is titration with *N*-bromosuccinimide (NBS) in the presence of potassium iodide and ether. At the end-point of the titration, when all the ascorbic acid present has been oxidised to DHAA, the excess NBS liberates iodine from the potassium iodide, which dissolves in the ether phase to give a visible brown colour. Very few of the methods commonly used determine any DHAA present; one that does not discriminate between the two compounds is the fluorimetric method using *o*-phenylenediamine.

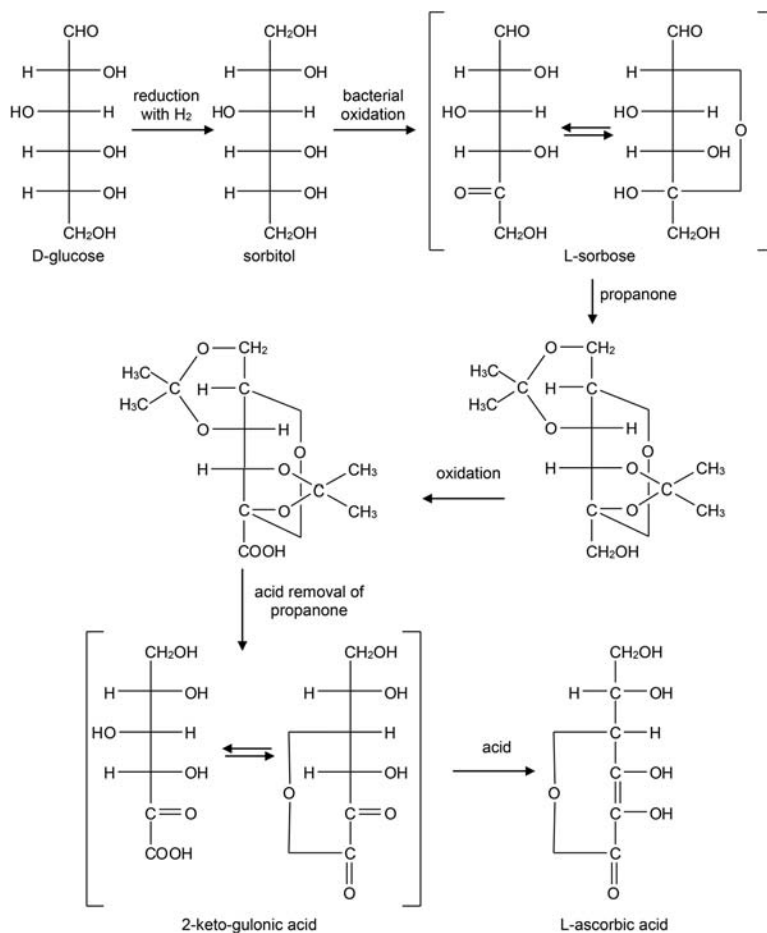
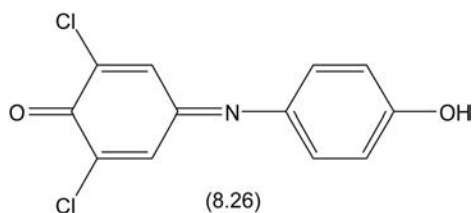
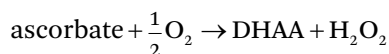


Figure 8.5 Commercial synthesis of ascorbic acid from glucose.



Information resulting from a variety of studies shows that ascorbic acid is one of the least stable vitamins. Losses during processing, cooking or storage can occur by a number of different routes, but few experiments have been devoted to discovering which routes are involved in particular foodstuffs. Leaching is obviously important in vegetable preparation if the cooking or processing water is discarded. Green vegetables may lose >50% of their vitamin C in this way if they are boiled for prolonged periods. Losses from root vegetables are usually much smaller, simply because they present a smaller surface area in relation to their weight.

In the presence of air, most actual degradation of the vitamin occurs through the formation of the less stable DHAA. Once formed, DHAA rapidly undergoes an irreversible ring-opening reaction to give 2,3-diketo-L-gulonic acid (DKGA), which has no vitamin activity. Oxidation to DHAA can occur by a variety of mechanisms. In plant tissues stored without blanching, especially if they have been sliced, peeled or otherwise damaged, the enzyme ascorbic acid oxidase is very active, catalysing the reaction:



The enzyme phenolase is also responsible for ascorbic acid losses when, as discussed in Chapter 6, ascorbic acid reduces *o*-quinones back to the original *o*-diphenols. Metal cations, particularly Fe^{3+} and Cu^{2+} , catalyse the oxidation and may cause serious losses in food products. Even in the absence of a catalyst, the oxidation occurs fairly rapidly at elevated temperatures. The formation of DKGA is virtually instantaneous at alkaline pH, rapid around neutrality, and slow under acidic conditions. The reason now becomes clear for not adding sodium bicarbonate to preserve the colour of green vegetables during cooking.

The vitamin is fairly stable in the acid environment of fruit juice processing. Its stability is enhanced by the presence of citrates and flavonoids, both of which can complex metal cations, but the juice should still be kept deaerated as far as possible. Ascorbic acid will also undergo breakdown under anaerobic conditions at elevated temperatures in much the same way as other sugars (see Chapter 2), except that in this case CO_2 is evolved.

The low cost of ascorbic acid and its obvious acceptability as a nutrient make it a valued food additive, for technological rather than nutritional purposes. In fruit and vegetable products, notably dehydrated potato, it is used as an antioxidant to prevent the browning catalysed by phenolase. It is used as a reducing agent to lower the concentration of nitrite needed to give a good pink colour in the manufacture of cured meats (see Chapter 9, Section 9.2). It is also used as a flour improver in modern bread-making (see Chapter 5, Section 5.5.5).

A comprehensive historical account of the discovery of each of the vitamins would be out of place in a text such as this, but no apology is offered for the inclusion of the following paragraphs, a useful reminder that scientists have not always taken themselves as seriously as they sometimes do today.

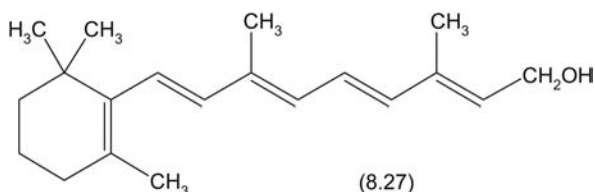
Chemists, who in exasperation may at times have referred to an unidentifiable sugar on their chromatograms as “@*!-knows”, may be comforted to know that this terminology has an honourable history. In 1928, Albert Szent-Györgyi set out to publish the details of a sugar acid with strong reducing properties that he had isolated, initially from the adrenal cortex of cattle and later from orange juice and the water in which cabbage had been boiled. In the draft of his paper he suggested the name “ignose” (Latin, *ignorare*, not to know). This, and his subsequent suggestion “godnose”, were both rejected by the editor of the *Biochemical Journal* on the grounds that they didn’t publish jokes. Szent-Györgyi reluctantly had to settle for “hexuronic acid”. His paper did hint at the possibility that this substance might actually be the antiscorbutic (*i.e.*

scurvy-preventing) factor already designated “vitamin C”, but this was not confirmed until 1932.

Once the structure was firmly established by Haworth in Birmingham, using the kilogram quantities Szent-Györgyi was able to isolate from a local vegetable, red pepper, the name ascorbic acid was adopted in recognition of its antiscorbutic properties. Food chemists should forgive Szent-Györgyi's later remark that “... vitamins were, to my mind, theoretically uninteresting. ‘Vitamin’ means that one has to eat it. What one has to eat is the first concern of the chef, not the scientist.”

8.9 Retinol (Vitamin A)

This vitamin is the first of the fat-soluble vitamins to be considered in this chapter. Its active form in mammalian tissues is the alcohol retinol (8.27), but most of the vitamin occurs in the human diet as precursors, described earlier in this chapter as provitamins, of which there are several.



Plant tissues contain the vitamin in the form of carotenoids. As was indicated in Chapter 6, any carotenoid that contains the β -ionone ring system, as do β -carotene (at both ends of the molecule), α - and γ -carotene and β -apo-8'-carotenal (at one end only), is converted by enzymes in the mucosa of the small intestine to retinol (see Figure 6.7). The retinol, plus any absorbed directly from the diet, is then esterified with fatty acids and the resulting retinyl esters are incorporated into the lipoprotein bodies known as chylomicrons for transport in the general circulation. The absorption of carotenoids and their conversion to retinol do not occur with total or uniform efficiency, which makes the estimation of the vitamin A activity of different foodstuffs difficult. There are three possible causes for this inefficiency: (1) the activity of the carotene dioxygenase is relatively low so that much of the dietary β -carotene remains unconverted; (2) other carotenoids that are not vitamin A precursors are suspected of inhibiting the reaction; and (3) some cleavage of β -carotene occurs at other sites, leading to apocarotenals that cannot be converted to retinol (see Chapter 6, Section 6.2).

The equivalence figures originally recommended by the Food and Agriculture Organization/World Health Organization (FAO/WHO) in 1967 continue to be in general use, *i.e.* 6 μg of β -carotene or 12 μg of other active carotenoids being regarded as the equivalent of 1 μg of retinol. In the years since then, and particularly since 1998, data have accumulated indicating that the efficiency of the conversion of β -carotene to retinol is highly dependent on the nature of the foodstuff supplying the β -carotene and is often significantly less than implied by the FAO/WHO figures. This can lead to an overestimation of the retinol equivalent content of many foods (Table 8.2). In particular, leafy green vegetables, although rich in β -carotene, are not good sources of vitamin A. Less

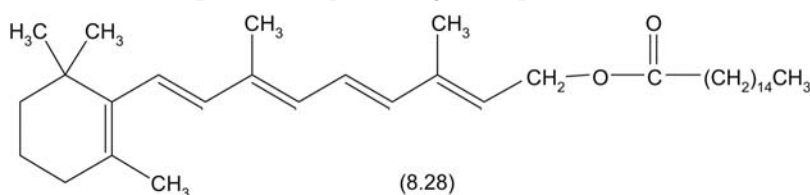
Table 8.2 Conversion factors for the provision of vitamin A (retinol) by β -carotene in different foodstuffs. (From G. Tang, *Am. J. Clin. Nutr.*, 2010, **91**, 1468S and T. Muzhingiri, *et al.*, *Am. J. Clin. Nutr.*, 2011, **94**, 510).

β -Carotene source	Conversion factor
All foods (FAO/WHO 1967 recommendation)	6:1
All foods (US Institute of Medicine 2001 recommendation)	12:1
Orange/yellow fruit (mango, ^a papaya, ^b orange) and sweet potato ^c	12–13:1
Leafy green vegetables (spinach, ^d Indian spinach, ^e cabbage)	20–30:1
Yellow maize	$\approx$ 3:1
Golden rice	3–4:1

^a*Mangifera* sp. ^b*Carica papaya*. ^c*Ipomea batatas*. ^d*Spinacea oleracea*. ^e*Basilla alba*.

than 10% of the vitamin may be absorbed when they are eaten raw, whereas about 50% is absorbed from cooked vegetables. On cooking, dietary carotenoids tend to dissolve in any lipids present and in this state their absorption into the bloodstream is much more efficient.^x

The vitamin A content of plant materials consists entirely of carotenes. Leafy green vegetables contain between 100 and 3000 retinol equivalents per 100 g, mostly as β -carotene. Carrots, the classic source, contain about 2000 retinol equivalents per 100 g. The other major plant source is red palm oil, whose β -carotene content is sufficient to give about 20 000 retinol equivalents per 100 g. In animal tissues the vitamin, always derived originally from plant sources, is stored and transported as the retinyl ester of long-chain fatty acids, mostly palmitate (8.28) and stearate. Dietary retinyl esters are first hydrolysed and then re-esterified by the intestinal mucosal cells during the process of absorption. A specific protein binds the retinyl esters in the liver, with the result that liver and liver oils are particularly good sources in the diet. Cod and halibut liver oils are well known for their vitamin A content, about 10^5 and 10^7 retinol equivalents per 100 g, respectively. The vitamin A content of mammalian liver varies widely, from 3000 in pig's liver and 17 000 in sheep's liver to 600 000 retinol equivalents per 100 g in polar bear liver. The relationship between the diet of these animals and the vitamin A content of their livers is obvious. Meat (*i.e.* muscle tissue) contains very little of the vitamin. Although milk itself has only a low concentration, dairy products in which the lipid phase of the milk is concentrated, such as butter and cheese, contain 500–1000 retinol equivalents per 100 g. Margarine sold in the UK is required by law to be supplemented with vitamin A, usually in the form of synthetic retinyl acetate or red palm oil, to give a level of 800–1000 retinol equivalents per 100 g, comparable with butter.



^xThis effect of cooking was previously noted in connection with the health benefits of dietary lycopene, see Chapter 6, Section 6.2.

The chemical synthesis of vitamin A was first achieved in 1947. It is now produced on a large scale commercially in the form of retinyl acetate in a simple process using β -ion-one as the starting material. As one might expect from its chemical structure, ensuring the resistance of vitamin A preparations to autoxidation is a major issue, especially in the presence of trace metals.

The function of vitamin A in the visual process is well known and the sequence of events in the “visual cycle” of the rod cells in the retina is shown in Figure 8.6. The inefficiency of the cycle inevitably results in losses and the supply of all-*trans* retinol is replenished from the circulation. The absence of adequate supplies results in a loss of vision in dim light, known as “night-blindness”. This was linked to a deficiency of the vitamin as early as 1925.^{xi}

Although the involvement of vitamin A in vision is well known, its principal functions are in the control of the growth and development of tissues. It functions in much the same way as the steroid hormones, in the sense of having different effects on different tissues. It binds to receptors on cell nuclei to regulate the transcription of specific genes. One of its specific roles is in the differentiation of immune system cells and deficiency

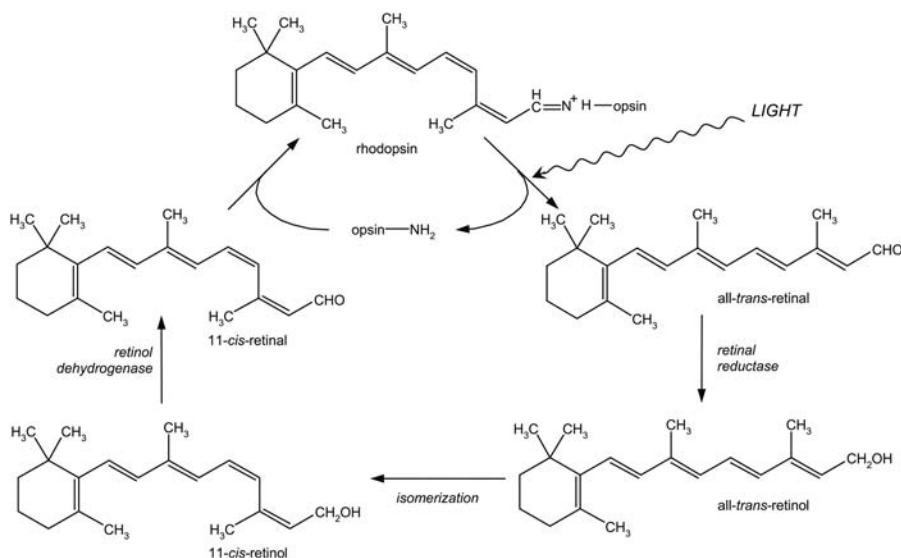


Figure 8.6 Vitamin A in the visual cycle. In the dark, 11-*cis*-retinal combines with the protein opsin to form rhodopsin. When this absorbs light (λ_{max} 500 nm) the *cis* bond isomerises to a *trans* bond and, ultimately, the all-*trans* retinal detaches from the protein. The complex processes that lead to the generation of nerve impulse are initiated during the course of this isomerisation. The 11-*cis*-retinal is then regenerated to recombine with opsin in darkness.

^{xi}During the Second world war, the UK government encouraged people to eat carrots (which, unlike dairy products and meat, were in plentiful supply) focussing on their β -carotene/vitamin A content. Posters hinted that RAF pilots were consuming large quantities of carrots to enhance their night vision when, in fact, their successes were due to developments in the radar detection of enemy aircraft. There is no evidence to suggest that the Luftwaffe were fooled by the British propaganda, but the British public did increase their carrot consumption significantly (see www.carrotmuseum.co.uk/history4.html, last accessed 2020).

can therefore lead to an increased susceptibility to infectious diseases. This occurs at degrees of vitamin deficiency insufficient to cause the characteristic impairment of vision. Other symptoms of deficiency include abnormal bone development, disorders of the reproductive system and xerophthalmia, a drying and degenerative condition of the cornea of the eye, which, if untreated, causes total blindness. There are indications of a role for retinol in the synthesis of the glycoproteins occurring in mucous membranes. Deficiency is known to lead to keratinisation of these membranes, resulting in the degeneration of the epithelia lining the digestive and respiratory tracts.

Only since the 1960s has it been fully appreciated that vitamin A deficiency afflicts millions of people in the developing countries of Asia. In these areas, neither dairy products nor green vegetables feature strongly in the diet of the poorer sectors of the population. A variety of methods have been explored, with little success, to supplement local diets with synthetic retinyl acetate. In 2000, a team led by Ingo Potrykus (in Zurich) and Peter Beyer (in Freiburg) successfully used genetic modification techniques that led to rice plants able to synthesise β -carotene in the endosperm of the rice grain as well the normal formation in the leaves. Unfortunately, the level of carotenoids initially achieved, $1.6 \mu\text{g g}^{-1}$, were not sufficient to justify widescale cultivation of the new strain, known as Golden Rice due to the yellow colour of the grain. The subsequent (2005) development of Golden Rice 2, using similar genetic modification techniques, massively increased the levels of total carotenoids (most of which is β -carotene) to up to $37 \mu\text{g g}^{-1}$. One serving of this rice (50 g dry weight) could provide about 1 mg of β -carotene, generating $>400 \mu\text{g}$ of retinol when consumed.^{xii}

Unfortunately, the adoption of Golden Rice 2 has been bitterly opposed, principally because its introduction breaches long-standing arguments against the agricultural application of genetically modified organisms. The official approvals of Golden Rice 2 for consumption in the USA, Canada, New Zealand and Australia (and possibly the UK in 2020) actually have little bearing on this issue because in none of these countries is rice the staple food that it is countries such as the Philippines or Bangladesh. Consumer acceptance may be another challenge because many people in Bangladesh prefer to eat white rice. At the time of writing, the Bangladeshi authorities were poised to give their approval for Golden Rice planting on a commercial scale.

Vitamin A deficiency is also widespread in Zimbabwe and elsewhere in sub-Saharan Africa. This is largely due to a low consumption of fruits and vegetables and foods of animal origin, coupled with dependence on maize as a major energy source. The varieties of maize cultivated for human consumption have a white endosperm, containing very little β -carotene. So-called “yellow maize” varieties, potentially rich sources of β -carotene, are traditionally grown only for feeding livestock (pigs). Cooked yellow maize has an excellent conversion factor for retinol formation and its use for human consumption could go a long way towards reducing the incidence of vitamin A deficiency in southern Africa.^{xiii}

^{xii}see G. Tang *et al.* β -Carotene in Golden Rice is as good as β -carotene in oil at providing vitamin A to children. *Am. J. Clin. Nutr.*, 2012, **96**, 658.

^{xiii}see T. Muzhingi, *et al.*, Yellow maize with high β -carotene is an effective source of vitamin A in healthy Zimbabwean men, *Am. J. Clin. Nutr.*, 2011, **94**, 510.

Considerable attention has been focused in recent years on the possibility that vitamin A might have an anticancer function. Laboratory experiments with unnaturally high doses of the vitamin have shown promise, but it has not proved possible to put these to therapeutic effect. Large doses simply lead to an increase in the liver reserves without action on the target tissues. The potential beneficial effect of vitamin A in this context is in its antioxidant activity. Both retinol and β -carotene are weak antioxidants (see Chapter 4, Section 4.3.3), but β -carotene also undergoes a specific reaction with singlet oxygen (see Chapter 4, Section 4.6.2). This quenching reaction may help to eliminate the dangerous by-products resulting from reactions involving oxygen in the body. There is growing support for the view that vitamin A, vitamin E (see Section 8.11), selenium (as a component of the enzyme glutathione peroxidase) and ascorbic acid function together as the body's antioxidant system. They should therefore be considered collectively when the relationship between health and the vitamin content of the diet is assessed.

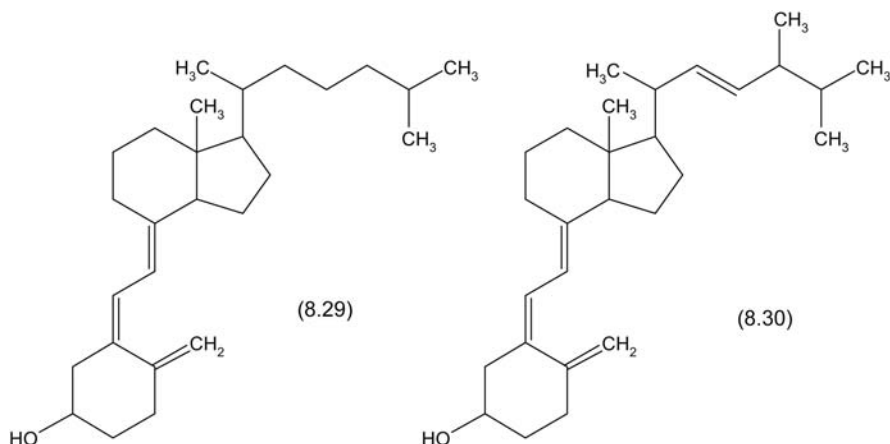
An unusual feature of retinol is its toxicity in excess. Most cases of hypervitaminosis have been in people who consume high levels of supplements, but it has also been reported that polar explorers have also been affected after the ill-advised consumption of polar bear flesh. As the recommended daily intake for the vitamin is only about 750 retinol equivalents, one modest portion of polar bear liver is equivalent to over two years' supply of retinol. Although the excessive consumption of polar bear liver is unlikely to present much of a hazard for most of us, it is recommended that pregnant women should not eat liver from any source to prevent excessive consumption of vitamin A. Fortunately, there is no risk of hypervitaminosis from excess β -carotene consumption because, as its level in the diet increases, the efficiency of its conversion to retinol falls.

Both retinyl esters and β -carotene are fairly stable in food products. Most of the information on stability concerns β -carotene. High temperatures in the absence of air, such as in canning, can cause isomerisation to neocarotenes (see Chapter 6), which only possess vitamin activity if one end of the molecule remains unaffected. Breakdown can be rapid in the presence of oxygen, especially in dehydrated foods in which a large surface area is exposed to the atmosphere. The hydroperoxides resulting from the autoxidation of polyunsaturated fatty acids will bleach carotenoids and can be presumed to have a similar destructive effect on retinyl esters.

The determination of the amount of vitamin A in a foodstuff is complicated by the diversity of forms in which it occurs. An indication can be gained from the absorbance of a solvent extract at 328 nm, the absorption maximum of retinol. Elaborate procedures are available to compensate for the interfering absorbance of other substances at this wavelength, but the problem can be avoided by using preliminary chromatography to separate and identify the different carotenoids and retinyl esters.

8.10 Cholecalciferol (Vitamin D, Calciferol)

Only one form of this vitamin, known either as cholecalciferol (8.29), vitamin D₃ or calciol, occurs naturally in the diet. A different form of the vitamin, ergocalciferol (vitamin D₂, ercalciol, 8.30), is used for pharmaceutical applications and for food supplementation.



There is an increasing tendency for physiologists, but not nutritionists, to regard cholecalciferol as a hormone rather than a vitamin. This is because it functions in the body in the same way as a hormone, rather than as an enzyme prosthetic group like most other vitamins. Furthermore, humans and other mammals have, under the right circumstances, a more than adequate capacity to synthesise it for themselves.

Animals are able to synthesise cholesterol and other steroids, one intermediate in the pathway being 7-dehydrocholesterol. This is located in the epidermal cells of the skin and is converted to cholecalciferol by the action of the UV component of sunlight. The course of the reaction is shown in Figure 8.7.

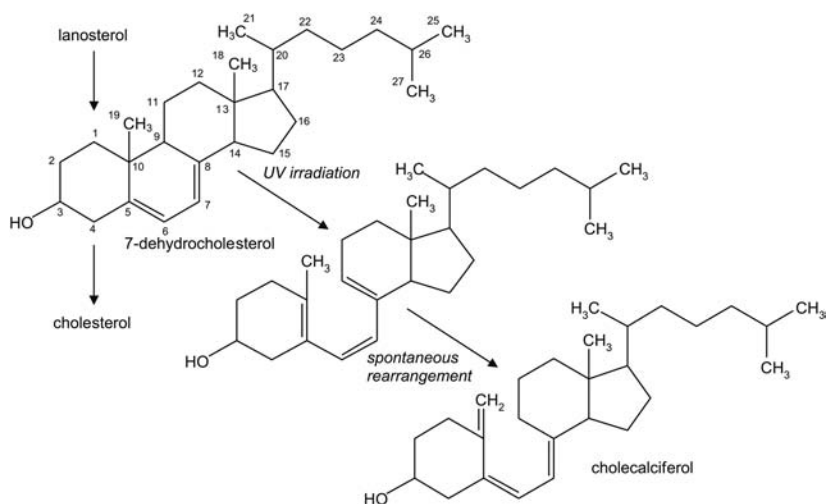


Figure 8.7 Formation of cholecalciferol in the skin. The standard numbering of the carbon atoms of steroids is used in 7-dehydrocholesterol, the immediate biosynthetic precursor of cholesterol. The optimum wavelength for the first reaction is 300 nm. The structure shown here for cholecalciferol may appear to differ from that in the text (8.29), but this is only because the single bond between carbons 6 and 7 has been rotated through 180° to illustrate more clearly the relationship between cholecalciferol and its parent compounds.

When human skin is adequately exposed to sunlight, the body is able to provide sufficient vitamin D for its needs (2.5–10 µg per day, with infants, children and pregnant or lactating women having the highest requirements). The body is able to maintain useful stores of the vitamin, but, unlike the other fat-soluble vitamins, it is not only stored in the liver, but is also found in the lipid elements of most tissues, particularly in adipose tissue. These reserves provide some protection against its reduced formation in the skin during the winter months, but without dietary supplies many people in northern latitudes still have a suboptimal vitamin D status for part of the year. At the latitude of the British Isles, sunlight contains no useful UV radiation from mid-October to the end of March. The levels of the physiologically active form of the vitamin, 1,25-dihydroxycholecalciferol in the blood of UK adults vary significantly with the seasons. In 2004, it was reported that for adult men the average concentrations vary between 65 nmol L⁻¹ in July to September and 41 nmol L⁻¹ in January to March.^{xiv} The high levels of skin pigmentation associated with ethnic groups originating in tropical regions prevents a high proportion of the UV light from reaching the epidermal cells in which cholecalciferol is formed. It may well be that the pale complexion of the northern European races is an adaptation to ensure the most efficient use of the little sunlight available.

The pattern of distribution of cholecalciferol in foodstuffs is strikingly similar to that of retinol. As with retinol, fish liver oils contain spectacular levels, *e.g.* mackerel liver oil has 1.5 mg per 100 g. The muscle tissues of fatty fish such as salmon, herring and mackerel are also valuable dietary sources (5–45 µg per 100 g), but mammalian tissues, including liver, provide <1 µg per 100 g. Milk contains as little as 0.1 µg per 100 g, but fatty dairy products, such as butter and cream, do contain useful amounts (1–2 µg per 100 g) and egg yolk has about 6 µg per 100 g. An unfortunate contrast with vitamin A is that plant materials do not contain significant amounts of vitamin D.

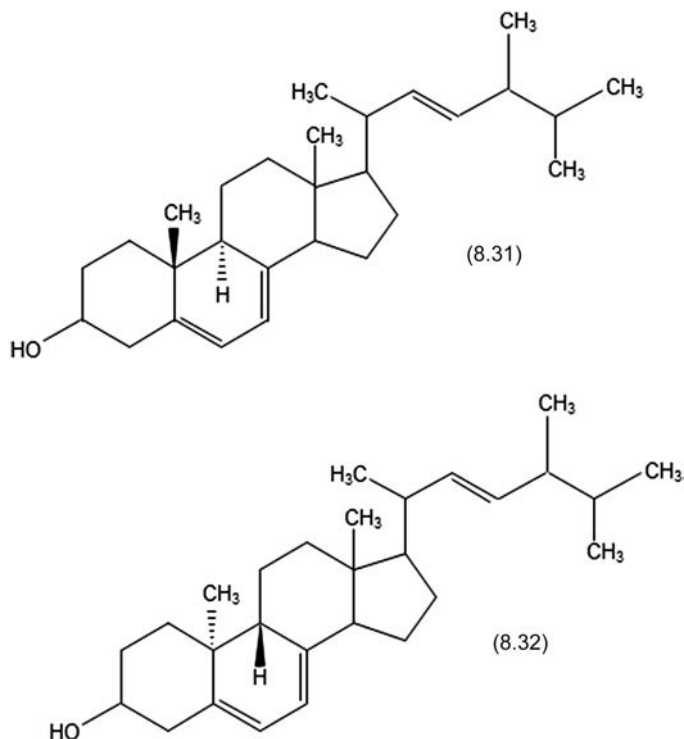
It is now common practice to enhance the vitamin D contents of certain foods, including breakfast cereals, milk (in the USA) and margarine. In the UK there is a legal requirement for the vitamin D content of margarine to be between 7.05 and 8.82 µg per 100 g,^{xv} a level ten times greater than that present in butter. These levels are normally expressed in terms of International Units, where 1 I.U. of vitamin D equals 0.025 µg of cholecalciferol or ergocalciferol. The growing unpopularity of fatty dairy foods (see Chapter 4) may eventually lead to a requirement for the vitamin D supplementation of milk in the UK. There have been reports of the widespread incidence of rickets among sheep on farms in Scotland and northern England.

Vitamin D₃ (cholecalciferol) is produced industrially by the UV irradiation of 7-dehydrocholesterol. The 7-dehydrocholesterol is extracted from the natural grease (lanolin) removed from wool before it is converted into textiles. Vitamin D₂ (ergocalciferol) is produced in a similar way using ergosterol from yeast or mushrooms as a starting material. Vitamin D₂ is similarly synthesised on a commercial basis from ergosterol (8.31)

^{xiv}D. Ruston, *et al.*, *The National Diet and Nutrition Survey: Adults Aged 19 to 64 Years, Volume 4: Nutritional Status (Anthropometry and Blood Analytes), Blood Pressure and Physical Activity*, TSO, London, 2004.

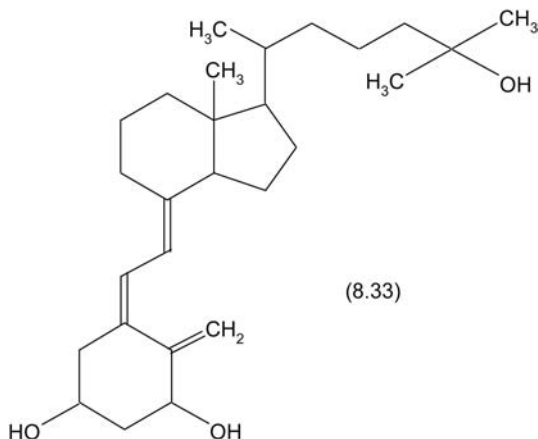
^{xv}This curious degree of precision has no nutritional significance; it is the result of converting the ounces of the original regulations to grams.

obtained from yeast. What was once known as vitamin D₁ is now unheard of outside chemical laboratories. It was the product of the experimental UV irradiation of ergosterol and found to consist of a 1:1 mixture of ergocalciferol and lumisterol (8.32), an isomer of ergosterol.



Rickets, a childhood disease closely linked to vitamin D deficiency, is a disorder of bone development. First described in 1645, it was always regarded as a disease of the urban poor. By the late nineteenth century, 80% of children in London showed some symptoms. Although some authorities guessed correctly that it was a “sun-light deficiency” disease, syphilis and hereditary factors were also blamed. The fact that poverty and nutritional deprivation could, in some senses, be regarded as inherited, escaped attention. Cod liver oil was used as a cure as early as 1848 (at Manchester Infirmary), but most of the medical profession remained sceptical until the 1920s.

Whether obtained from the diet, or formed in the skin, cholecalciferol is converted to 25-hydroxycholecalciferol (calcidiol) in the liver. This is released into the bloodstream and taken up by the kidneys, where it is hydroxylated for a second time to yield the physiologically active compound 1,25-dihydroxycholecalciferol (calcitriol, 8.33). This is one of three hormones (the others are calcitonin and parathormone) that together control calcium metabolism. Calcitriol promotes the synthesis of the proteins that transport calcium and phosphate ions through the cell membranes. A lack of calcitriol prevents the uptake of calcium from the intestine and the resulting shortage of calcium for bone growth is manifested as rickets.



Another feature of this vitamin that it shares with retinol is toxicity in excess. Babies and young children were at one time the most likely to be affected following confusion between halibut liver oil and cod liver oil, which contain 3000 μg per 100 g and 250 μg per 100 g, respectively. A daily teaspoonful of cod liver oil, about 5 mL, would provide a useful 10–15 μg supplement, whereas the same amount of halibut liver oil would supply 150 μg , a dangerous overdose if repeated regularly. Massive overdoses cause calcification of soft tissues such as the lungs and kidneys, but in babies even modest overdoses can cause intestinal disorders, weight loss and other symptoms.

The determination of cholecalciferol is extremely difficult at the levels occurring in most foodstuffs. Biological assays using rats reared on a rickets-inducing diet were essential until the development of gas chromatography and HPLC. As a result, very little work has been done on the behaviour of cholecalciferol in food. In general, it is believed to be fairly stable to heat processing and only liable to oxidative breakdown in dried foods such as breakfast cereals.

8.11 Vitamin E (α -Tocopherol)

Of all the vitamins, this one and the one concluding this chapter, vitamin K, have defeated efforts to replace their old alphabetical designations with more scientific terminology. In both cases this is because no single chemical name is both sufficiently comprehensive and at the same time sufficiently exclusive.

The E vitamins are a group of derivatives of 6-hydroxychroman carrying a phytyl side-chain. As shown in Figure 8.8, the tocopherols have a fully saturated side-chain, whereas in the tocotrienols the side-chain is unsaturated. Variations in the pattern of methylation of the chroman nucleus give the α -, β -, γ - and δ -tocopherols and tocotrienols. All eight of the compounds shown in Figure 8.8 are found in nature, but only the α -, β - and γ -tocopherols and the α - and β -tocotrienols are widespread. Inspection of the formulae in Figure 8.8 shows that carbons 2, 4' and 8' of the tocopherols and carbon 2 of the tocotrienols each carry four different substituents, making them asymmetrical (see Chapter 2, Section 2.1) and raising the possibility of a large number of possible optical isomers. In fact, the asymmetrical centres of all the tocopherols and tocotrienols found in nature have the same configuration, shown in Figure 8.8. The commercial, chemical

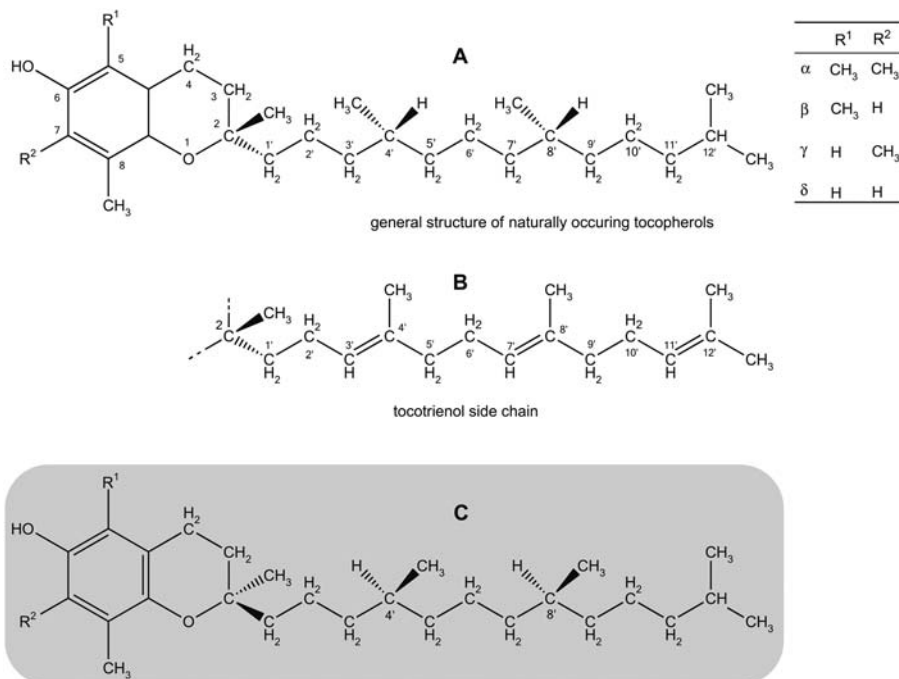


Figure 8.8 Vitamin E structures. In A and B all the asymmetrical centres (carbons 2, 4' and 8' in tocopherols, carbon 2 in tocotrienols) are shown in the configuration found in nature. In C (shaded), all three asymmetrical centres are shown in the opposite configuration. The configurations in A and B are all designated as (*R*), whereas those in C are (*S*). (These designations, similar to the prefixes *D* and *L* used with sugars and amino acids, are derived from the Latin *rectus* – right-handed – and *sinister* – left-handed.) Depending on the details of the particular synthesis, chemically manufactured α -tocopherol is always a mixture of different optical isomers, e.g. (*RSS*), (*RSR*), (*RRS*), (*RRR*). The synthetic vitamin is therefore a *racemic mixture*, which is usually prefixed (*DL*) to distinguish it from the natural product (*D*)- α -tocopherol, i.e. (*RRR*)- α -tocopherol.

synthesis of α -tocopherol is important as it becoming widely used as a fairly natural antioxidant for food lipids (see Chapter 4, Section 4.3.3).

The evaluation of much of the data on the occurrence of vitamin E is complicated by the variations in vitamin activity between the different tocopherols and tocotrienols. Expressions of the total tocopherol content are clearly of no value in this situation and an International Unit was adopted by the WHO in 1947. This was defined as the average daily dose of the vitamin required to prevent foetal resorption and sterility in rats. This happened to correspond closely to 1 mg of the best standard tocopherol preparation available at the time. As highly purified samples of all the various isomers and derivatives of tocopherol have become available, the original International Unit has become redundant and is now regarded as obsolete. It is now usual to express vitamin E in terms of “ α -tocopherol equivalents” i.e. the sum of:

- 1.0 $\times$ mg of α -(*D*)-tocopherol,
- 0.5 $\times$ mg of β -(*D*)-tocopherol,
- 0.1 $\times$ mg of γ -(*D*)-tocopherol,
- 0.3 $\times$ mg of α -(*D*)-tocotrienol,

when the vitamin E contents of seed oils and other foods are reported. The DL-racemic mixtures have about three-quarters of the activity of the corresponding pure D-isomers. Unofficial International Units, now simply treated as equal to 0.67 α -tocopherol equivalents, are used in some contexts, especially the marketing of vitamin E supplements.

The most important sources of vitamin E in the diet are the plant seed oils (50–200 mg α -tocopherol equivalents per 100 g). Most other plant tissues contain <0.5 mg per 100 g. Among the vegetables, leafy green species are the richest sources, *e.g.* spinach about 1.7 and cabbage about 7 mg α -tocopherol equivalents per 100 g. The older dark green outer leaves of cabbages contain much higher levels (the figure quoted here) rather than the almost white inner tissues, from which the vitamin is almost absent.

Animal tissues, including liver, milk and eggs, contain similarly low levels. Unlike other fat-soluble vitamins, vitamin E is not found in particularly large amounts in fish liver oils; cod liver oil, for example, has only about 25 μ g per 100 g.

The tocopherols are the natural antioxidants of animal tissues and possibly also those of plants. Vegetable oils, such as those listed in Table 8.3, are also protected by their tocopherol content. Antioxidants block the free radical chain reactions of lipid peroxidation (see Chapter 4). The tocopherols have a special affinity for the membrane lipids of the mitochondria and endoplasmic reticulum of animal cells. It has been suggested that the shape of the tocopherol side-chain allows a close association with the arachidonic acid component of the phospholipids in these membranes. One could speculate that the differing vitamin activities of the various isomers of α -(D)-tocopherol reflect the potential closeness of this association. Mitochondrial membranes from animals reared on diets deficient in vitamin E are exceptionally prone to peroxidation. In view of the antioxidant role of vitamin E, it might be expected that dietary supplements would be beneficial in reducing the likelihood of cardiovascular disease. Unfortunately, this attractive hypothesis has not been borne out by research, which has tended to show

Table 8.3 Typical data on the vitamin E, tocopherol and tocotrienol content of a selection of vegetable oils. These figures are based on data from the *Handbook of Vitamins*, ed. L. J. Machlin, Dekker, New York, 2nd edn, 1990. They should be regarded as typical rather than absolute or average values; particular samples can vary considerably from these figures. Readers requiring more detailed and comprehensive data on a wider range of oils should consult the 2016 review by Shahidi and Costa da Camargo (see Further Reading).

	(D)-Tocopherols				Total tocotrienols
	α -	β -	γ -	δ -	
	mg per 100 g				
Cottonseed oil	39	—	39	—	—
Olive oil	5	—	—	—	—
Palm oil	26	—	32	7	46
Soybean oil	8	—	59	26	—
Wheatgerm oil	119	71	26	27	21
Maize (corn) oil	11	5	60	2	—

that the intake levels required before measurable effects on clinical markers of cardiovascular disease are detected are extremely high, of the order of 1500 α -tocopherol equivalents per day.

A deficiency in dietary selenium can cause symptoms in farm and laboratory animals similar to those arising from vitamin E deficiency. The only known physiological role of selenium is in the active site of glutathione peroxidase, the enzyme responsible for the breakdown of hydrogen peroxide and organic peroxides. This enzyme therefore constitutes a second line of defence against the consequences of lipid autoxidation.

In spite of the tendency of some people to regard vitamin E as beneficial in all aspects of human sexual behaviour, no such deficiency condition has ever been identified in humans under otherwise normal circumstances. It is now well established that to maintain satisfactory levels of the vitamin in tissues, the minimum intake is related to the quantity of polyunsaturated fatty acids in the diet. A diet high in vegetable oils, which are rich in vitamin E, will also be rich in the polyunsaturated fatty acids that these oils contain (see Chapter 4).

Determination of the vitamin E content of foodstuffs is complicated by the diversity of its various forms, each with different vitamin activity. Straightforward chemical tests for total tocopherols can be carried out, but they are clearly valueless. Chromatographic methods can, however, distinguish between the different forms and are now well established.

It is difficult to draw generally applicable conclusions about the stability of vitamin E in foodstuffs during storage or processing. The most significant losses of tocopherols occur when food materials that also contain polyunsaturated fatty acids are exposed to air and the vitamin exercises its antioxidant function. One example is the manufacture of breakfast cereals, when drum-drying or extrusion cooking may be involved. The vitamin is fairly stable in extracted vegetable oils unless conditions arise which allow autoxidation of the polyunsaturated fatty acids. Its presence in the oil will delay the onset of rancidity, but inevitably the build-up of peroxides will eventually overcome the effect of the tocopherols and cause their oxidation to compounds lacking antioxidant, and vitamin, activity. Vitamin activity is rapidly lost from the oil content of commercial frozen products, such as potato chips which have been deep-fried in fat. Low-temperature storage does not, however, prevent the loss of much vitamin activity.

8.12 Vitamin K (Phylloquinone, Menaquinones)

The alphabetic designation of this vitamin has been maintained in view of the lack of suitably concise chemical names for the various substances with vitamin K activity. The K vitamins are chemically menadione (2-methyl-1,4-naphthoquinone, 8.34) or its derivatives. Phylloquinone (8.35), known as vitamin K₁, has a phytyl side-chain. The vitamin K₂ series, the menaquinones, have side-chains of varying lengths up to 13 isoprenoid units, but those most commonly encountered have between four and ten, in particular vitamin K₂, which has seven (8.36).

Vitamin K deficiency results in failure of the blood-clotting mechanism. Many substances that antagonise the activity of vitamin K are known. Most are derivatives of coumarin, *e.g.* dicoumarol, 3,3'-methylene-bis-4-hydroxycoumarin (8.39), and warfarin, 3-(α -acetylbenzyl)-4-hydroxycoumarin (8.40). Dicoumarol is produced when clover for animal feeds is spoiled by fermentation and causes disease in cattle. Warfarin is used as a rat poison and, at rather lower doses, in human medicine to prevent blood-clotting in patients with cardiovascular disease.

8.13 Special Topics

8.13.1 Details of the Coenzyme Functions of Thiamin and Pyridoxine

The role of thiamin pyrophosphate (TPP) in oxidative decarboxylation is shown in Figure 8.9. Although the details of enzyme reaction mechanisms are not essential for students of food chemistry, this is a valuable illustration of the reason why vitamin

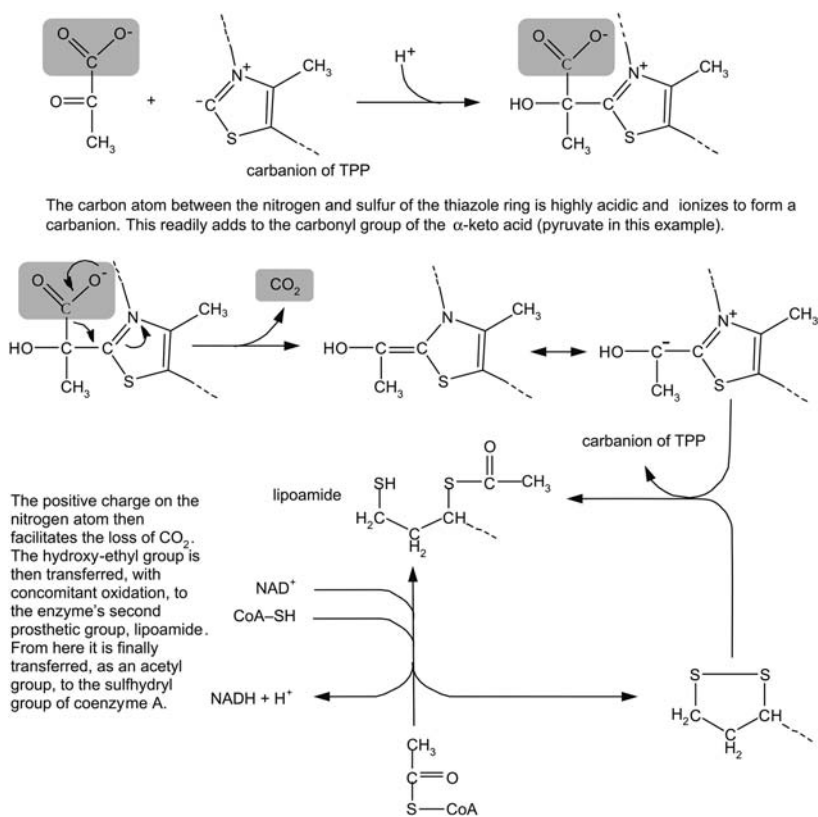


Figure 8.9 Mechanism of oxidative decarboxylations involving thiamin, illustrated here by the conversion of pyruvate to acetyl coenzyme A for entry into the Krebs cycle or fatty acid synthesis. Lipoamide is the other prosthetic group required in this type of decarboxylase. NAD^+ and NADH are, respectively, the oxidised and reduced forms of coenzymes based on the vitamin niacin, described in Section 8.4.

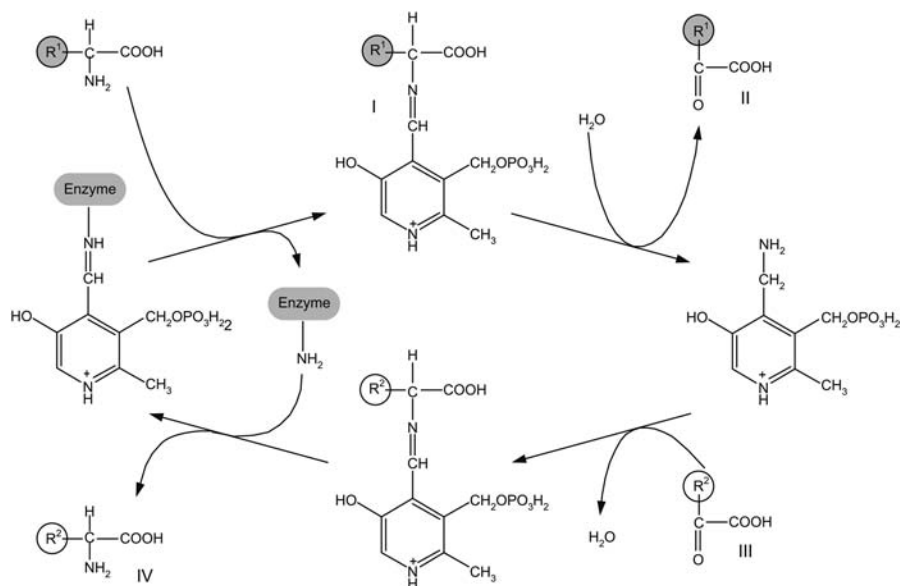


Figure 8.10 Mechanism of a transamination reaction. The ϵ -amino group of a lysine in the active site of the enzymes carries the pyridoxal phosphate. This is displaced by the incoming amino acid to form a Schiff base (I). The hydrolysis of the weakened N-C bond then liberates the α -keto acid (II). The sequence is then reversed; the second α -keto acid (III) enters, and the second amino acid (IV) is formed. The overall result is a reaction such as: glutamate + oxaloacetate $\rightleftharpoons$ α -ketoglutarate + aspartate.

structures are so often complex. It is also worth noting that the essential component of coenzyme A, pantothenic acid, is also a vitamin (see Section 8.7).

The reactions involving pyridoxal phosphate are another example of the complexity of the role of vitamins in metabolism. The reaction sequence is a transamination, a typical pyridoxal phosphate-requiring reaction, and is shown in Figure 8.10.

8.13.2 Further Details of the Role of Ascorbic Acid

Although a full treatment of the reactions in which ascorbic acid participates is more properly the concern of biochemists, it is instructive for food chemists to have a systematic overview of the full range of the potential activities of vitamin C.

Ascorbic acid is an effective scavenger of the various free radical forms of oxygen that arise in tissues, notably as by-products of oxygen metabolism or the autoxidation of unsaturated fatty acids (see Chapter 4). As shown in Figure 8.11, the free radicals' unpaired electrons are, in effect, transferred to the ascorbic acid. Ascorbic acid is then regenerated by reaction with other reducing agents such as glutathione or NADH.

Ascorbic acid is an important cofactor in many reactions catalysed by oxygenases. These are reactions in which an oxygen atom is incorporated into a substrate in the form of a hydroxyl group. In monooxygenase reactions, shown in Figure 8.12, one of the two oxygen atoms emerges from the reaction as water. However, in dioxygenase

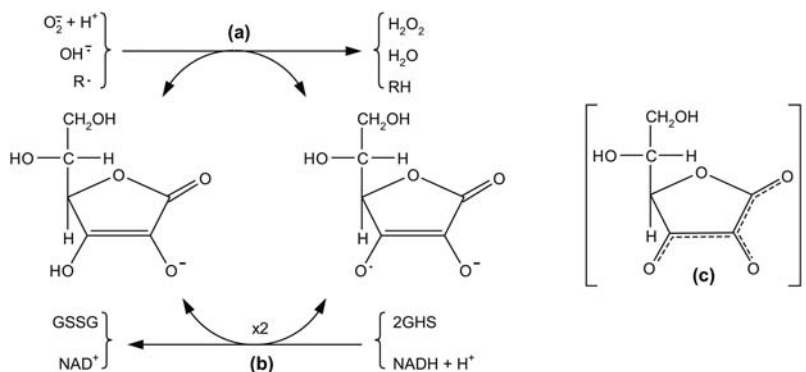


Figure 8.11 (a) Reaction of ascorbic acid with free radicals and (b) regeneration by reducing agents such as reduced glutathione (GSH) or reduced nicotinamide dinucleotide ($NADH + H^+$). (c) The stability of the ascorbic acid free radical is the result of extensive delocalisation, as indicated in the alternative structure.

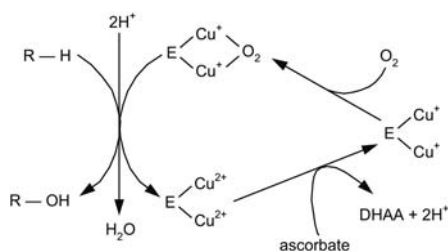
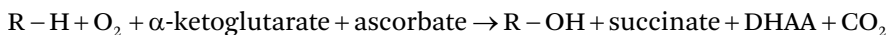
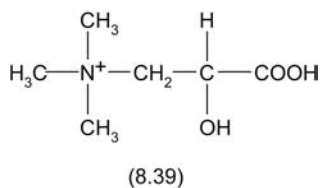


Figure 8.12 Role of ascorbic acid and copper in monooxygenase reactions.

reactions, the second oxygen atom is used in the oxidation of the auxiliary substrate, α -ketoglutarate, to succinate:



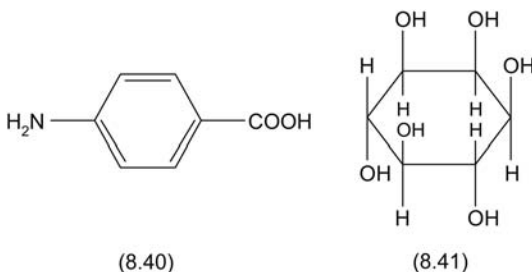
The hydroxylation of proline side-chains in collagen (8.24) referred to earlier in this chapter is a dioxygenase reaction, whereas the conversion of dopamine to noradrenaline (8.25) is a monooxygenase reaction. Two essential stages in the biosynthesis of carnitine (8.39) are catalysed by dioxygenases. Carnitine is an essential component of the system for transporting long-chain fatty acids across mitochondrial membranes, and in vitamin C deficiency its depletion may well be the basis of some of the symptoms of scurvy.



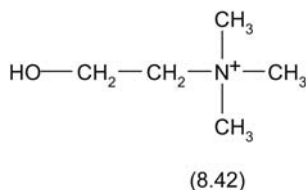
8.13.3 Non-Vitamins

A number of substances, not mentioned so far in this chapter, are sometimes described as vitamins or listed with them. These are sometimes terminological hangovers from the 1930s, but there are others with even less credibility that in no way correspond to the definition of a vitamin recorded at the beginning of this chapter.

Carnitine (8.39) is required for the growth of mealworm larvae (*Tenebrio molitor*) and *p*-aminobenzoic acid (PABA, 8.40) is a growth factor for a variety of microorganisms. There is, however, no evidence that either of these are required by humans. *myo*-Inositol (8.41), the only one of nine isomers of inositol occurring widely in nature, has been shown to be a growth factor necessary for a few types of human cells growing in culture, but there is no evidence for its requirement *in vivo*. There is therefore no reason to regard any of these three substances as vitamins, nor for them to be adopted as dietary supplements.



Choline (8.42) has been listed as a vitamin from time to time, but its status has now been clarified. The body uses considerable quantities of choline, in the form of phosphatidyl choline, lecithin, (see Chapter 4, Section 4.5) as a component of cell membranes. Although humans are able to synthesise choline by the addition of methyl groups to phosphatidyl ethanolamine, this process still requires a source of methyl groups. These can be supplied by the essential amino acid methionine, but a normal diet is unlikely to provide a useful surplus of methionine beyond that required for synthesising protein. As adults we require at least 0.5 g of choline per day. Growing infants and children may require higher levels in relation to their body weight. Fortunately, any normal diet supplies at least 1 g of choline per day. The amounts required and its function as a tissue component mean that it can be considered as an essential nutrient alongside the essential fatty acids (Chapter 4) and essential amino acids (Chapter 5), but not as a vitamin.



Three other substances are often referred to as vitamins, although they are outside the realms of conventional nutrition. Orotic acid (8.43), an intermediate in the biosynthesis of pyrimidines, is sometimes listed as “vitamin B₁₃”. The supposedly scientific name pangamic acid is otherwise known as “vitamin B₁₅”, but both these names have

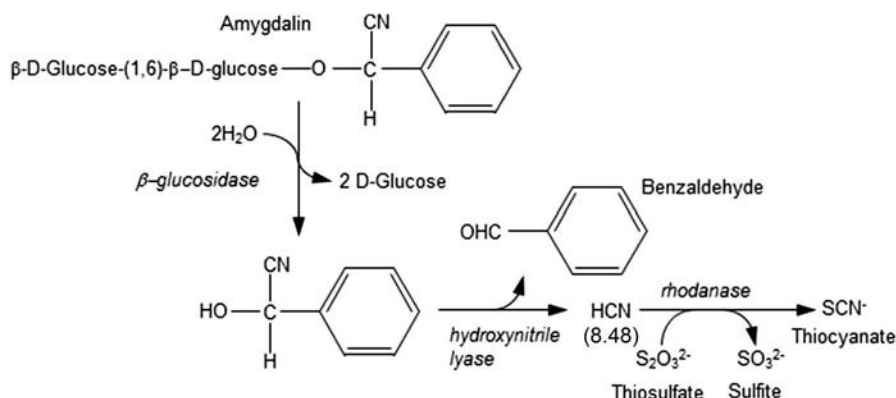
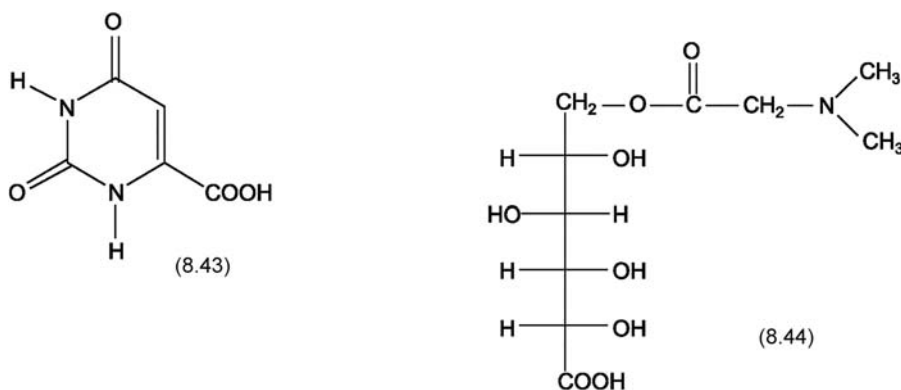


Figure 8.13 Enzymic breakdown of amygdalin and detoxification of the resulting cyanide ion.

been applied to a variety of different substances, most notably D-gluconodimethylaminoethanoic acid. (8.44). “Vitamin B₁₇” was first encountered in Chapter 2 as amygdalin and, under the name laetrile, it has achieved notoriety as a supposed cancer cure. The theory, unsupported by any experimental evidence, was that cancer cells are especially rich in the β -glucosidases that can catalyse its breakdown, ultimately releasing cyanide, which could supposedly kill the cancer cells selectively. In actual fact, the levels of cyanide released would rapidly diffuse away from the tumour cells and be rapidly detoxified by conversion to thiocyanate by the enzyme rhodanase, which is widely distributed in animal tissues (Figure 8.13).



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9 Preservatives

Microorganisms, particularly bacteria, yeast and moulds, have nutritional requirements remarkably similar to our own. Unless they use photosynthesis (as some bacteria do), their energy is obtained from the oxidation or fermentation of organic compounds.ⁱ Similarly, organic compounds are the source of carbon, nitrogen and other elements for the biosynthesis of cell material. It is therefore hardly surprising that, given the opportunity, few types of microorganisms are unwilling to utilise the same nutrients that make up a typical human diet. The attractiveness of human foodstuffs to microorganisms is enhanced by two factors: (1) their tendency to be at moderate pH and temperature; and (2) they offer a wide enough range of oxygen concentrations to tempt both obligate anaerobes and aerobes.

It is therefore to be expected that food technology has always had a preoccupation with the control of food-borne microorganisms. The word “control” rather than “eliminate” is an acknowledgement of the fact that the total elimination of microorganisms from food is often not even attempted and, indeed, it would be undesirable in certain foodstuffs.

There are four distinct aspects of food science to which microbiologists can contribute. The most important is the issue of safety. Foodstuffs are ideal carriers of pathogenic bacteria, *e.g.* *Salmonella* sp. They are also ideal substrates for the growth of bacteria and fungi, such as *Clostridium botulinum* and *Aspergillus flavus*, which secrete toxins. A second aspect of food microbiology is that of biodeterioration. If food materials were not vulnerable to degradation by the extracellular enzymes secreted by invading bacteria and moulds, then there would be little prospect of success for the parallel processes of digestion that occur in our own alimentary canal.

The other two aspects of food microbiology are more positive in nature and concern the use of microbial activity in food production. Many processes, often under the blanket description of fermentation, have been practised for thousands of years. Examples of our exploitation of biodeterioration include pickling and cheese-making. Microbial action reduces the raw material to a more stable product in which there is much less scope for further breakdown. This usually happens because microbial activity has been

ⁱSome bacteria, not directly relevant to human food, obtain their energy from the oxidation of inorganic compounds.

accompanied by the loss of water and/or the accumulation of ethanol, lactic acid or acetic acid (ethanoic acid).

The fourth aspect of food microbiology, which attracts less interest today than in previous years, is the use of microorganisms themselves as food. Although we have always eaten fungi such as mushrooms, scientists have more recently explored the use of single-celled microorganisms. These can be used directly as human food, but, with one notable exception, the plan was for the protein to be extracted and used as a food supplement for livestock. Strains of microorganisms were developed that could feed readily on methanol and other by-products of the oil industry. Subsequent increases in world oil prices have ruined the economics of most single-cell protein production. However, one notable exception is the mycoprotein known commercially as Quorn™ (R. Angold, *et al.*, *Food Biotechnology*, in Further Reading). The carbon source for the production of Quorn™ is the starch generated as a by-product of the commercial production of wheat gluten.

Of these various aspects of food microbiology, it is the use of chemical substances to deter unwanted microbial activity that is of greatest interest to chemists. Even with unwanted microorganisms, we can often only achieve the inhibition of growth. As we have seen elsewhere in this book, the heat-processing methods required to obtain sterility – in other words, the complete elimination of *viable* vegetative cells and spores – are seldom compatible with either maximum nutritive value or desirable textures and flavours. Furthermore, the essential similarity between the metabolic processes of microorganisms and those of humans means that chemical sterilants (*e.g.* high concentrations of chlorine or phenol) will never be acceptable as food ingredients. The advantage of a chemical additive approach to the reduction of microbial activity is that many products need to remain stable for a period after the package has been opened. A jar of jam or a bottle of tomato ketchup will be subjected to repeated and massive recontamination once it comes under the influence of junior members of the household.

It is actually fairly unusual for a single antimicrobial procedure to be used on its own to protect a food product. Wherever possible, several relatively mild procedures are combined to maximise the inhibition of microbial activity, at the same time minimising their adverse effects on flavour and nutritional value. For example, the long-term storage of meat, fish or vegetables at ambient temperature requires canning, with the temperature in the centre of the can maintained above 115 °C for more than one hour. Otherwise, the surviving spores of *C. botulinum* would find the anaerobic, nutrient-rich and neutral pH environment perfect for germination, growth and toxin production. By contrast, the natural acidity of fruit eliminates the risk of botulism and the more modest heat treatments we associate with home-bottling are adequate. As we shall see in our discussion of individual food preservatives, these are often used in combination with other antimicrobial procedures. For example, the safety of cooked ham depends on: (1) the salt content, to maintain low water activity; (2) cooking, to destroy most of the vegetative bacterial cells and some spores; and (3) the inclusion of nitrites to inhibit spore germination and bacterial growth.

The use of preservatives other than sodium chloride and smoke as recognised food additives is covered by legislation in Europe and elsewhere. The principal preservatives approved for use in the European Union and other countries around the world are listed in Table 9.1.

Table 9.1 Antimicrobial preservatives permitted in Europe and elsewhere. In most countries, legislation restricts the range of foods in which particular preservatives may be used and also lays down maximum concentrations and other conditions. The websites from which the information in this table were obtained are listed in Appendix II. “Y” means “Yes, the preservative is permitted”.

Common name	E or INS No.	Permitted in					
		EU and UK	USA	Canada	Australia and New Zealand	Japan	South America ^a
Sorbic acid	E200	Y	Y	Y	Y	Y	Y
Sodium sorbate	201		Y	Y	Y		Y
Potassium sorbate	E202	Y	Y	Y	Y	Y	Y
Calcium sorbate	E203	Y	Y	Y	Y	Y	Y
Benzoic acid	E210	Y	Y	Y	Y	Y	Y
Sodium benzoate	E211	Y	Y	Y	Y	Y	Y
Potassium benzoate	E212	Y		Y	Y		Y
Calcium benzoate	E213	Y			Y		Y
Ethyl- <i>p</i> -hydroxy-benzoate	E214	Y				Y	Y
– Sodium salt	E215	Y					Y
Propyl- <i>p</i> -hydroxy-benzoate	E216		Y		Y	Y	Y
– Sodium salt	E217						Y
Methyl- <i>p</i> -hydroxy-benzoate	E218	Y	Y		Y		Y
– Sodium salt	E219	Y					Y
Sulfur dioxide ^b	E220	Y	Y	Y	Y	Y	Y
Sodium sulfite	E221	Y	Y	Y	Y	Y	Y
Sodium bisulfite	E222	Y	Y	Y	Y		Y
Sodium dithionite	—			Y		Y	
Sodium metabisulfite	E223	Y	Y	Y	Y		Y
Potassium metabisulfite	E224	Y	Y	Y	Y	Y	Y
Potassium bisulfite	—		Y				
Potassium sulfite	225				Y		Y
Calcium sulfite	E226	Y					Y
Calcium bisulfite	E227	Y		Y			Y
Potassium bisulfite	E228	Y	Y	Y	Y		Y
Biphenyl	E230	Y					
Orthophenyl phenol	E231	Y					
Sodium orthophenyl phenol	E232	Y					
Nisin	E234	Y	Y		Y	Y	Y
Natamycin	E235	Y	Y	Y	Y	Y	Y
Hexamethylene tetramine	E239	Y					Y
Dimethyl dicarbonate	E242	Y	Y		Y		Y

Potassium nitrite	E249	Y	Y	Y	Y		Y
Sodium nitrite	E250	Y	Y	Y	Y	Y	Y
Potassium nitrate	E251	Y	Y	Y	Y	Y	Y
Sodium nitrate	E252	Y	Y	Y	Y	Y	Y
Lactic acid	E270	Y	Y		Y	Y	Y
Propionic acid	E280	Y	Y	Y		Y	Y
Sodium propionate	E281	Y	Y	Y		Y	Y
Calcium propionate	E282	Y	Y	Y	Y	Y	Y
Potassium propionate	E283	Y					Y

^aThe Mercosur group of countries: Argentina, Brazil, Paraguay, Uruguay and Venezuela.

^bIn UK legislative documentation sulfur compounds continue to be spelt with *ph* rather than *f*.

9.1 Sodium Chloride

Common salt, sodium chloride, was undoubtedly the first antimicrobial substance to be used in foodstuffs. One can be confident that in early civilisations it was used as a preservative rather than for flavouring. Salting is the traditional method of preserving meat, often in combination with smoking and drying. Modern technology has provided more rapid methods of getting the salt into the meat, but the essentials have remained unchanged for centuries. Solutions containing 15–25% salt are used to bring the water activity, a_w , down to about 0.96.ⁱⁱ This has the effect of retarding the growth of most microorganisms, including the majority of those responsible for meat spoilage. With the advent of other preservative methods, notably canning and refrigeration, the importance of salting has diminished, but not without some salt-preserved meat products such as ham, bacon and salt beef remaining firmly established in our diet. As the necessity for a high salt content for preservation has decreased, our taste has adapted to bacon and ham that is less salty and less dry.

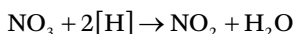
The modern understanding of the health implications of high levels of sodium chloride in our diet have resulted in strenuous efforts being made to reduce salt levels in preserved and processed food. Advances in the technology of refrigeration and other techniques are making the levels of salt used simply for its antimicrobial properties less critical, but its contribution to the flavour makes it difficult to eliminate. The responses of legislators, including those in the UK and USA, to pressure to reduce the salt content of manufactured foods, notably bread, are considered in Chapter 11.

9.2 Nitrites

Very little meat is now preserved using common salt alone. At some distant point in history, it was realised that it was the unintended presence of saltpetre (sodium nitrate, NaNO_3) as an impurity in crude salt that gave a longer life as well as an attractive red or pink colour to preserved meat. Subsequently, nitrates and/or nitrites became an almost indispensable component of the salt mixtures (known as “pickles”) used for curing bacon and ham. In spite of the antiquity of the process, it is only in recent decades that the special antimicrobial properties of nitrites and their mechanism of action have been recognised. There are many subtle variations in curing procedures, but the well-known Wiltshire cure is typical. Sides of pork are injected with 5% of their weight of a pickling brine containing 25–30% sodium chloride, 2.5–4% sodium or potassium nitrate and sometimes a little sugar. The sides are then submerged in a similar solution for a few days. After removal, they are stored for one to two weeks to mature. If prolonged storage is called for, a second preservative, smoke, is applied. By the time the maturation stage is complete, the salts will have become evenly distributed throughout the muscle tissue and a complex series of reactions will have produced the characteristic red colour of uncooked bacon and ham. The essential features of these reactions are as follows.

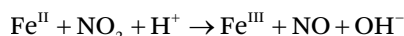
ⁱⁱThe water activity of an aqueous solution is an expression of the availability of the water, rather than its concentration. It is equal to its vapour pressure divided by the vapour pressure of pure water at the same temperature. This concept is considered in greater detail in Chapter 13.

- (i) Some of the nitrate present is reduced to nitrite:



by either salt-tolerant microorganisms in the brine or the respiratory enzymes of the muscle tissue.

- (ii) The nitrite oxidises the iron of the myoglobin in the muscle to Fe^{III} iron (see Chapter 5):

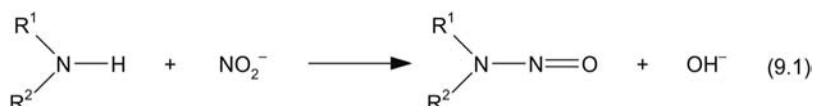


In other words, myoglobin (Mb) is converted to metmyoglobin (MMb) and nitrogen oxide (*i.e.* nitric oxide) is formed.

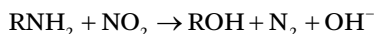
- (iii) The nitrogen oxide reacts with the iron MMb to form nitrosyl metmyoglobin (MMbNO).
- (iv) The MMbNO is immediately reduced by the respiratory systems of the muscle tissue to nitrosyl myoglobin (MbNO), the red pigment present in uncooked bacon and ham.

The distribution of electrons around the iron atom of MbNO is similar to that in oxymyoglobin (MbO_2), hence the similarity in colour. When bacon is grilled or fried, or when ham has been boiled, the nitrosyl myoglobin is denatured and a bright pink pigment, nitrosylhaemochromogen, is formed. There is some uncertainty about its structure, but it is believed that the denaturation of the globin allows a second nitrogen oxide molecule to bind to the iron in place of the histidine side-chain.

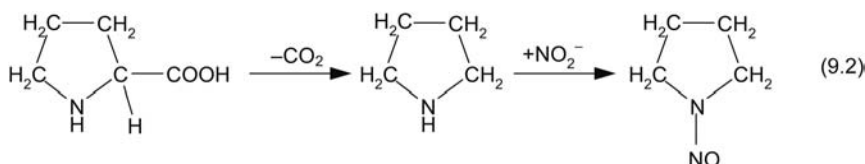
In recent years, the use of nitrates in food has been regarded with increasing suspicion as a result of the possible formation of nitrosamines, which are potent carcinogens. Secondary amines react readily with nitrous acid to form stable *N*-nitroso compounds (9.1):



The reaction with primary amines, which are, of course, abundant in meat as free amino acids, leads simply to deamination:



The reaction with amino acids is important because it eliminates some of the excess nitrite in cured meat. Secondary amines are much less abundant in meat, but are assumed to arise as a result of microbial action, especially by anaerobes. The decarboxylation of proline, which occurs spontaneously at the high temperatures involved in frying, leads to the formation of the important nitrosamine *N*-nitrosopyrrolidine (NOPyr, (9.2)):



Although nitrosamines have been detected in many cured meat products, such as salami and frankfurters, it is clear that their presence is most significant in cured meat cooked at high temperatures, such as fried bacon. Levels of NOPyr in fried bacon are consistently about $100 \mu\text{g kg}^{-1}$, along with lower levels of *N*-nitrosodimethylamine (NDMA). Although the carcinogenicity of these volatile nitrosamines has been confirmed in laboratory animals, there is little evidence that the consumption of cured meats is actually responsible for causing cancer in humans. Studies in Finland have, however, shown that, over a long period, there is a positive correlation between NDMA intake and the incidence of colorectal cancer. In this study, a high intake of smoked or salted fish was found to be a more significant factor and there was no correlation with the consumption of cured meat. The potential hazard is enough to ensure that steps remain in place to reduce the consumption of *N*-nitrosamines.

Beer is another product in which the nitrosamine content has received attention. Studies in the late 1970s showed that levels of NDMA of about 2–3 ppb were common. These arose during the normal stage of processing when malted barley is heated and dried. This operation, known as kilning, is needed to prevent continued germination once the starch-degrading α - and β -amylases have been synthesised in the grain. Traditionally, the malt was exposed directly to flue gases from a solid fuel- or oil-fired furnace. Nitrogen oxides generated in the flames of the furnace were transferred to the germinated barley by direct exposure to the flue gases. When it was realised that malt kilning was to blame, changes were made in the construction of the dryers, which, with the incorporation of sulfur dioxide into the brewing process to inhibit nitrosation, have effectively eliminated the NDMA problem.

Improvements in processing controls are making a steady reduction in residual nitrite levels possible, but the most important measure is the inclusion of ascorbic acid in curing salt mixtures. Ascorbic acid is beneficial in two ways. First, being a reducing agent, it enhances (either directly or indirectly) the rate of the key reducing reactions in the formation of MbNO, thereby allowing lower levels of nitrites or nitrates to be used in the pickles. Second, it actually inhibits the nitrosation reaction.

If the only value of nitrite was as a colouring agent, even a remote possibility of a hazard in its use for curing would warrant its prohibition. (The question of whether nitrite actually contributes to the flavour of cured meat remains unresolved.) However, the antimicrobial properties of nitrites are extremely valuable and would justify its inclusion even if it had no effect on colour. It had been known for some years that the growth of many types of anaerobic bacteria, including the organism causing botulism, *C. botulinum*, is prevented in cured meat by an unidentified product of the interaction between the residual nitrite and the meat during the time the meat is cooked. If a ham were given sufficient heat treatment to ensure that all the spores of *C. botulinum* had been killed, then it would be unacceptably overcooked. Canned stewing beef, which is given this degree of heat treatment, provides an illustration of the kind of texture the ham would have. This effect of the residual nitrite in ham was termed the “Perigo effect” after its discoverer and a great deal of work was carried out to identify what became known as the “Perigo inhibitor”. It is now well established that, during cooking, much residual nitrite is broken down to nitrogen oxide.

This is not liberated from the meat, but becomes loosely associated with some of the exposed amino acid side-chains and iron atoms of the denatured meat proteins. From this reservoir, nitrogen oxide is available for the inhibition of sensitive bacteria. It appears that nitrogen oxide is a specific and potent inhibitor of at least one enzyme (pyruvate:ferredoxin oxidoreductase) that has an essential role in the energy metabolism of anaerobes such as *C. botulinum*.

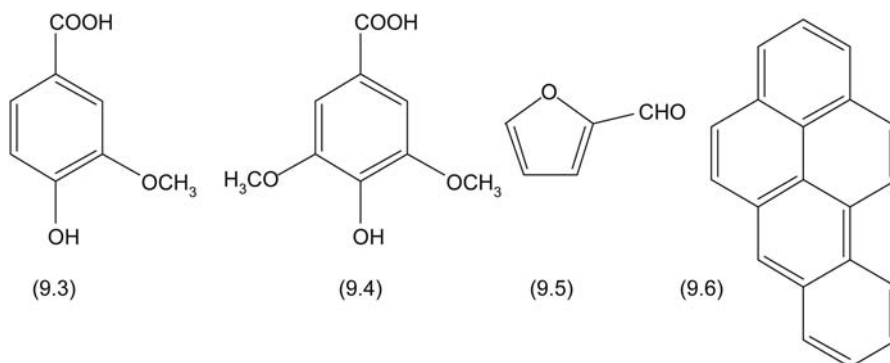
The use of nitrites therefore presents those concerned with food safety with a paradox. On the one hand, we have nitrite the preservative, in use for centuries and now known to be essential for microbiological safety. On the other hand, we have nitrite the colouring agent, suspected of giving rise to carcinogens. Apart from the obvious solution of eliminating cured meats from our diet, the most we can do is to ensure that residual nitrite levels are kept below the minimum at which toxins are produced, *i.e.* about $50 \mu\text{g g}^{-1}$. In 2020, a traditional British style of bacon cured with salt, but no nitrate, and flavoured with fruit and spice extracts began to be marketed.

It should be remembered that nitrates are naturally present in other foods, particularly vegetables, and even in drinking water. Nitrates can be reduced to nitrite by our intestinal bacteria and therefore also have the potential to cause problems, even for those who do not eat cured meat (see Chapter 10).

9.3 Smoke

Smoke is another preservative traditionally associated with meat and fish. We can be fairly sure that the flavour produced by wood smoke was regarded initially as merely a valuable side-effect of drying over a wood fire. The specific action of the smoke, beyond the dehydration resulting from the heat, almost certainly went unrecognised. Meat and fish are now rarely preserved by drying alone and refrigeration has made the preservative action of smoke less important than the flavour it gives. When meat is dried for long-term preservation, as, for example, in the production of South African biltong, the strips of meat (beef, ostrich or antelope) are marinated in vinegar and salted before drying, each of them effective antibacterial procedures in their own right.

Smoke consists of two phases: a disperse phase of liquid droplets and a continuous gaseous phase. In smoking, the absorption of gas-phase components on the food surface is considered to be much more important than the actual deposition of smoke droplets. The gas phase of wood smoke has been shown to include >200 different compounds, including formaldehyde (methanal, HCHO), formic acid (methanoic acid, HCOOH), short-chain fatty acids, vanillic (9.3) and syringic (9.4) acids, furfural (9.5), methanol, ethanol, acetaldehyde (ethanal), diacetyl (butanedione), acetone (propanone) and 3,4-benzpyrene (9.6). Of these, the most important antimicrobial agent is almost certainly formaldehyde. The short-chain fatty acids impart a degree of tartness to the flavour and also help in forming the dry skin on the surface of smoked items. The various phenols are the major source of smoke flavour and also provide some antimicrobial and antioxidant activity.



The detection of known carcinogens, such as 3,4-benzpyrene (benzo[*a*]pyrene, or B[*a*]P) and many other polynuclear aromatic hydrocarbons (PAHs), in wood smoke has caused concern about the safety of smoked foods.ⁱⁱⁱ

Apart from a relatively small number of food products where any departure from traditional smoking procedures would be regarded as sacrilege, the potential hazards of smoking have been minimised by the use of so-called “liquid smoke”. Liquid smoke preparations are manufactured by cooling the smoke generated by smouldering wood and collecting the material that condenses at temperatures of about 65 °C. After further fractional distillation (if required), the condensate is extracted with water, which leaves behind the potentially carcinogenic PAHs and other water-insoluble undesirables, but dissolves the valuable flavour and antimicrobial compounds.

The value of liquid smoke preparations is illustrated by current European Union legislation. Although it may represent only a small proportion of the total PAHs, the BaP level is the accepted marker for these carcinogens. The maximum permitted level of BaP in traditional smoked foods, including meat, fish and derived products, is 5 µg kg⁻¹. However, in foods flavoured by liquid smoke preparations, the corresponding figure is as low as 0.03 µg kg⁻¹. European Union legislation (2013) authorises a limited number of commercial liquid smoke products, manufactured in Europe and the USA, for use as food flavourings. The chemical composition of these is only prescribed in fairly broad terms, such as totals of water, organic acids, carbonyl compounds and phenols, but the source materials – that is, the wood (always hardwood, never coniferous) species – is specified in detail. Beech (*Fagus sylvatica* and *F. grandifolia*) and oak (*Quercus alba* and *Q. rubra*) are the most commonly used, but proportions of hickory (*Carya ovata*), hornbeam (*Carpinus betulus*), maple (*Acer saccharum*), ash (*Fraxinus americana*), birch (*Betula* sp.) or cherry (*Prunus serotina*) are used by particular manufacturers.

9.4 Sulfur Dioxide

Sulfur dioxide (SO₂) has been used in wine-making for hundreds of years to control the growth of unwanted microorganisms. It was originally obtained by the rather hazardous process of exposing the “must”, the unfermented grape juice, to the fumes of

ⁱⁱⁱFurther information on benzpyrene in food can be found in Chapter 10.

burning sulfur. The gas itself is rarely used today and has been replaced by a number of compounds that generate sulfur dioxide, particularly sodium sulfite (Na_2SO_3), sodium hydrogen sulfite^{iv} (NaHSO_3) and sodium metabisulfite (sodium disulfite, $\text{Na}_2\text{S}_2\text{O}_5$). The relationship between these compounds, sulfur dioxide and sulfurous acid are outlined in Figure 9.1. The antimicrobial activity of these compounds increases dramatically as the pH decreases and it has therefore been assumed that the antimicrobial activity is due to undissociated sulfurous acid. However, in recent years the presence of undissociated sulfurous acid in aqueous sulfur dioxide has been called into question, with the sulfur dioxide now thought to be present simply as a complex of formula $\text{SO}_2 \cdot \text{H}_2\text{O}$. It is therefore now thought that sulfur dioxide itself is probably the effective antimicrobial agent.

Total sulfur dioxide levels of about 100 ppm are added to wine musts to achieve a differential effect. The desirable wine yeast *Saccharomyces cerevisiae* is able to grow and ferment sucrose to ethanol, but some undesirable yeast species, such as *Kloeckera apiculata*, and bacteria producing lactic acid are suppressed. Sulfur dioxide inhibits many of the NAD-dependent dehydrogenases in yeast and bacteria, but is particularly effective against glyceraldehyde dehydrogenase in yeast and malate dehydrogenase in *Escherichia coli*. Both of these enzymes have central roles in energy metabolism. About two-thirds of the total sulfur dioxide in a must or wine is bound to anthocyanins or other flavonoids (see Chapter 6), sugars (9.7) and other aldehydes:

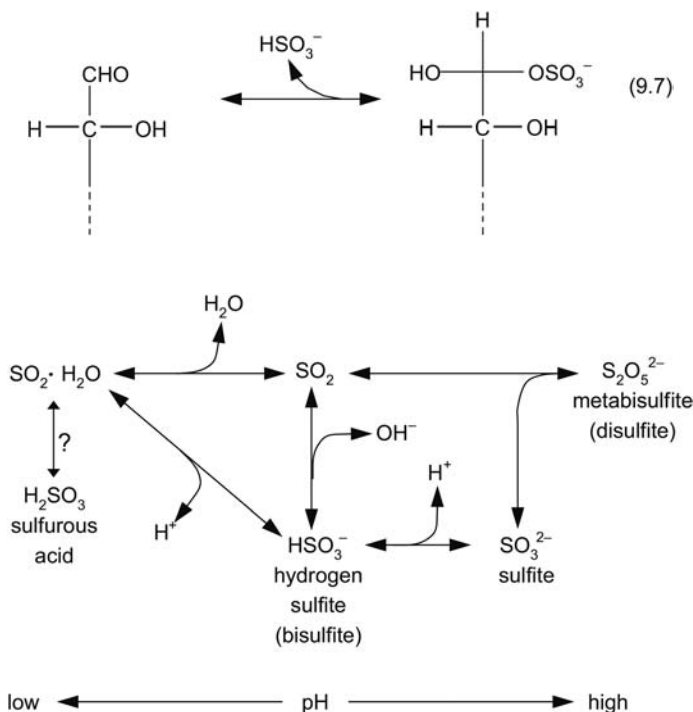


Figure 9.1 Structural relationships among compounds that generate sulfur dioxide.

^{iv}Sodium hydrogen sulfite is still often referred to by its now obsolete name of sodium bisulfite.

In addition to the added sulfur dioxide, many strains of wine yeasts produce sulfur dioxide themselves by the reduction of sulfates present in grape juice. When the wine is bottled, further sulfur dioxide is added to prevent the secondary fermentation of residual sugar in the bottle. In Europe, the legal limits for sulfites (as sulfur dioxide) depend on the type of wine: red, 160 ppm; white and rosé, 210 ppm; and sweet wines (*i.e.* containing some unfermented residual sugar), 400 ppm. There are similar, but not identical, limits in the USA and Australia. In recognition of the possible problems that sulfites may pose for people with asthma, their addition to wine at levels >10 ppm must be declared on the label.

A wide range of other foodstuffs, particularly fruit- and vegetable-based products, contain sulfites, either added as a preservative or as a residue from processing operations. The use of sulfite as an antioxidant was considered in Chapter 6.

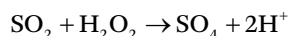
Sulfur dioxide is generally regarded to pose no toxicity hazard at the levels usually found in foodstuffs. Of all the preservatives, sulfur dioxide and its relatives are consumed in the largest amounts. Although there is no data more recent than 1986, UK consumption is not expected to have changed significantly since then. At that time, the average consumption per person was estimated to be 18.2 mg per day, with alcoholic drinks, sausages, hamburgers and dried fruit being the major sources. The margin between this level of intake (and more especially intakes at the top end of the range) and the Acceptable Daily Intake (ADI, see Chapter 10, Section 10.6) is not large. In 2016, the European Food Safety Authority concluded that an ADI of 0.7 mg SO₂ equivalent per kg body weight (corresponding to 245 mg for a typical 70 kg adult) per day was acceptable. This figure was based on the observation of a “no observed adverse effect level” (NOAEL) of 70 mg SO₂ equivalent per kg body weight during a long-term toxicity study in rats. The UK authorities do not consider current levels of sulfur dioxide intake to be a cause for concern.

Recently, levels at the upper end of the normal range in food have been shown to cause symptoms similar to a severe allergic response in a small proportion of people with asthma. It remains to be seen whether this will be sufficient for a reappraisal of the Generally Recognised as Safe (GRAS) status of sulfur dioxide in the USA. The use of sulfur dioxide can present a more significant nutritional problem because the sulfite ion reacts destructively with the vitamin thiamine (see Chapter 8). Vegetables such as potatoes are often stored in sulfite solutions during the intermediate stages of processing and can lose considerable proportions of their thiamine content. It is for this reason that many countries prohibit the use of sulfur dioxide in foodstuffs providing an important source of thiamine in that country's diet.

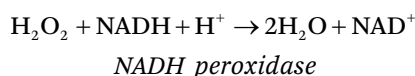
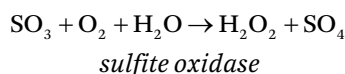
A further drawback to the use of sulfur dioxide and sulfites is their taste. Above 500 ppm, most people are aware of the disagreeable flavour caused by sulfur dioxide and some people can detect it at much lower levels. Some white wines are actually characterised by their slight sulfur dioxide flavour, notably Gewurztraminer from the Alsace region of France. Very little is known for certain about how sulfur dioxide inhibits microbial growth. The most probable explanation is that it forms addition compounds with the aldehyde groups in key metabolic intermediates or coenzymes, but these have not been identified.

Sulfur dioxide is one of the easier preservatives to determine in foodstuffs and drinks, and one which has not yet been taken over by the chromatographers. In the most

popular version of the Monier–Williams method, the sample is placed in a distillation flask and acidified to convert any disulfites *etc.* to sulfite/SO₂ and to liberate the sulfur dioxide from complexes with aldehydes. The volatile sulfur dioxide is then removed by distillation under reflux, retaining the volatile organic acids. The volatile sulfur dioxide is trapped in a hydrogen peroxide solution:



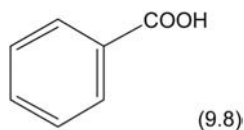
and this allows titrimetric estimation. Sulfur dioxide can also be conveniently measured enzymically using a combination of the two enzymes sulfite oxidase and NADH peroxidase:



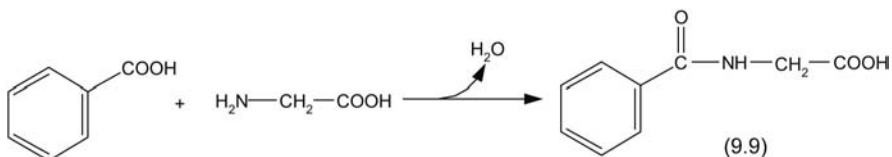
A concentration of 0.10 mM sulfite gives a decrease in the absorbance at 340 nm (due to NADH, see Chapter 8, Section 8.4) of 0.622, providing ample sensitivity for food analysis purposes. A chemical method that lends itself to automation depends on the reaction of Ellman's reagent, 5-5-dithiobis-(2-nitrobenzoic acid), with sulfite. The yellow end-product, 5-mercaptanitrobenzoate (MNB), absorbs light with a λ_{max} value at 410 nm.

9.5 Benzoates

Benzoic acid (9.8) occurs naturally in small amounts in many foods, particularly in fruit, and notably in cloudbberries, *Rubus chamaemorus* (at about 0.8%), cranberries (*Vaccinium* sp.) and prunes. However, the benzoates used as additives are the products of chemical synthesis. The sodium salt is much more soluble in water than the free acid and therefore this is the form usually used as a food preservative.



The undissociated acid is active against microorganisms, particularly yeasts and bacteria, through its inhibitory effect on the activities of the enzymes of the tricarboxylic acid cycle (*i.e.* the Krebs or citric acid cycle), α -ketoglutarate dehydrogenase and succinate dehydrogenase. The use of benzoic acid is restricted to acidic foods (pH 2.5–4.0), such as fruit juices and similar beverages, because it is only active in the protonated form. No deleterious effect on humans has been detected at the levels used (0.05–0.1%). Benzoates do not accumulate in the human body, but are converted by condensation with glycine into hippuric acid (*N*-benzoylglycine, 9.9) and are excreted in urine.



During the 1990s, concerns began to be expressed about the possible connection between the appearance of benzene, a recognised carcinogen, in some soft drinks and the use of benzoates as antimicrobial additives. Benzene levels of about $5\text{--}20\ \mu\text{g L}^{-1}$ (ppb) have been found in drinks such as colas containing ascorbic acid and benzoates. Although it was initially assumed that the benzene had originated from contaminated raw materials or during processing, the real source has now been identified. Under acidic conditions, which are normally the case in soft drinks, low concentrations of ascorbic acid in the presence of oxygen and trace amounts of transition metal ions (e.g. Fe^{III} or Cu^{II}) can give rise to hydroxyl radicals ($\text{OH}^\bullet$). Exposure to heat and light promote this reaction. The hydroxyl radicals then react with benzoic acid, resulting in its decarboxylation to benzene. Details of these reactions are shown in Figure 9.2. The benzoic acid content of cranberries has already been mentioned and, depending on the details of processing, drinks containing cranberry juice may also contain trace amounts of benzene.

Although some concern has been expressed in the popular press in the USA and the UK, the authorities have pointed out that the benzene emitted in automobile exhausts causes about $200\ \mu\text{g}$ to be routinely inhaled daily by city-dwellers. It has been calculated that one would have to consume at least $20\ \text{L}$ (5.5 gallons) per day of a drink containing benzene at $10\ \mu\text{g L}^{-1}$ to equal the amount of benzene breathed in from urban air every day.

Esters of *p*-hydroxybenzoic acid with ethanol (9.10), methanol or other alcohols, together with their sodium salts, are known collectively as “parabens”. Like the benzoates, they are effective inhibitors of mould and yeast growth, although they are less

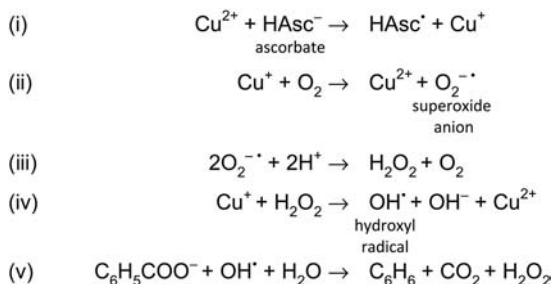
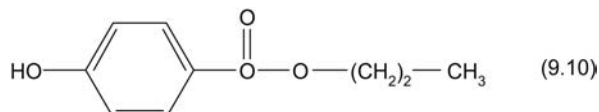


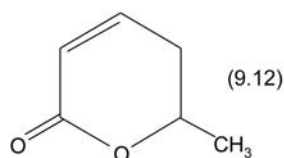
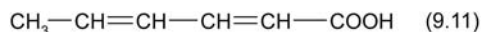
Figure 9.2 Reactions leading to the spontaneous formation of benzene in soft drinks. Reproduced from: L. K. Gardner and G. D. Lawrence, Benzene production from decarboxylation of benzoic acid in the presence of ascorbic acid and a transition-metal catalyst, *J. Agric. Food Chem.*, 1993, **41**, 693, with permission from American Chemical Society, Copyright 1965 and R. W. Matthews and D. F. Sangster, The measurement by benzoate radiolytic decarboxylation of relative rate constants for hydroxyl radical reactions, *J. Phys. Chem.*, 1966, **69**, 1938, with permission from the American Chemical Society, Copyright 1965.

useful against bacteria. Compared with benzoic acid, the esterification of the carboxyl group ensures that they remain non-ionised up to pH values of about 8.5. Although there is no certainty, it appears that they are able to disrupt the cytoplasmic membrane of target cells. The listings in Table 9.1 show that different authorities around the world have tended to come to different opinions regarding acceptability. For example, although some are permitted in Europe (E214, E215, E218 and E219), this permission only applies to their use in the jelly coatings of meat products (such as pâté) and cereal- or potato-based snacks and coated nuts. Parabens preservatives are used extensively in non-food products, such as cosmetics and antiperspirants.



9.6 Other Organic Acids

A number of other organic acids are useful preservatives. Sorbic acid (9.11) is a particularly effective mould inhibitor. Although it is normally manufactured by chemical synthesis, a natural precursor, parasorbic acid (sorbic acid lactone, 9.12) occurs in the berries of the rowan tree (*Sorbus aucuparia* or mountain ash). It appears to act as an inhibitor of a number of enzymes important in intermediary metabolism, including enolase and the tricarboxylic acid cycle dehydrogenases. As with benzoates, it is the undissociated form rather than the anion that is effective. However, the higher pK_a value (5.8) of sorbic acid compared with benzoic acid (5.2) makes it useful at pH values up to 6.5. This means that it can be used in a wider range of food products, including processed cheese, low-fat spreads such as margarine, and flour confectionery (*e.g.* cakes and pastries). It cannot be used in bread, however, due to its inhibitory effect on yeast. There is growing interest in using it to replace at least some of the nitrite in cured meat products because it does show some activity against bacteria. At the levels normally used (up to 0.3%), no toxic effect has been detected. It has been suggested that it is readily metabolised by mammals *via* a similar route to naturally occurring unsaturated fatty acids.



It might be assumed that the association of acetic acid with preserved foods, such as pickles, was a consequence of its acidic character, but in fact it is an effective inhibitor of many types of spoilage bacteria and fungi at concentrations too low (0.1–0.3% in the undissociated form) to have any effect on the pH of the foodstuff. Apart from its presence in vinegar-based food products, acetic acid is becoming increasingly popular as

an inhibitor of moulds and the formation of “rope”^v in bread. One of its special virtues, and the one which has led to it superseding propionic acid and its salts, is that it can be added as vinegar, which can then be listed as a simple ingredient on the packaging and not described as an “additive” or “added chemical”.

Like acetic acid, propionic acid ($\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$) is found naturally in many foodstuffs. Some Swiss cheeses contain as much as 1%. It has a similar spectrum of antimicrobial activity to that of acetic acid and has many applications similar to those of sorbic and acetic acids.

There is a surprising lack of information about the mechanisms by which organic acid preservatives actually inhibit microbial growth. The most probable site of action is the cell membrane, where it is envisaged that such molecules will form close associations with the polar membrane lipids. In doing so, they could disrupt the normal processes of active transport into the cell.

9.7 Nisin and Natamycin

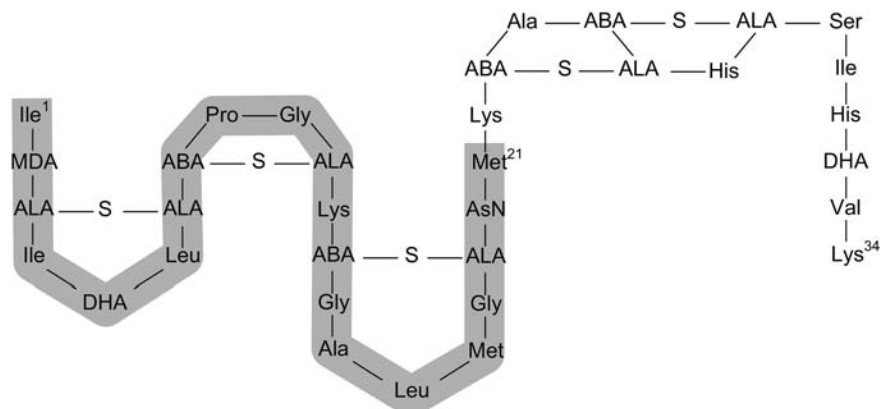
These two antibiotics are unlikely preservatives. The better known of the two, nisin, is a polypeptide consisting of 34 amino acids in a chain, with a number of small loops formed by sulphhydryl bridges involving unusual amino acids such as lanthionine. Although of interest to biochemists, the structure of nisin, beyond the simplified diagram shown in Figure 9.3, need not concern us further.

Nisin was identified in the 1930s as a problematic inhibitory substance occurring naturally in many dairy products. It was found to be produced by some strains of the bacterium *Streptococcus lactis*. This organism commonly occurs in milk and was found to inhibit the growth of other bacteria (but not fungi or yeasts) important in cheese-making, including both the desirable lactobacilli and the undesirable clostridia. The origins and activity of nisin mean that it could strictly be regarded as an antibiotic, but because it has never been used in the treatment of disease, the term is not normally used.

Nisin is now manufactured by growing selected strains of *S. lactis*. It is likely to prove valuable in a wide range of foodstuffs in which spoilage is caused by gram-positive bacteria, but at present it is mainly used in processed cheese. It prevents the growth of bacteria such as *Clostridium butyricum*, the spores of which can survive the 85–105 °C melting temperature reached in cheese processing. In common with antibiotics and enzyme preparations, it is usual to quantify nisin in units of activity rather than absolute units of weight: 1 g of pure nisin is equivalent to 4×10^7 international units (IU). For most applications, levels of nisin giving 250–500 IU g⁻¹ are required.

Nisin functions by binding to a specific protein in the cell membrane of susceptible bacteria and, in doing so, it forms a pore in the membrane, allowing ions to leak across. Nisin has highly specific requirements for binding to the target cells and this is one reason for the absence of unwanted effects in humans. Being a protein, it is completely

^vRope occurs in bread when spores of *Bacillus mesentericus* survive the high temperature in the centre of the loaf and then germinate once the loaf cools. The bacteria then attack the starch to produce a foul-smelling mass of sticky dextrins along the centre of the loaf: the “rope”.



MDA = β -methyldehydroalanine, $\text{CH}_3\text{CH}=\text{CNH}_2\text{COOH}$

DHA = dehydroalanine, $\text{CH}_2=\text{CNH}_2\text{COOH}$

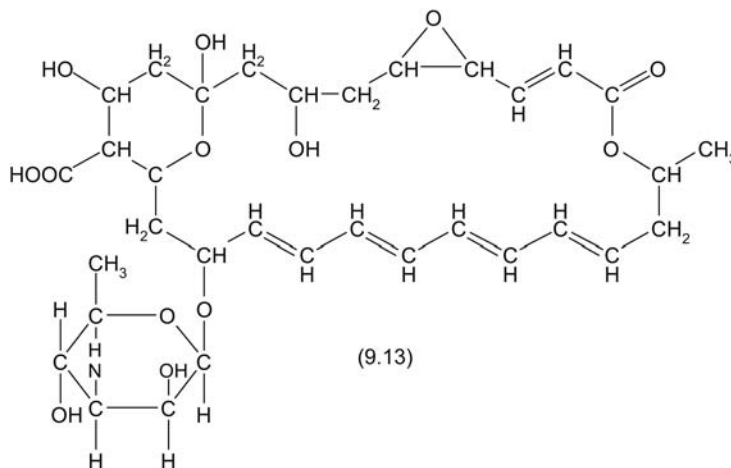
ALA-S-ALA = lanthionine, $\text{S}[\text{CH}_2\text{-CH}(\text{NH}_2)\text{-COOH}]_2$

ABA-S-ALA = β -methyl-lanthionine, $\text{HOOCCH}(\text{NH}_2)\text{CH}_2\text{SCH}(\text{CH}_3)\text{CH}(\text{NH}_2)\text{COOH}$

Figure 9.3 Amino acid sequence of nisin. The amino acids are numbered in the conventional way from the NH_2 terminal. The usual abbreviations for amino acids are used, plus those shown. The first 21 amino acids (shaded) are predominantly hydrophobic and form a region of the molecule able to bind with the membrane lipids of susceptible microorganisms.

digested to its component amino acids when it reaches the human alimentary tract and there is no report of it causing any allergic response. It has a very long history of natural occurrence in dairy products.

Natamycin (9.13) is an antifungal antibiotic (it is a polyene), which has a limited clinical application in ophthalmology and a restricted application as a food preservative. It is permitted as a surface treatment for cheese to deter unwanted mould growth. It is produced by a number of *Streptomyces* species, including the splendidly named *S. chattanoogaensis*.



9.8 Irradiation

It might be argued that the use of ionising radiation to reduce the microbial content of foodstuffs belongs within the province of microbiology or physics. However, chemists are concerned with the effects of irradiation on nutrients and other components of foodstuffs and should therefore have some appreciation of the process.

The possibility that food might be preserved by irradiation was first recognised in the early 1950s. Irradiation was seen as a method of killing contaminating pests and microorganisms without damaging the physical structure, flavour or nutritive value of food. In spite of varying degrees of acceptance by the authorities in many regions, including the European Union and USA, it remains a potential, rather than widely used, process. This official acceptance has not, so far, been matched by public opinion or by the food industry. Neither is the author qualified to comment on the potential economic costs or benefits of this technology.

The different levels of irradiation that can be used are outlined in Table 9.2, although the novel names that have been devised for some of treatment levels are rarely encountered.

Although X-rays or electron beams from accelerators could provide the necessary radiation, the ideal penetration into food material is given by the γ -rays emitted by the radioactive decay of either ^{60}Co (1.17 and 1.33 MeV) or ^{137}Cs (0.66 MeV). The mechanism by which irradiation kills microorganisms or induces other biological effects depends in the first instance on the absorption of energy by the atoms of the irradiated material. In the case of food materials, the vast majority of the atoms present are of low atomic number. In a typical “wet” biological material, hydrogen (about 10%), carbon (about 12%), nitrogen (about 4%) and oxygen (about 73%) account for 99% of the elements present. None of these elements is converted into unstable radioactive isotopes by radiation at the energy levels used. It follows that any induced radioactivity will be at a very low level, much lower than that resulting from the naturally occurring radioisotopes of heavier elements.

In the majority of foods, the energy of irradiation is absorbed by the water present according to the Compton effect. The incident photon of an γ -ray transfers some of its energy to an orbital electron, which is then ejected from the absorbing atom. The residual energy is emitted as a photon of reduced energy. When water is the absorbing

Table 9.2 Levels of irradiation treatment.

Process	Treatment ^a (kGy)	Effect
Inhibition	0.02–3.0	Delayed sprouting and ripening of vegetables and fruit
Disinfestation	0.1–1.0	Elimination of insect pests from stored food materials
Radurisation	1–5	Reduction of numbers of major spoilage bacteria and fungi
Radicidation	2–8	All vegetative microbial cells (but not spores) eliminated
Decontamination (hygienisation)	3–30	Reduction of total microbial load in food ingredients and diet sterilisation for specialised clinical applications
Radapperrisation	~50	Effective sterilisation

^a1 kilogray (kGy) = 1000 gray (Gy). 1 Gy corresponds to 1 J kg⁻¹ of energy absorbed from ionising radiation. 1 Gy = 100 rad (1 rad = 100 erg g⁻¹).

substance, the loss of an electron leads to a range of free radicals, ions and other products, including $\cdot\text{OH}$, $\text{H}\cdot$, H_2 , H_2O_2 and H_3O^+ . The ejected electron is immediately hydrated (indicated by e_{aq}^-) and gives rise to further free radicals. It is these various free radicals that account for the chemical and biological effects of irradiation. The distinction between irradiation and processes, such as heating, for delivering energy to food materials is that irradiation focuses a high proportion of the energy into a small number of chemically reactive molecules. By contrast, heat energy is dispersed throughout all the molecular species present in the material.

The lethal effect of irradiation on the reproductive capability of microorganisms is attributed to the effect of free radicals on the vulnerable purine and pyrimidine bases of nucleic acids. The dose required to kill vegetative bacteria lies between 0.5 and 1.0 kGy; bacterial spores require doses in the range 1.0–5.0 kGy. The greater complexity of higher organisms leads them to be affected by much lower lethal doses: 10–1000 Gy for insects and 5–10 Gy for mammals. A more detailed consideration of the effects of radiation on microorganisms is the concern of microbiologists.

Other important biological effects of irradiation are those that affect the development of fruit and vegetables. Depending on the fruit and the precise dose, small amounts of radiation can either accelerate or retard the onset and speed of ripening. Sprouting in stored potatoes can also be inhibited. The actual mechanism of these effects is obscure, which is hardly surprising when one considers how little is known of the biochemical control of plant development in general, even under conventional conditions.

It is the side-effects of irradiation on the chemical components of food that are our primary concern. In general, the effect on carbohydrates resembles that of heating under alkaline conditions (see Chapter 2). Attack by free radicals causes fragmentation to compounds of low molecular weight, such as formaldehyde and glyoxal. The glycosidic bonds of oligo- and polysaccharides are also vulnerable; in model systems, amylose solutions have been shown to lose most of their viscosity with radiation doses of 20 kGy. The total amount of carbohydrate breakdown products obtained is small and they do not appear to include any that are not also associated with the effect of heat.

Much the same situation applies to amino acids and proteins. Losses of amino acids are small, particularly in the dry state, when the direct interaction of the γ -ray photons with the target molecules occurs; this is not the case with water-derived products. As with polysaccharides, some depolymerisation occurs in aqueous systems. Although this can have a deleterious effect on some physical properties, such as the whippability of egg white, the nutritional value is largely unaffected.

Lipid materials, particularly unsaturated fatty acids, are much more vulnerable to irradiation. The susceptibility of lipids to free radical attack was described in Chapter 4 and experiments have shown that irradiation can initiate autoxidation along classic lines with all the expected end-products. If oxygen is abundant, very high levels of fatty acid hydroperoxides are formed. When fatty food materials are irradiated in an anaerobic environment and this is subsequently maintained, the extent of autoxidation and the development of rancidity are greatly reduced.

With the notable exceptions of thiamine, retinol and the tocopherols, the loss of vitamins during the irradiation of foods is only slight. Many vitamins, *e.g.* ascorbic acid, are unstable in simple aqueous solutions, but are stabilised by close association with food

components such as sugars and proteins. Substantial losses of thiamine occur during irradiation due to attack by both $\cdot\text{OH}$ radicals and e_{aq}^- at the $\text{C}=\text{S}$ and $\text{C}=\text{O}$ double bonds. In addition to retinol, carotenoids also suffer from irradiation-induced autoxidation in fatty foods, but are more stable in vegetable foodstuffs. Similar autoxidation reactions can overwhelm tocopherols.

The conclusion to be drawn is that treatments of up to 5–10 kGy can greatly reduce the rate of microbial spoilage and have little adverse effect on all but fatty foods. In general, the irradiation of foods while they are frozen minimises any deleterious effects on the food components without significant reduction in the antimicrobial effect. High doses have a similar effect on the chemical components of food to that of sterilisation by heat. In spite of the reassurance these observations might provide for consumers, there is considerable opposition to the use of irradiation in food preservation. Much of this opposition is based on the unfounded fear of induced radioactivity. However, legislators and consumer organisations are more concerned about the risk that unscrupulous food processors may attempt to describe foods as “fresh” when the popular understanding of this adjective does not apply. Successful legislation will require a reliable test that can indicate whether a foodstuff has been irradiated. Several methods have been developed in recent years, but none is universally applicable to all foods.

In spite of the observation that up to 300 mg kg^{-1} of radiolytic products may be produced per 10 kGy of radiation, the nature of these products, as discussed in the preceding paragraphs, varies with the composition of the food. Heat processing can also generate so many similar compounds that the number qualifying as “unique radiolytic products” (URPs) is, in fact, very small. A failure to detect URPs cannot be taken as evidence that food has not been irradiated. A valuable screening technique is to examine the microbial flora present in a suspect food. Too few bacteria or, more precisely, many more dead bacterial cells detected by the direct epifluorescence filter technique than those remaining alive, strongly suggests that irradiation has been used.

Free radicals generated by irradiation can remain trapped in foods containing dry rigid matrices, such as bone (including fragments), shell (from shellfish), and certain nuts and seeds. Their unpaired electrons can be detected by electron spin resonance, but the instrumentation involved is expensive and the technique highly skilled. Most plant-based foods carry traces of the silica present in soil particles, even after washing, and irradiation energy is trapped by the crystalline matrix of silica. Heating releases this energy as light and the thermoluminescence produced can be measured and related to the irradiation dose.

When foods contain a certain amount of lipids, it is worthwhile looking for fatty acid breakdown products, provided these can be distinguished from those arising from heat treatment or oxidative rancidity (see Chapter 4). The amounts of long-chain unsaturated hydrocarbons, such as tetradecene ($\text{C}_{14:1}$) and hexadecadiene ($\text{C}_{16:2}$), have been shown to increase in relation to the irradiation dose. They can be separated from the foodstuff by vacuum distillation and then identified and quantified by gas chromatography linked with mass spectrometry.

Another type of fatty acid/triglyceride breakdown product that qualifies as a URP is the alkylcyclobutanones, the formation of which is shown in Figure 9.4. These can also be detected by gas chromatography.

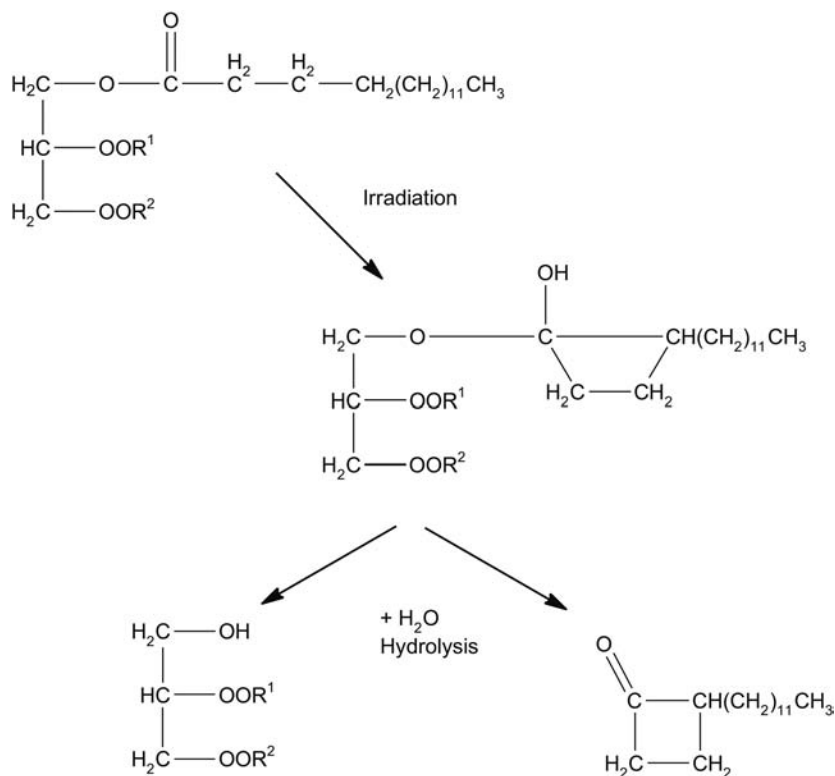


Figure 9.4 Formation of 2-alkylcyclobutanes from triglycerides during the irradiation of food. The example shown is the formation of 2-dodecylcyclobutanone from a triglyceride containing palmitic acid.

One effect of irradiation on DNA is to create breaks in the polymer chain, leading to the formation of small fragments. These fragments can be detected by a technique known as microelectrophoresis. Although repeated freezing and thawing also damages DNA, the pattern of fragments produced by irradiation is sufficiently distinctive for this to be a valuable procedure for detecting irradiation.

Despite exhortations from the authorities and the food industry that losses of flavour and nutritional value caused by low doses of irradiation are trivial, these have not been sufficient to quell public unease. Although the use of irradiation is increasing in many parts of the world, *e.g.* irradiated fresh meat is on sale in the USA, the food industry in the UK is possibly more cautious of public opinion. It has been said that in bringing irradiated products to the marketplace, the major food retailers in the UK are in a desperate race “to be the first to be second”. At the present time herbs and spices are the only irradiated products on sale in the UK. One source of consumer concern, alluded to earlier, is that unscrupulous food suppliers might pass off less than fresh foods by using irradiation to eliminate their high microbial load without reversing the other signs of excess age, such as nutrient losses and the formation of bacterial exotoxins (see Chapter 10) or off-flavours.

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10 Undesirables

The previous chapters of this book will have left the reader in no doubt that the food we eat contains many substances that are neither directly nutritive nor useful in any other way. While we can assume that our bodies are indifferent to many minor food components, the rule that substances that are not friends are likely to be enemies is a helpful guide. This chapter discusses many of the substances we find in our food that we would probably be better off without. There are many others, considered in previous chapters, that can have adverse effects under some circumstances, but also have some redeeming nutritional value.

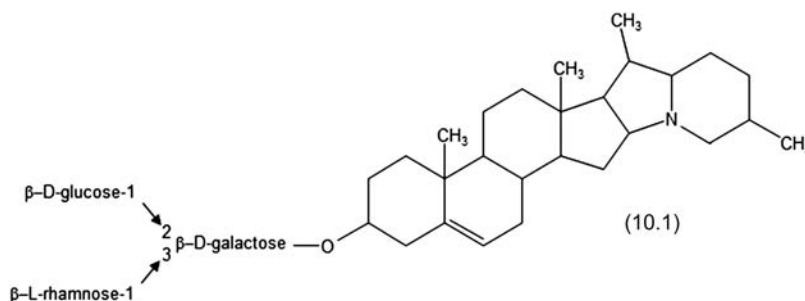
So far in this book substances have been classified either in terms of their chemical structure (Chapters 2–5) or according to their function (Chapters 6–10). Neither of these approaches is satisfactory when we consider the undesirable components of food. The enormous diversity of their chemistry, and the nature of their undesirable physiological effects, mean that it is better to classify them by origin. This gives us the four broad groups listed here and the overall arrangement of the material in this chapter:

- endogenous toxins – substances that are normal, natural components of the foods we eat;
- microbial toxins – substances that are secreted by contaminating moulds or bacteria;
- toxic residues – substances that are carried over into food materials from procedures applied by humans to the living plants or animals we eat;
- toxic contaminants – substances that arise during, or are derived from, food processing, preservation or cooking operations.

10.1 Endogenous Toxins in Foods of Plant Origin

It is widely assumed that if a foodstuff is “natural”, then it will be safe. Inevitably, this assumption leads to the suggestion that afflictions of humankind that can be traced back to diet will disappear as soon as we stop eating “unnatural” foods and stop processing natural foods in unnatural ways. For the author, there is no clearer demonstration of the naivety of this attitude than the abundance of naturally occurring toxins in supposedly natural plant food materials, including those we frequently choose to eat. In the face of the evidence it is hard to maintain the view that plants were placed on this planet with the primary objective of sustaining human life. The physiological similarities between humans and the animals we eat mean that endogenous toxins are likely to be encountered much more often in the plants that we eat than in the animals, and more frequently in invertebrates or fish than in mammals. This section examines a representative selection of toxins found in the plants we commonly eat.

One of the best-known plant toxins is solanine, which occurs in potatoes. This steroidal glycoalkaloid (10.1) is not only found in potatoes (along with smaller amounts of related alkaloid derivatives), but also in other members of the Solanaceae family, such as the aubergine (*Solanum melongena*) and the highly poisonous nightshades (e.g. *Atropa belladonna*). Potatoes usually contain 2–15 mg solanine per 100 g fresh weight. When potatoes have been exposed to light and turned green, the level of solanine may reach 100 mg per 100 g, mainly concentrated beneath the skin. The growing popularity of commercially prepared potato products in which the skin is intended to be eaten is a cause for concern because, even without obvious signs of greening, the total concentration of glycoalkaloids in the skin can reach 60 mg per 100 g. Other metabolically active tissues, such as the sprouts on potatoes, may contain even higher concentrations. Solanine is not one of the more potent toxins, although over the years a number of fatalities have been reported in the scientific literature.

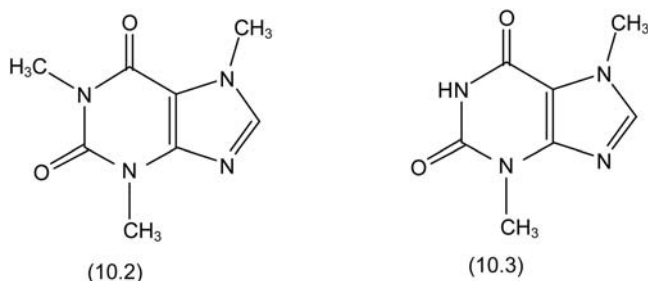


Solanine is an inhibitor of the enzyme acetylcholinesterase, a key component of the nervous system, and signs of neurological impairment have been recorded after ingestion of the toxin at a level of about 2.8 mg kg^{-1} . The relatively high doses required for toxic effects are considered to be the result of its poor absorption from the gastrointestinal tract because experimental animals show much greater sensitivity when the toxin is injected directly into their bloodstream.

The widespread awareness of the general public to the dangers of eating greened potatoes restricts the incidence of solanine poisoning to low levels. This has been reinforced

by the observation that potatoes with solanine above about 20 mg per 100 g have an unacceptable bitter taste. As solanine is heat stable and fairly insoluble in water, it is not lost during normal cooking procedures. The usually accepted safety limit for total glycoalkaloids is 200 mg kg⁻¹, but lower limits have been suggested. This suggestion has arisen from the observations of wide variations in glycoalkaloid levels found even in samples from the same batch of potatoes, and also since the milder, but no less welcome, symptoms of solanine poisoning, such as abdominal pain and vomiting, may go unreported or be mistakenly attributed to food-borne microorganisms.

Caffeine (10.2), already mentioned in Chapter 6, is a purine alkaloid. Another important member of the same group, known as the methylxanthines, is theobromine (10.3). It could be argued that these substances, which are found in tea, coffee, cocoa and cola beverages, should not be classified as undesirables because they are usually regarded as stimulants rather than toxins, although they could never be regarded as nutrients.



Roasted coffee beans contain between 1 and 2% caffeine, but the level in the beverage itself is highly dependent on the method of preparation and the strength. Values between 50 and 125 mg per cupful are normal. Black leaf tea (see Chapter 6) contains about 3–4% caffeine, giving around 50 mg per cut,ⁱ and rather less theobromine, about 2.5 mg per cupful. Cocoa powder contains around 2% theobromine and 0.2% caffeine. The levels in plain chocolate are about a quarter of these figures, and in milk chocolate about one-tenth. The caffeine content of cola drinks is restricted to a maximum of 200 mg L⁻¹, although the average value is nearer to 65 mg L⁻¹. In recent years, energy drinks containing caffeine have become popular. These often include extracts of the South American plant guarana (*Paullinia cupana*), which supplies additional caffeine to give a total concentration in the 200–300 mg L⁻¹ range.

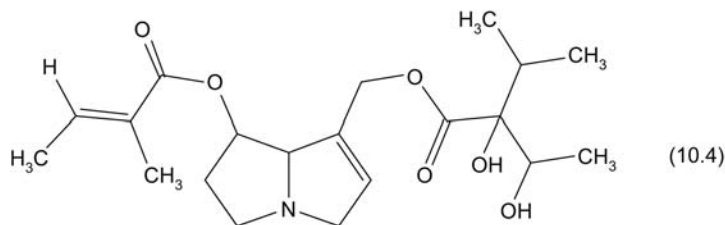
Nearly all physiological studies on methylxanthines have concentrated on caffeine. The stimulant action of caffeine is the result of its promotion of the synthesis or release of the catecholamine hormones epinephrine and norepinephrine (better known as adrenalin and noradrenalin, respectively) into the bloodstream. Most of the other effects of coffee on the levels of glucose, triglycerides and cholesterol in the blood can be traced back to the release of these hormones. The diuretic effect of caffeine is well known, of course, but these details are outside the scope of this book. Although the various effects of caffeine tend to lie within the normal range, even for quite heavy

ⁱAs the proportions of water and tea leaves, ground coffee, *etc.* in beverages are far from fixed, the apparent imprecision of the “cupful” is not so unreasonable. In the USA, where the “cup” is still a commonly used unit in domestic recipes and some nutrition data, 1 cup = 240 mL (equivalent to 190 g of granulated sugar or 120–140 g of flour).

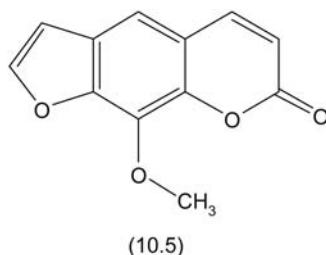
coffee-drinkers, it remains questionable whether they are beneficial in the long term. There have been too few cases of death directly attributable to caffeine consumption to provide useful data about its acute toxicity, but doses from 150 to 200 mg kg⁻¹ are known to have proved fatal in humans. This level of intake will hardly ever be achieved, even by obsessive coffee-drinkers. A vast amount of work has been carried out, using both animal experimentation and epidemiological methods, to establish whether caffeine consumption can have adverse effects on human reproduction. At the present time, caffeine has been exonerated from causing either birth defects or low birth weights, but its ordinary stimulant effects are still not regarded as being beneficial during pregnancy. In Europe, drinks, other than tea or coffee, that contain caffeine at levels >150 mg L⁻¹ (as consumed) must carry a label stating “High caffeine content. Not recommended for children or breast-feeding or pregnant women” as well as the actual caffeine content expressed in mg per 100 mL.

For many consumers the answer to the possible adverse effects of caffeine is to consume instant coffee in its decaffeinated form. In this case, the caffeine is removed by solvent extraction before the beans are roasted. Water is first added to the beans to bring their moisture content up to about 40%. They are then extracted in a counter-current system with the solvent methylene chloride (dichloromethane, CH₂Cl₂) at temperatures between 50 and 120 °C, conditions fairly selective for caffeine. Any residual solvent and moisture are then driven off and the beans are roasted. An alternative, extremely selective, solvent for caffeine is liquid carbon dioxide at high pressure (120–180 times atmospheric pressure, commonly described as supercritical). The beans must still be moist and the caffeine is removed from the circulating supercritical CO₂ by adsorption onto activated charcoal. This process has the advantages that the removal of desirable flavour elements is minimised and that there is no question of the possible toxicity of the residual solvent.

Another group of alkaloids, the pyrrolizidine alkaloids, are more often associated with herbal remedies rather than with foods. Their very occasional appearance in milk or honey when the cows or bees concerned have fed on unsuitable plants barely deserves a mention in this book. However, there is one plant material, comfrey (*Symphytin* spp.), which is often used as the basis of herbal tea, a product lying on the borderline between food and medicine. Comfrey contains at least 12 different pyrrolizidine alkaloids. Symphytine (10.4) is one of the most abundant of these and shows a chemical feature characteristic of these compounds: fused five-membered rings with a shared nitrogen atom. A cup of herbal tea made from comfrey root could contain as much as 9 mg total pyrrolizidine alkaloids. With so many different compounds occurring in such a wide range of plants, reliable data on the toxicity of pyrrolizidine alkaloids are scarce. Symphytine is thought to be carcinogenic and pyrrolizidine alkaloids are usually assumed to be the blame in cases of liver damage associated with the over-use of herbal preparations.



Plant substances that have adverse effects on humans are often found to be natural insecticides, *i.e.* substances synthesised to discourage insect pests. A good example of this phenomenon is the psoralens found in celery. Psoralens are a group of substances (also known as furocoumarins) that occur widely in the plant kingdom. Their significance as components of edible plants first was first observed in the USA in 1986 when numerous reports of severe skin rashes and related conditions among farm workers, packers and shop workers were traced back to them having handled a new variety of celery (*Apium graveolens* var. *dulce*), bred for increased resistance to insect pests. Psoralens, notably 8-methoxypsoralen (10.5), were found in the green leafy parts of this type of celery at levels around 6200 ppb, compared with 800 ppb in other varieties. The psoralens from the plant are absorbed through the skin and enter the nuclei of the skin cells. Here, they interact with DNA, finding their way into the space between neighbouring pairs of thymidine residues, a process known as intercalation. Subsequent irradiation with UV light, as experienced by anyone working outdoors, causes the formation of a link between the psoralen molecule and the pyrimidine ring of the thymidine. Long wavelength UV (320–400 nm) is the most potent. Although human systems for the repair of damaged DNA can recognise psoralen's effects, some damage still results. Suffice it to say that this variety of celery is no longer grown. "Margarita"ⁱⁱ dermatitis is caused by the 8-methoxypsoralen found in limes (*Citrus aurantiifolia*). The people most often affected are sunbathers who have whiled away their time on the beach drinking cocktails, leaving fresh lime juice on their skin.



Chocolate is also well known for its ability to cause migraine headaches in some people. This is due to its content of phenylethylamine, a vasopressor (*i.e.* it raises blood pressure). Vasopressor amines occur widely in foods; some of the more notable are shown in Figure 10.1. They may be normal products of the amino acid metabolism of the plant or, in the case of cheese and wine, the microorganisms involved in fermentation. The human body is usually well equipped to deal with these amines. The activity of the enzyme monoamine oxidase, situated in the mitochondria of many tissues, converts them into the corresponding aldehydes. Clearly, some people are either particularly sensitive to these amines or have difficulty oxidising them. Inhibitors of monoamine oxidase are important drugs (*e.g.* in the treatment of depression), so patients taking

ⁱⁱ A margarita is a cocktail of lemon or lime juice with tequila.

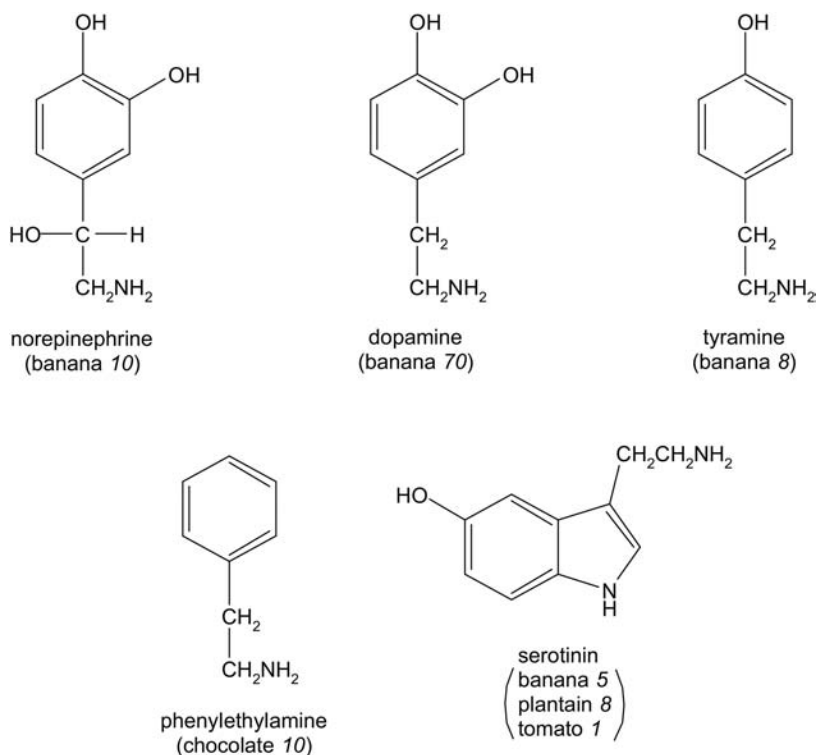


Figure 10.1 Vasopressor amines. The figures in italics are typical concentrations of the amine in the food material indicated, expressed in mg per 100 g fresh weight. Phenylethylamine is also found in significant amounts in many cheeses and red wines.

they are advised to avoid these foods. The usual effect of these amines is constriction of the blood vessels in various parts of the body. The general result is hypertension, but, in the brain, vasoconstriction causes intense headaches. There is also some support for the suggestion that the prevalence of a type of heart disease (endomyocardial fibrosis) in West Africa may be caused by the large amounts of plantain, a relative of the banana, in the diet. A typical plantain-based diet could easily give a daily intake of 200 mg serotonin.

Another African staple food, cassava (manioc, *Manihot esculenta*), poses a different toxicological problem, that of cyanogenesis. The glycoside amygdalin was briefly described in Chapter 2. This is just one of a group of glycosides that occur in many plant families, but particularly in the Rosacea and Leguminosea families. Although the presence of amygdalin in the stones of some fruit does not present much of a hazard, the higher levels of α -hydroxyisobutyronitrile and related compounds in cassava do. Fresh cassava may give rise to 50 mg hydrogen cyanide per 100 g due to the activity of two enzymes: β -glucosidase and hydroxynitrile lyase (see Figure 8.13). Both occur in plant tissues and become active when the tissue is damaged during harvesting or preparation for cooking. Although animals do not produce either of these enzymes for themselves, there is evidence that intestinal bacteria are able to release the hydrogen cyanide from cyanogenic glycosides. It is a well-established tradition in West Africa for those who

depend on cassava to allow a fermentation period between slicing or shredding the tubers and before cooking, which allows the endogenous enzymes to release the HCN, which, being volatile, is eliminated. An outbreak of suspected cyanide poisoning that occurred in western Uganda in 2017, and included two fatalities, was traced to a batch of cassava flour made from wild cultivars that had a high cyanogen content.

Despite these defensive food preparation procedures, it is becoming clear that chronic cyanide poisoning, caused by the ingestion of low levels of HCN over prolonged periods of time, is widespread in parts of the world dependent on cassava. The possibility that low levels of serum cyanide may afford a degree of protection against the malarial parasite was discussed in the context of the bitter taste of cyanogenic glycosides in Chapter 7. Two common diseases, one a degenerative neurological condition (ataxia) and the other a form of blindness (tropical amblyopia) have been attributed to an interaction between cyanide from cassava and a deficiency of the vitamin cobalamin in the diet. Another problem is caused by the body's use of the enzyme rhodanase to detoxify small amounts of cyanide by converting it into thiocyanate (CNS). Where the cyanide intake is high, *e.g.* in those parts of eastern Nigeria where dried, unfermented cassava is popular, high levels of thiocyanate are found in the blood. Thiocyanate is known to interfere with iodine metabolism, resulting in the goitre common in eastern Nigeria.

Another important food crop that can contain dangerous levels of cyanogenic glycosides is the lima bean (*Phaseolus lunatus*), which is grown in many parts of the world. The difficulty of preparing these beans in a way that eliminates their toxicity has led to the breeding of bean varieties with reduced levels of glycosides.

Cyanogens are not the only toxins found in legumes. Another group are the protease inhibitors. Legume seeds, including peas, beans, soya and peanuts, all contain proteins that can inhibit the proteases of the mammalian digestive tract, notably trypsin and chymotrypsin. The inhibition is the result of a one-to-one binding that blocks the active site of the enzyme. These proteins fall into two groups: those with molecular weights of around 22 000 that are specific to trypsin; and those with molecular weights of between 6000 and 10 000, which can inhibit both trypsin and chymotrypsin. Experiments with animals fed on diets consisting largely of unheated soya bean meal show reduced efficiency in utilising proteins (see Chapter 5). If the diet is continued, the pancreas, the organ that synthesises trypsin and chymotrypsin, shows a marked increase in size, presumably a compensatory mechanism.

When legumes are heated, the efficiency with which they are utilised as sources of dietary protein increases, but experiments with soya have shown that even prolonged heating does not allow the theoretical efficiency to be reached. This is because the losses of essential amino acids caused by the Maillard reaction eventually become significant.

Any examination of the effects of protease inhibitors in legumes is complicated by the presence of a second group of toxic proteins, the lectins. The lectins are characterised by their ability to bind to the surface of certain animal cells, particularly red blood cells, causing them to clump together – hence their alternative name, haemagglutinins. Because individual lectins show a high degree of specificity for particular types of animal cells, *e.g.* red blood cells of single blood groups, they have become popular laboratory tools with animal biochemists and physiologists. Injected directly into the bloodstream, some can be extremely potent poisons, with toxic doses in the region of 0.5 mg kg^{-1} .

Lectins are proteins with molecular weights around 10^5 . As a result of their usefulness as biochemical tools, some have been studied in great detail, but until recently the results have not had much relevance to food science. The natural role for lectins is to provide a degree of protection for the host plant from insect pests through their inhibition of the insect's digestive enzymes. During the 1990s, the possibility was explored of introducing the genes for lectins into valuable food crops, using recombinant DNA techniques, to improve their pest resistance, but (to date) none of the resulting genetically modified varieties appear to have been developed commercially.

Lectins may represent a significant fraction of the total protein in legumes, amounting to 3% in the case of soya beans. Experimental animals fed diets containing as little as 1% unheated soya beans show impaired growth, but some other legumes can be lethal at this level. The differences appear to be caused by the variations in the susceptibility of different lectins to break down by digestive enzymes. The lectin in red kidney beansⁱⁱⁱ is one of the more heat resistant. Different authorities recommend 10–30 minutes of boiling for red kidney beans to eliminate the toxin. Cooking at lower temperatures, *e.g.* at 80 °C for several hours in a slow cooker, may actually increase the activity of the toxin by making it more available. White kidney beans contain about one-third of the level found in the red variety. Beans of other species, such as broad beans (*Vicia faba*), contain <10% of the red kidney bean level.

The symptoms of lectin poisoning vary, but include vomiting and diarrhoea, as well as reduced uptake of nutrients from the digestive tract believed to be caused by damage to the intestinal mucosa. The relevance of lectin toxicity to humans is debatable. From time to time there are bursts of enthusiasm in the media for eating raw vegetables, which, as we have seen, can be a potential hazard. As long as the contribution of uncooked legumes, other than kidney beans, to one's total protein intake remains small, the danger of lectin-induced illness is likely to be slight. Fortunately for advocates of the culinary value of barely cooked sprouted beans, massive breakdown of lectins occurs as the seeds germinate.

Soya beans are the best-known source of another group of biologically active substances that occur fairly widely in plants: the phytoestrogens. It is debatable whether they should be considered under the heading “Undesirables”, but in the absence of a specific discussion of what are sometimes referred to as “nutraceuticals”, this chapter is as good a home for them as any other. They were discovered in the 1940s as a cause of fertility problems in Australian sheep that had consumed a diet rich in a species of clover, now recognised to be a major source of a phytoestrogen known as coumestrol. This substance is unusual in that it binds to the oestrogen receptors in the body with an affinity comparable to that of oestradiol, whereas the phytoestrogens in soya, the only ones of significance in human diets, have binding affinities between one-thousandth and one ten-thousandth of that of oestradiol. As illustrated by the structures shown in Figure 10.2, phytoestrogens have their critical phenolic and hydroxyl groups in a similar structural relationship to those in oestradiol.

ⁱⁱⁱ*Phaseolus vulgaris*. Red and white kidney beans are two cultivars of this species. Amongst other cultivars, the following are well known: flageolet beans, green beans, pinto beans and haricot or navy beans. The “baked beans” canned in tomato sauce, popular in the UK, are haricot or navy beans.

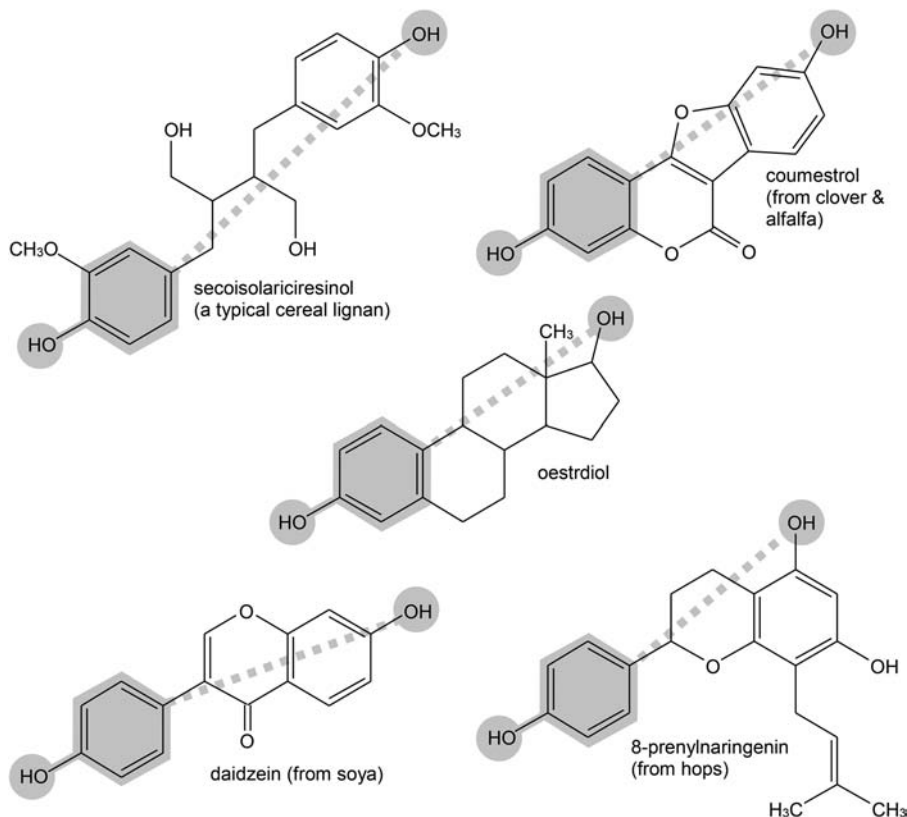


Figure 10.2 Structures of phytoestrogens compared with the human hormone oestradiol.

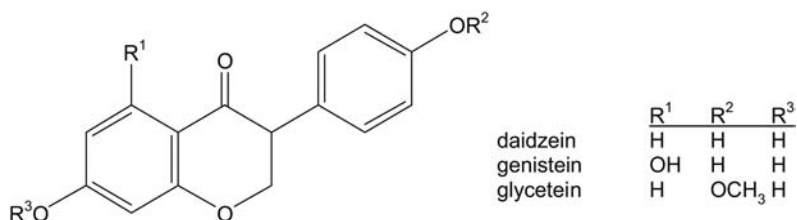


Figure 10.3 Principal isoflavanoid phytoestrogens of soya beans. In the raw beans, there is usually a glucose residue attached at position 3. These glycosides are known as daidzein, genistein and glycitein, respectively. Glycitein is not considered to be oestrogenic.

The legume phytoestrogens are classed as isoflavones.^{iv} The structures of the principal soya bean phytoestrogens are shown in Figure 10.3. In Europe, most soya-based foods are consumed as part of vegetarian or vegan diets, whereas in the Far East and

^{iv}Isoflavones differ from other flavonoids in that their B ring (see (6.10)) is attached at the 3-position rather than the 2-position of the central oxygen-containing ring.

among communities of Far Eastern origin in North America and elsewhere, fermented soya products (*e.g.* soy sauce, tofu, miso and tempeh) are components of virtually everyone's diet. Soya milk, popular in Europe with consumers as an alternative to cow's milk, is made by grinding soybeans in water to a slurry, followed by filtration and boiling. Heat treatment is essential to inactivate the soybean trypsin inhibitor and the enzyme lipoxygenase (see Chapter 7, Section 7.2.3). Soy-based products were reported in 2001 to constitute nearly one-third of the infant formula milks consumed in the USA.

The total amounts of phytoestrogens found in soya beans can vary widely, between 20 and 300 mg kg⁻¹, mostly as genistein or daidzein (Table 10.1). Levels in foods other than legumes rarely exceed 1 mg kg⁻¹. Total phytoestrogen levels in soya-based infant formula milk products vary, but are usually in the region of 9 mg L⁻¹ when made up. Adults consuming omnivorous European-style diets have been estimated to consume up to 0.8 mg of phytoestrogens daily, although the figure for adult vegetarians may be 100 times this figure. The daily phytoestrogen intake of Chinese and Japanese adults has been estimated to between 20 and 200 mg.

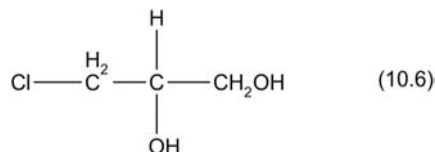
A variety of potential health benefits have been ascribed to phytoestrogens. These include lowered risks of osteoporosis, heart disease, and breast and prostate cancer. However, phytoestrogens also have the potential to disrupt the endocrine system, leading to the possibility of adverse effects on health. The actual mechanisms by which phytoestrogens may exert their effects are far from fully understood. An authoritative opinion on potential benefits or otherwise of phytoestrogen consumption is outside the scope of this book, so readers seeking such opinions are strongly recommended to consult the 2017 review by Rietjens, Louisse and Beekmann (see Further Reading). Other significant issues for European soya consumers are the increasing dependence on genetically modified soya varieties grown in North America and the environmental implications of Amazon rain forest clearance for soya cultivation.

As an aside to these problems with soya, this is an appropriate point to mention the contamination of some soya-based products with 3-monochloropropane-1,2-diol (3-MCPD, (10.6)), particularly soy sauce. Traditionally, soy sauce is manufactured by a natural microbial fermentation in which the soybeans and other ingredients are slowly hydrolysed to the amino acids that provide its distinctive flavour. Today, this process is accelerated by an initial treatment of the soybeans with hydrochloric acid. This hydrolyses the proteins, liberating free amino acids for the bacterial fermentation, but the hydrochloric acid also interacts with the fatty acids in soya to generate small amounts

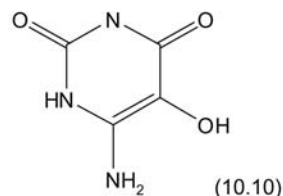
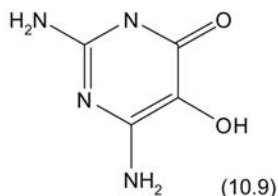
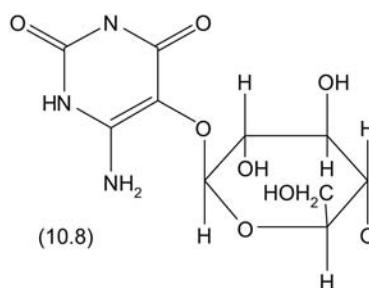
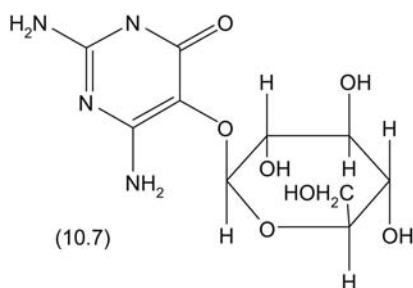
Table 10.1 Phytoestrogen content of soya-based food products. Adapted from data given in *Phytoestrogens and Health* (Committee on the Toxicity of Chemicals in Food Consumer Products and the Environment, Food Standards Agency, 2003).

Food	Total phytoestrogen content (mg per 100 g)
Soya beans	14–300
Soya milk	5–10
Soya flour	130–200
Soya protein-based burgers, sausages, <i>etc.</i>	8–15
Fermented foods, <i>e.g.</i> tempeh, tofu, miso	15–70
Soy sauce	0.1–1.5
Non-dairy infant formula	≤20

of MCPD. Current European Union regulations prohibit the importation of soy sauce containing levels of MCPD $>20 \text{ mg kg}^{-1}$. However, the No Observable Effect Level (NOEL; see Section 10.6) has been established at 1.1 mg per kg of body weight per day, with a recommended Tolerable Daily Intake^v (TDI) of 2 mg per kg body weight.



Another legume, the fava bean, *Vicia faba*,^{vi} is also well known for its toxicity. In some parts of the world, consumption can lead to a potentially fatal haemolytic anaemia (*i.e.* the premature destruction of red blood cells) known as favism. The association of fava beans with attacks of what we now term favism was known to the physicians of Ancient Greece and also to the mathematician Pythagoras, who published a “rule” or “consultation” stating “κνάμων απέχεσθαι”, which translates roughly to “stay away from fava beans”. The problem is caused by two pyrimidine glycosides, vicine (10.7) and convicine (10.8). These occur at levels of about 700 and 200 mg per 100 g, respectively, in the bean’s cotyledons, the main edible portion. During the normal processes of digestion, these are broken down by microorganisms in the gut to yield their corresponding aglycones: divicine (10.9) and isouramil (10.10). Although fava beans are widely grown in many of the temperate zones of the world, favism is mainly encountered in territories bordering the Mediterranean, such as Greece, southern Italy, Turkey, Cyprus, Sardinia, Algeria, Lebanon and Egypt. Favism also occurs in the Near East (Iraq and Iran) and parts of India.



^vThe TDI is an estimate of the amount of a substance (expressed on a body weight basis) that can be ingested daily over a lifetime without appreciable risk.

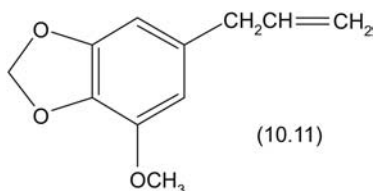
^{vi}The species *V. faba* is normally divided into two subspecies, *V. faba minor*, the fava bean proper, and *V. faba major*, known in the UK as the broad bean. Both subspecies contain similar levels of pyrimidine glycosides.

The common feature of all these regions is the prevalence of malaria. In these regions, a significant proportion of the population have an inherited condition that results in reduced levels of the enzyme glucose-6-phosphate dehydrogenase (G6PDH) in their red blood cells (erythrocytes). This reduced enzyme activity results in these cells providing an unfavourable environment for the development of the malarial parasite, giving those affected considerable resistance to the disease. A downside of this protective effect is that its beneficiaries have an increased susceptibility to an adverse effect on the stability of their erythrocytes caused by divicine and isouramil and are thus vulnerable to attacks of favism. It is now established that the fava bean glycosides reduce the erythrocytes' hospitality to malarial parasites to a similar extent as G6PDH deficiency.

One might expect the incidence of favism to have led to vulnerable populations ceasing to cultivate fava beans, but this has not occurred for two reasons. First, these beans are an excellent source of protein and the plants thrive in difficult environmental conditions. Second, the presence of divicine and isouramil affords a useful degree of protection against malaria, especially in people with reduced G6PDH. The risks of favism to people with the lowest levels of G6PDH can be assumed to be, in evolutionary terms, a price worth paying.^{vii}

The actual mechanism of the effects of divicine and isouramil on the metabolism of erythrocytes is far from simple and detailed consideration is given in the first of the Special Topics at the end of this chapter.

The concluding toxin in this section is myristicin (10.11). This occurs in significant levels in nutmeg and, in smaller amounts, in black pepper, carrots and celery. There is sufficient myristicin in 10 g of nutmeg powder to induce similar symptoms to a large intake of ethanol: initial euphoria, hallucinations and, eventually, narcosis. Higher doses induce symptoms similar to those of ethanol poisoning, including nausea, delirium, depression and stupor. Of course, the amount of myristicin in the quantities of nutmeg used as a flavouring are well below those affecting the brain, but it is interesting that, in the UK at least, the consumption of nutmeg-flavoured foods during pregnancy has traditionally been regarded as ill-advised.

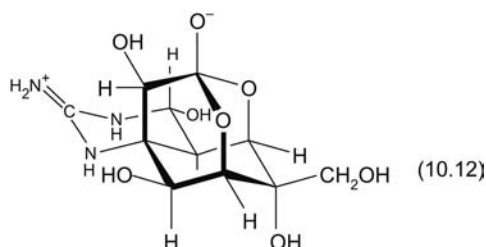


10.2 Endogenous Toxins in Foods of Animal Origin

The reason for the relative shortness of this section has already been mentioned. Most animal toxins of significance to human eating habits are found in taxonomic groups distant from humans, such as the crustaceans, but one of the most interesting occurs

^{vii}The relationship between the incidence of sickle cell anaemia in parts of Africa and resistance to the malarial parasite is another example of an evolutionary compromise.

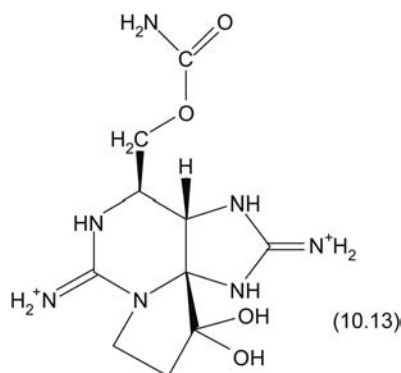
in a number of fish species, notably the infamous puffer fish (*Lagocephalus sceleratus*). The toxin tetrodotoxin (10.12) occurs in various organs of the puffer fish, including the liver and ovaries (at around 30 mg per 100 g) and, to a lesser extent, in the skin and intestines. The minimum lethal oral dose of tetrodotoxin is estimated to be between 1.5 and 4.0 mg. The muscles and testes are generally free from the toxin and it is these that form a delicacy popular in Japan. Unfortunately, great skill is required by the cook to separate the deadly parts of the fish from those that are merely risky. Stringent licensing of expert cooks reduces the risk, but fatalities still occur.



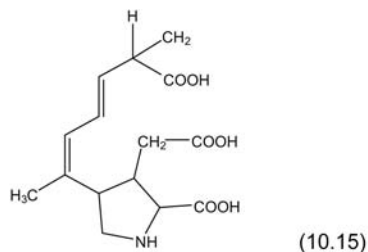
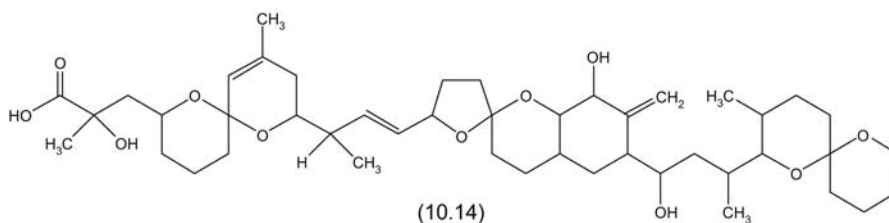
The toxin is rapidly absorbed and the first symptoms may become apparent as soon as ten minutes after eating the toxic fish, although an interval of an hour or so is more usual. The effect of the toxin is to block the movement of sodium ions across the membranes of nerve fibres. The result is that nerve impulses are no longer transmitted and a range of distressing nervous symptoms is experienced, developing into total paralysis and respiratory failure that can lead to death within 6–24 hours. No specific treatment has been discovered and, in the light of the continued enthusiasm for eating these fish, all that can be done is to ensure the best possible training of Japanese chefs. Of the other toxins associated with marine foods, most occur in shellfish such as mussels, cockles, clams and scallops. However, these toxins were not synthesised by the shellfish, but by the marine microorganisms, phytoplankton, that the shellfish consume. At certain times of year the coastal waters in the hotter parts of the world develop a striking reddish colour due to the proliferation of red-pigmented dinoflagellates. These “red tides” are alluded to in the Old Testament (Exodus 7:20–21) and may have given the Red Sea its name. Problems also occur in cooler regions, such as the coasts of Alaska and Scandinavia, and the dinoflagellates are not always red-pigmented. Dinoflagellates containing toxins quickly find their way up the food chain to shellfish caught for human consumption. There are a number of different classes of these shellfish toxins, but the best known are those that cause paralytic shellfish poisoning (PSP), *i.e.* paralysis of the nervous system. Death may follow rapidly after the consumption of only a few milligrams of toxin.

One of the best known PSP toxins is saxitoxin (10.13). Most of the others differ only in minor variations in the groups attached around the rings. Several different variants may be encountered in a single outbreak of PSP. The relationship between red tides and the toxicity of shellfish appears to have been recognised for many centuries in coastal communities and, as a result, the number of outbreaks of poisoning is limited. However, the number of fatalities that may occur in a single outbreak of PSP is such that the authorities in regions such as the Pacific coast of North America maintain routine checks on toxin levels in shellfish catches. A serious outbreak of PSP, fortunately not

resulting in fatalities, occurred in 1968 when 78 people were taken ill after eating mussels from sites on the northeast coast of England. Since that time, shellfish in this area have been regularly monitored, although on only one subsequent occasion, during ten weeks in the summer of 1990, were levels >80 mg per 100 g of mollusc flesh recorded and warnings issued.

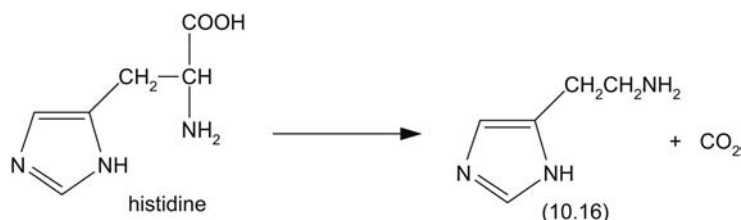


Diarrhetic shellfish poisoning (DSP), involving a rapid onset of diarrhoea, nausea and vomiting lasting up to three days, occurs mostly in Japan, but cases have been reported worldwide. Although no case has been recorded in the UK, the toxins concerned have been detected in shellfish, *e.g.* queen scallops (*Aequipecten opercularis*), in British waters. The best known of the DSP toxins is okadaic acid (10.14), again produced by species of dinoflagellates. Cases of amnesic shellfish poisoning (ASP) were first identified in Canada. ASP can cause severe neurological symptoms that can lead to permanent brain damage. The toxin involved, domoic acid (10.15), is produced by various species of diatom.



A different type of poisoning is associated with fish belonging to the family Scombridae. This family includes many commonly eaten fish, including mackerel, tuna and sardines. When freshly caught the fish are not toxic, but, if held at temperatures $>10^\circ\text{C}$,

for several hours, high levels of histamine (10.16) are sometimes found. Histamine is formed from the amino acid histidine, which occurs naturally in the muscle. The reaction is catalysed by a decarboxylase produced by a number of common bacteria, but that of *Proteus morganii*, an otherwise fairly innocuous marine microorganism, has been particularly incriminated. Despite levels of histamine >100 mg per 100 g being produced by the bacteria, obvious putrefaction of the fish may not have taken place. The consumption of the large doses of histamine that might occur in contaminated fish does not necessarily have adverse effects because histamine is poorly absorbed from the intestines. Nevertheless, the collection of symptoms that are encountered from time to time, including severe headaches, palpitations, gastrointestinal upsets, skin flushes and erythaema, correspond closely to the effects of the direct injection of histamine into the bloodstream. In addition, the symptoms are characteristically relieved by antihistamine drugs. It is assumed that people affected by scombrototoxic fish have some minor intestinal lesion or disorder that has allowed histamine into their bloodstream.



Histamine is classified as a biogenic amine, *i.e.* a low molecular weight amine with recognised biological activity. Other biogenic amines, which occur in plant materials and are grouped together by their tendency to increase blood pressure in humans, were described in Section 10.1. Such biogenic amines are not uncommon in foods of animal origin whenever microorganisms have become involved, as in the spoilage of meat and the maturation of cheese. Histamine, tyramine, cadaverine [$\text{H}_2\text{N}(\text{CH}_2)_5\text{NH}_2$, derived from the amino acid lysine] and putrescine [$\text{H}_2\text{N}(\text{CH}_2)_4\text{NH}_2$, from glutamine] are all abundant in cheese, typically at levels between 100 and 500 mg kg⁻¹. The levels of amines in meat are generally around one-tenth of these figures.

10.3 Mycotoxins

The preceding chapter explained the susceptibility of human and animal foodstuffs to contamination by microorganisms. Although the value to humans of the fermentative microorganisms involved in dairy products *etc.* is generally recognised, most people will still reject food that is obviously mouldy. This rejection may well be made on what appear to be aesthetic grounds, but in actual fact the growth of many species of fungi on foods and food raw materials is accompanied by the production of toxins that can be extremely dangerous. Perhaps the most famous (“infamous”?) of the diseases caused by fungi is ergotism. This reached epidemic proportions throughout much of Europe in the Middle Ages, particularly where rye was an important cereal crop. During the twentieth century smaller outbreaks occurred in both the UK and France. It is now established

that the cause of the disease is the infection in the field of rye and other cereals by the commonly occurring mould *Claviceps purpurea*, or ergot. At one stage during its life cycle the mould produces structures known as sclerotia, which are hard, purplish-black masses of dormant cells. About the same size as a cereal grain, these may be harvested inadvertently along with the grain and, unless recognised and removed, will be milled into the flour. The sclerotia have been found to contain at least 20 different toxic alkaloids, the most abundant of which are peptide derivatives of lysergic acid (Figure 10.4).

The biological effects of the ergot alkaloids appear to be due to the collective results of different individual alkaloids, each acting on different features of human physiology. As a result, the symptoms will vary from outbreak to outbreak depending on the range of toxins produced by the particular strain of the mould. The onset of the disease is usually slow but insidious, and is generally catastrophic in outcome. Through as yet poorly understood effects on both the nervous and vascular systems, the affected person initially experiences general discomfort, followed by intense burning pains in their hands and feet. These give way to a total loss of sensation in their limbs, followed by gangrenous withering and blackening and leading to a loss of the limbs. These symptoms are often accompanied by mental derangement and gastrointestinal failure before death. It is hardly surprising that this awful disease was given the name “St Anthony’s Fire” in the Middle Ages.

The comparative rarity of the disease today is the result of several factors. Modern fungicides reduce the incidence of infected plants to a minimum and modern grain-drying systems largely prevent the post-harvest spread of the mould. All grain shipments are inspected for signs of the black sclerotia and modern grain-cleaning methods are able to separate the heavier grains from the lighter sclerotia.

To date, about 150 different mould species have been shown to produce toxins when they grow on human or animal foodstuffs. Cereals are their most likely targets. Most of these moulds are members of one of the three genera: *Fusarium*, *Penicillium* or *Aspergillus*. It would be impractical to consider even a small selection in the detail we have

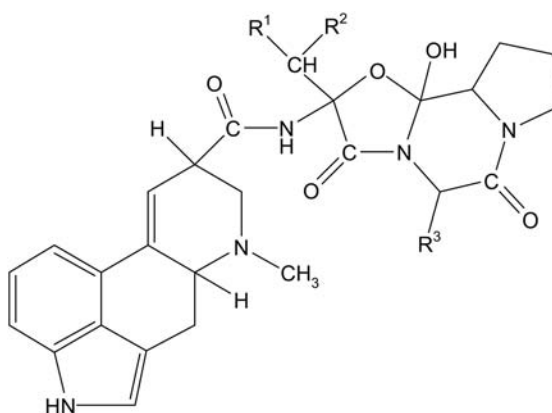


Figure 10.4 Ergot alkaloids. In ergotamine, one of the most abundant ergot alkaloids, R¹, R² and R³ are H, CH₃ and C₆H₅, respectively. Others have various combinations of H or CH₃ at R¹, R² and R³, and C₆H₅CH₂, (CH₃)₂CHCH₂ or CH₃CH₂ at R³.

given to ergot, but there is one aspergillus species, *Aspergillus flavus*, which merits particular attention. In 1960, thousands of turkeys were killed in England following an outbreak of what became known as Turkey X disease. The cause was traced to Brazilian groundnut (*i.e.* peanut, *Arachis hypogaea*) meal that had become infected with *A. flavus* and it was not long before the group of toxins shown in Figure 10.5 was identified. Although there are differences in the relative toxicity of particular aflatoxins towards individual animal species, it is now clear that aflatoxin B1 is one of the most potent liver carcinogens known. A diet containing as little as 15 ppb will, if fed over a few weeks, induce tumour formation in most experimental animals.

As might be expected, data on the effects of these toxins on humans are scarce, but they are recognised as being acute hepatotoxins. They are carcinogenic in many, but not all, animal species. In humans, long-term exposure is likely to cause liver necrosis and they may be involved in the development of liver cancer. There are also links to the occurrence of hepatitis B. A further complication is that the mechanism of aflatoxin-induced carcinogenesis is very different in humans from that in rodents. In the tropics, numerous cases have now been recorded of fatalities due to acute hepatitis and related disorders that could be linked to the consumption of mouldy cereals,

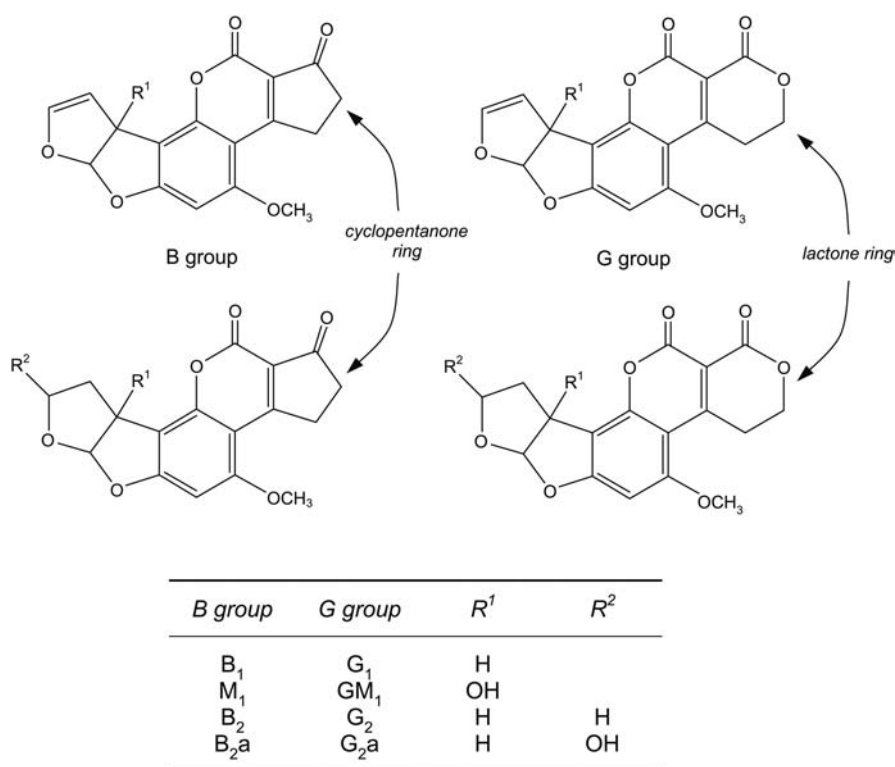


Figure 10.5 Mycotoxins of *Aspergillus flavus*. The aflatoxins are bifuranocoumarins fused in the B group to a cyclopentanone ring or in the G group to a lactone ring. Similar variations in the substitution pattern of the bifuranocoumarin system occur in both groups, as shown in the inset table. Hydroxylation at R2 eliminates toxicity.

especially rice, subsequently shown to contain between 0.2 and 20 mg kg⁻¹ of aflatoxins. Of much greater importance are the epidemiological studies that have been carried out in many parts of the world, notably Africa and the Far East. These studies have shown a clear positive correlation between the incidence of liver cancer within a community and the level of aflatoxins in its staple foodstuffs. Although most grains appear to be vulnerable to aflatoxin contamination, there is no doubt that peanuts and derived products such as peanut butter can present particular hazards. Levels of aflatoxins in peanut butter produced in some developing nations may routinely reach 500 µg kg⁻¹. As a result of such widespread contamination, it is not uncommon to find the total daily aflatoxin intake in some developing nations being estimated at about 5 ng per kg body weight.

As the climate of Western Europe tends not to favour the formation of aflatoxins, the regulatory authorities in the UK and the European Union focus their attention on a small range of imported products, including peanuts and peanut butter, dried figs, animal feedstuffs and some other types of nuts known to be vulnerable to contamination. The legal limit for aflatoxin contamination in the European Union is 4 µg kg⁻¹. Elsewhere limits are often higher, *e.g.* 20 µg kg⁻¹ in the USA. There are similar limits on aflatoxins in animal feedstuffs in the European Union, the USA and elsewhere. One problem facing the authorities in detecting contaminated foodstuffs is the wide variation between samples. For example, some years ago an analysis of 36 separate 1 kg samples selected at random from a consignment of dried figs imported into the UK showed that the majority were below 4 µg kg⁻¹, but three were >50 µg kg⁻¹ and one of these had >2 mg kg⁻¹!^{viii}

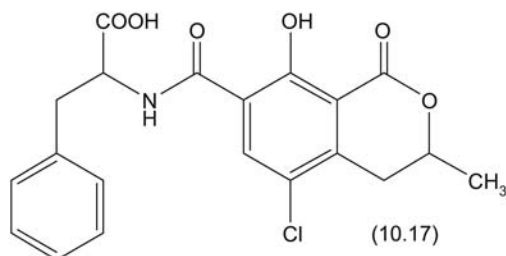
Whenever dairy cattle are fed contaminated animal feedstuffs, aflatoxins can be expected in their milk. Aflatoxins B1 and G1 are converted into M1 and GM1 (M indicates milk), respectively, by liver enzymes, which then appear at levels of about 0.05–0.5 µg L⁻¹ in liquid milk. It is remarkable how much of the aflatoxin in a cow's diet subsequently appears in its milk: about 1% of the level in the feed. This may not seem much, but it is sufficient for aflatoxins to present a potentially serious problem for the dairy industry. The concentrations of aflatoxins that come through into meat are less than one-thousandth of feedstuff levels.

The accumulation of detailed knowledge of aflatoxin distribution achieved by the authorities in many parts of the world has been greatly facilitated by the relative ease with which aflatoxins can be detected and quantified. Aflatoxins fluoresce strongly in UV light. At around 365 nm, the B-types give a blue and the G-types a green fluorescence. Solvent extraction has to be followed by a fairly elaborate clean-up procedure before thin-layer chromatography or high-performance liquid chromatography can be used for detection at the milligram level. Determinations down to 10 ppb (based on the original food) are now possible using techniques such as enzyme-linked immunosorbent assay (ELISA).

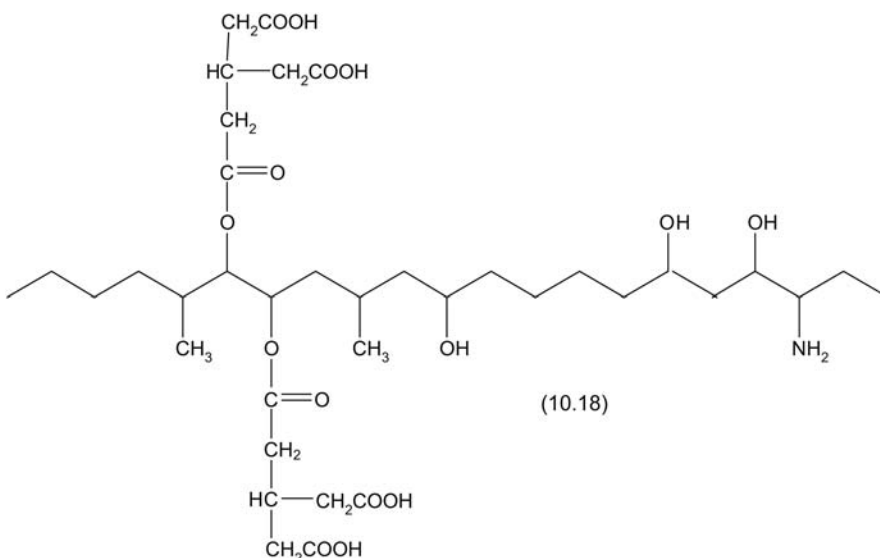
Food scientists now recognise the potential hazard caused by a number of other mycotoxins. Ochratoxins, notably ochratoxin A (10.17), were first identified as products of *Aspergillus ochraceus*, which occurs mostly in warm climates. In temperate climates

^{viii}Quoted in Ministry of Agriculture, Fisheries and Food, *Food Surveillance Paper No. 36, Mycotoxins: Third Report*, HMSO, 1993.

other aspergilli and penicillia, notably *Penicillium verrucosum*, are more important in cereals and cereal products. Ochratoxins have been implicated in kidney damage in pigs, but the toxin is destroyed by the rumen bacteria of cattle. A serious form of kidney disease affecting humans that is endemic in parts of the Balkans has also been associated with ochratoxins. The total intake of ochratoxins from a typical UK diet is very low because they come either directly from cereal products or from pigs' kidneys or black pudding made from pigs' blood, two tissues in which the toxin accumulates.

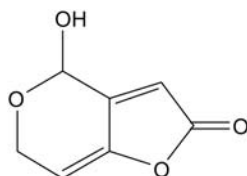


In the USA, the maize (*i.e.* corn) fungi *Fusarium verticilloides* and *Fusarium proliferatum* are a cause for concern. Contamination with these pathogens can lead to the presence of mycotoxins known as fumonisins, the best known of which is fumonisin B1 (10.18). They are unusual in being water-soluble and can be carried over into corn-based food products. They are known to cause a wide range of symptoms in livestock and are also suspected of being human carcinogens. In recent years, the levels of fumonisins detected in corn samples has, however, decreased considerably, possibly due to climate change.



Patulin (10.19) is classified as a mycotoxin, but is not considered to be dangerous. Although it has been found to be mutagenic in laboratory tests with bacteria, animal studies have not shown it to be either carcinogenic or teratogenic. The possibility has even been explored that it might have some useful antibiotic activity. Patulin is particularly associated with apple juice, in which its presence tends to be regarded as a marker

for the use of mouldy fruit rather than as a health hazard in its own right. It is also found in home-made jam that has mould growing on its surface. Patulin is produced by a number of species of *Aspergillus* and *Penicillium* (particularly *Penicillium expansum*) that cause rotting of stored fruit. Most clear apple juice sold in the UK has $<10 \mu\text{g kg}^{-1}$, but cloudy apple juice often contains patulin above this level. The difference is caused by the tendency of patulin to associate with the particulate material in cloudy juice that is removed when the juice is filtered.



(10.19)

The increasing awareness of the health problems that mycotoxins can cause has led to food manufacturers and processors taking a number of preventive steps. The need to discard mouldy cereals and nuts is obvious, but there are many food products that traditionally require moulds to ripen them. These include the blue cheeses and many fermented sausages, such as salamis. The approach now being adopted with these products is to forsake “natural” contamination/inoculation from the factory walls, employees or machinery, and to use instead pure starter cultures of mould strains that are known not to produce toxins.

10.4 Bacterial Toxins

The growth of moulds on food is often obvious to the naked eye. The growth of bacteria, on the other hand, is usually far from obvious and the first we may know of toxins being produced is when we start to suffer their effects. When considering the diseases caused by food-borne bacteria, it is essential to distinguish between infection and intoxication. Infection results when harmful bacteria present in our food are ingested. Once in the gastrointestinal tract, they proliferate and produce toxins that cause the symptoms of the particular disease. Diseases caused by *Campylobacter jejuni* and the innumerable species and subspecies of the genus *Salmonella* are among the most important of these food-borne infections. Our concern in this section is with the intoxications that result from the growth of harmful bacteria in food before it is consumed. Although the toxins secreted may well be deadly, it is curious, although of little consolation, that the conditions encountered in the intestines of the consumer may render the bacteria themselves harmless, whereas the toxins they secrete are unaffected.

The most feared of the bacterial food intoxications is botulism, caused by the toxin secreted by *Clostridium botulinum*.^{ix} The causative organism is commonly found in soil throughout the world, although it is an obligate anaerobe and only proliferates in the

^{ix}*Botulus* is Latin for sausage.

absence of oxygen. Unlike the spores, its vegetative cells are not particularly resistant to heat, but even the least resistant *C. botulinum* spores are able to withstand heating at 100 °C for two to three minutes. The heating regimes required for food preservation by canning are designed to eliminate all reasonable risk of *C. botulinum* spores surviving. Experience has shown that, for safety, the entire contents of a can must reach 121 °C for at least three minutes. Times approaching half an hour are required if the temperature only reaches 111 °C. To achieve temperatures like these at the centre of a large can may require prolonged heating of the can as a whole. Acidic foods, such as fruit or pickled vegetables, present no hazard because their pH is too low for growth of the bacteria and production of the toxin. However, home-bottling of non-acidic foods, including vegetables such as beans or carrots, is risky unless salt is added at high concentrations in the form of a pickling brine to bring the concentration to at least 15% w/v NaCl. Other factors that prevent bacterial growth are a reduction in water activity (a_w , see Chapter 13) to <0.93 or the presence of nitrites (see Chapter 9). If heating is inadequate and the other conditions are favourable, any spores carried into the can with the raw food materials will eventually germinate, multiply and secrete the toxin.

The species *C. botulinum* is subdivided into several types (A–G) that can be distinguished serologically, but more importantly, differ in the lethality of their toxins and in their geographical distribution. For example, in the USA type A predominates west of the Rocky Mountains, whereas to the north in Canada and Alaska type E, and elsewhere in North America type B, predominate. Only type B is found in soil samples collected in Western Europe. Throughout the world type E is associated with intoxication from seafood. Types C and D are most often associated with botulism in domestic animals rather than humans. Ducks are particularly vulnerable because of their occasional consumption of rotting vegetation from the anaerobic depths of ponds.

As with the mycotoxins, and for similar reasons, data are scarce on the amounts of botulinum toxin that produce adverse effects in humans. Estimates of the minimum lethal dose are around 1 µg for an adult, *i.e.* approximately 1.4×10^{-2} µg kg⁻¹. This amount of toxin is small, both in absolute terms and also relative to the productivity of the bacteria. Levels of toxin around 50 mg mL⁻¹ have been encountered in infected cans of beans. Laboratory cultures using ideal media may contain concentrations 100 times greater than this. Of the most common toxin types (A, B and E), B appears to be the most toxic and E the least. The variations in toxicity are principally due to differences in the stability of the toxins in the intestine and different rates of absorption into the bloodstream. By direct injection the toxicity is about 10³–10⁶ times higher.

Botulinum toxin consists of a complex of two distinct protein components: a haemagglutinin and a neurotoxin of molecular weights 5×10^5 and 1.5×10^5 , respectively. After absorption, the toxin is transported throughout the body by the blood and lymphatic systems. It reaches the nerve endings, where it binds, apparently through the haemagglutinin component, and blocks the transmission of nerve impulses to the muscles. The result is that the first signs of neurological damage appear about 12–36 hours after ingestion of the toxin: dizziness and general weakness, followed over a day or so by generalised paralysis, then death by respiratory and cardiac failure. When the affected person is known to have consumed food contaminated with the toxin, but before extensive symptoms have appeared, it is possible to retrieve the situation by the administration

of the antitoxin for the specific toxin involved. Unfortunately, the difficulty of rapidly identifying the causative toxin type means that guesswork informed by the geography and probable identity of the guilty foodstuff may be the best indicator of which antitoxin to use.

The intractability of botulism means that it is much better to prevent the disease than to attempt to cure it. Although rarely seen in the UK, there are typically about 40 outbreaks per year in the USA. In the main this difference can be ascribed to the greater enthusiasm for home-based food preservation in rural America, rather than to any lack of diligence on the part of the authorities or food manufacturers. Although the spores of *C. botulinum* are resistant to heat, the toxins themselves, in common with most other biologically active proteins, are quickly denatured at high temperatures. Any low-acid, high-water activity home-bottled or canned food can be boiled for 10 minutes before eating to ensure freedom from this deadly toxin. On rare occasions, cases of botulinum infection are encountered in infants when the conditions in the infant's intestines have allowed bacterial growth and toxin secretion to occur.

In contrast with the rarity and severity of botulism, the consequences of consuming food contaminated by the toxin from *Staphylococcus aureus* are rarely fatal, but will be experienced by almost everyone at one time or another. The staphylococci are a ubiquitous group of microorganisms found in air, dust and natural water. Of the staphylococci, the species *S. aureus* is particularly associated with humans and is found on the skin and in the mucous membranes of the nose and throat of a high proportion of the population. We usually remain unaffected by the presence of this microbial guest. However, human carriers are an extremely efficient distribution system. Flakes of skin shed naturally from the hands can transfer the organism to anything we touch and the micro-droplets from a sneeze will ensure more widespread distribution.

On reaching a food material that offers a favourable environment, this species will start to grow and, at the same time, secrete an enterotoxin. There are at least five different types of enterotoxin produced by various strains of *S. aureus*. All are proteins with molecular weights of about 32 000. Although very similar in their clinical effects, the different toxins produced by particular strains show a wide variability in their stability to heat. The least stable, type A, loses 50% of its activity after 20 minutes at 60 °C, whereas the most stable, type B, still retains >50% activity after five minutes at 100 °C. All these enterotoxins show considerable resistance to the proteolytic digestive enzymes of the stomach and small intestine. At the molecular level, little is known of the mechanism of the action of the staphylococcal enterotoxins. What is known is that 1 µg is sufficient to cause the development of symptoms within an hour or so. The affected person experiences vomiting and sometimes diarrhoea, accompanied by a selection of symptoms that may include sweating, fever, hypothermia, headache and muscular cramps. Only very rarely has the toxin proved fatal and the symptoms usually abate after a few hours.

The physiological requirements of *S. aureus* indicate which foods it is likely to be found in. Nutritionally, it requires most of the amino acids and some of the B vitamins. Oxygen enhances growth, but is not essential. Although growth and toxin production are optimal at about 36 °C, there are indications that toxin can be produced at temperatures as low as 15 °C and as high as 43 °C. Toxin is not produced below pH 5 or in salt concentrations >10% w/v. These requirements make cooked meats such as sliced ham,

prepared custard and hamburger patties (prior to grilling) prime targets. Any food not consumed straight after cooking is vulnerable, especially if it is not adequately refrigerated. Studies of food items incriminated in outbreaks of *S. aureus* poisoning have shown numbers of organisms in the range 10^7 to 10^9 g⁻¹. The discrepancies between the requirements for optimal growth and optimal toxin production, and also between the thermal stabilities of the microorganism and its toxin, mean that there is no correlation between cell count and toxin level. Responsibility for the prevention of *S. aureus* poisoning thus lies with food handlers, retailers and caterers rather than with food processors or manufacturers.

As might be expected, the determination of *S. aureus* enterotoxin levels in foodstuffs is not straightforward. Animal assays have been necessary, feeding suspect foods to kittens because they are especially sensitive and show similar symptoms to humans. Serological methods can be applied, but although they are sensitive (down to 0.05 µg mL⁻¹), they take several days to complete.

10.5 Allergens

Most of the undesirable substances that have been the subject of this chapter so far do not discriminate between the people they affect, although the youngest, oldest or those with frailty may be more vulnerable. However, a small, but not insignificant, number of people are affected by certain food components to which most of us are totally indifferent: the food allergens. When doctors and scientists talk about an allergy they have a very specific phenomenon in mind. Surveys conducted in the 1990s showed that as many as one in five people in the UK consider themselves allergic (in the sense of experiencing an unpleasant reaction of some type or another) to certain foods. However, the same surveys showed that when the correct diagnostic tests are applied to these people, only one in ten actually demonstrates a measurable reaction (*i.e.* 2% or less of the total adult population). Up to 8% of infants may show a genuine food allergy. Although most of the people who believed that they had a food intolerance identified food additives as the culprits, the estimated incidence of reactions to food additives is in fact much rarer, estimated to affect only 1 in 10 000 people. To be regarded as valid, any diagnosis of a food allergy must be based on a double-blind, placebo-controlled oral food challenge. Data obtained in the USA from large-scale surveys of 0–17 year olds showed an increase in the incidence of food allergy from 3.4% in 1997–1999 to 5.1% in 2009–2011, with similar increases, especially in cases of peanut allergy, found in studies elsewhere in the world.

Allergy (sometimes referred to as hypersensitivity) is defined as an abnormal reaction of the immune system to a foreign (but not infectious) material (the allergen), leading to reversible or irreversible injury to the body. Immune reactions are normally classified into four types. Reactions involving food components are almost always Type I, involving IgE antibodies. Types II and III also involve antibodies, but are not concerned with reactions to food components. Type IV reactions develop slowly and are caused by migrating lymphocytes (white blood cells). The only Type IV reaction of interest to us is coeliac disease, in which the presence of wheat gluten leads to damage to the lining of the small intestine.

Food allergy is associated with a wide range of symptoms, including skin reactions, such as eczema (atopic dermatitis) and urticaria, and gastrointestinal responses, such as vomiting and diarrhoea. In anaphylaxis, an exaggerated immune response resulting from re-exposure to an allergen, these symptoms can be very severe and also involve the respiratory system, with tissue swelling causing obstruction to the airways. All these allergic reactions follow the release of histamine and other inflammatory mediators, provoked by the presence of the allergen. In food allergy, this immune response is directed against a specific protein in the diet. It is important to recognise that the allergen must penetrate the lining of the digestive tract for an allergic reaction to take place. A combination of digestive enzymes, the mucous membrane lining and lymphoid tissue provides a barrier that normally protects us from the allergens and invasive micro-organisms we all consume. Most of the common types of food allergy are only seen in infants and young children, but “growing out” of the problem is more likely to be the result of a gradual strengthening of these barriers rather than the body becoming, by some mechanism, accustomed to the allergen.

In general, allergens need to have molecular masses >5000 and $<70\,000$. Molecules below this range only provoke a reaction when they are bound to a larger protein; above this range they are too large to interact with the immune system. In most cases the allergenic effect of proteins is not lost when the protein is denatured (see Chapter 5, Section 5.2). This suggests that the immunological reaction is directed towards relatively short sections of the molecule containing a particular amino acid sequence, rather than dependant on the tertiary structure or overall shape of the molecule. The location and amino acid sequences of many of these sections, known as epitopes, have been established for many allergenic proteins, *e.g.* those in cow's milk.

Cow's milk allergy (CMA) is well known, affecting up to 7% of infants, but very few older children. CMA should not be confused with cow's milk intolerance (CMI, often known as lactose intolerance). CMI is caused by the inability to absorb lactose due to the lack of the enzyme lactase, as described in Chapter 2. Any of the milk proteins could be the culprit in a particular case of CMA. However, studies of large numbers of people affected by CMA have shown that the most abundant milk proteins, α -lactalbumin, β -lactoglobulin and the caseins (see Chapter 5), are the major allergens.

There are considerable similarities between the amino acid sequences of the versions of these proteins that occur in the milk of cows, sheep and goats, which means that many people with CMA are also likely to react to these milks. Babies consuming only breast milk may not be immune if their mother routinely drinks large amounts of cow's milk. Some cow's milk proteins can be absorbed through the mother's gut mucosa more or less intact and are then secreted in their breast milk. The proportions of these potentially allergenic proteins varies considerably between the three species, offering the possibility that replacing cow's milk with that from another species might provide relief from the symptoms of CMA. In fact, the only practical alternative is often goat's milk, not least because of its availability. The protein involved in a significant proportion of CMA cases is α_{s1} -casein. An unusual characteristic of the genetics of goats is that individual goats (apparently regardless of breed) fall into one of two categories in terms of the proportion of α_{s1} -casein in the total casein content of their milk, about 2% or 18%. Either figure compares favourably with the

corresponding figure of 35–40% for cow's milk. This is the protein involved in a significant proportion of CMA cases and many children affected by CMA can tolerate goat's milk. The food industry's use of milk proteins, in the form of sodium caseinate or whey proteins, as functional ingredients in a wide range of food products can make the avoidance of CMA symptoms much more difficult than simply not consuming obvious dairy products such as milk and cheese. Soya milk, a preparation of proteins extracted from soya beans, does not cause such cross-reactions, but infants who are badly affected by CMA often go on to become allergic to soya proteins. This is because the damage to the gut lining caused by the CMA can lead to interactions between soya proteins and the immune system.

Coeliac disease is the clinical name given to an allergy to wheat proteins, more specifically the gliadin fraction (see Chapter 5, Section 5.5.5) of the gluten proteins. People with coeliac disease also react to the corresponding proteins in barley and rye, and sometimes oats. Coeliac disease occurs throughout the world wherever wheat is the staple cereal and tends to cluster in families, suggesting that there is a strong genetic component. The disease appears when infants are weaned and wheat-based foods are introduced to their diet. However, the effects of the disease do not normally become obvious until after the child's first birthday. More rarely, it may not be properly diagnosed until the child reaches adulthood. Along with the appearance of antibodies to gliadin proteins in their blood, there is severe damage to the cells lining the jejunum (part of the small intestine). This damage leads to greatly impaired digestion and absorption of nutrients, resulting in a general failure to thrive, together with anaemia, lethargy, gastrointestinal disorders and other problems. The precise sequence of events in coeliac disease is still not well understood, but it appears to have much in common with other chronic autoimmune inflammatory diseases.

The complete removal of wheat from the diet, as well as the other cereals mentioned here, is the only satisfactory remedy. As with the allergens in cow's milk, the allergenicity of the gliadins is unaffected by cooking. Fortunately, both maize and rice are safe for people with coeliac disease and this makes it possible to provide gluten-free cereal products to take the place of bread, cakes and pastries. The use of wheat flour in many processed food products that are not obviously cereal in character poses special problems and emphasises the importance of accurate food labelling.

Several other foodstuffs are well known for their allergenicity, although the number of people affected is small. Egg proteins (notably ovomucoid in the white, see Chapter 5, Section 5.5.3) can produce similar symptoms to those invoked by milk. The allergenicity of ovomucoid survives cooking. Traces of two other egg proteins, ovalbumin and ovotransferrin, have been found in raw chicken meat, but fortunately their allergenicity does not survive cooking.

Nut allergies, especially to peanuts (also known as groundnuts, *Arachis hypogaea*) have attracted particular attention from the scientific and medical communities and also the general public. Far more cases of peanut allergy occur than allergies to tree nuts such as almonds (*Prunus dulcis*), Brazils (*Bertholletia excelsa*) and cashews (*Anacardium occidentale*), although much greater quantities of peanuts and derived products, such as peanut butter, are consumed. The edible part of the peanut, the cotyledon, contains around 25% protein. This protein fraction includes several members of an

important family of plant storage proteins, the cupins,^x which are particularly associated with legumes such as peanuts and soya beans, and also almonds, walnuts and cashews. In the case of peanuts, two proteins, known as arachin and conarachin, dominate the edible portion of the nut and are the usual culprits in peanut allergy. Immunologists refer to conarachin and arachin as the *ara h 1* and *ara h 3* allergens, respectively. They are particularly heat resistant and can be detected in products containing only small amounts of peanut flour.

Peanut allergens can suddenly produce serious symptoms in susceptible individuals, including anaphylaxis, and these can be severe enough to be life threatening. Studies of the general population have suggested an incidence of peanut allergy of 5 to 6 per 1000 in the UK and USA. Peanut allergy accounts for a major proportion of food induced anaphylactic reactions and more than 50% of food allergy fatalities. It has been estimated that approximately 95% of peanut allergy cases involve reaction to the *ara h 1* allergen.

The increase in peanut sensitivity in recent decades has been speculatively ascribed to the growing popularity of peanut products and their inclusion in young children's diets. However, the issue of the consumption of foods containing peanuts in infancy actually promoting the development of peanut allergy has been questioned. Observations first published in 2015 suggested that the early introduction of peanuts to infants with severe eczema or allergy to eggs significantly decreased the frequency of the development of peanut allergy. The possible mechanism of this process, and its clinical application, are clearly outside the scope of this book and readers with a particular interest in this topic are recommended to consult the 2018 review by Du Toit and colleagues in the Further Reading section.

Allergic reactions to peanuts may be provoked in susceptible people by doses of as little as 100 mg of peanut protein, equivalent to no more than 400 mg of peanut meal. One study suggested that sufficient amounts of peanut protein may remain in the saliva of someone who has just eaten a peanut butter sandwich to make it unwise for a peanut allergy sufferer to be kissed by them.

The potential problems posed by peanut allergens have made their detection and quantification in foods a major objective for food analysts. Two methods are in regular use. The most popular is sandwich ELISA, a type of enzyme-linked immunosorbent assay. The other, which detects fragments of peanut DNA corresponding to the allergenic protein, uses the polymerase chain reaction. (This is the same technique as that used in forensic science to correlate DNA residues left at crime scenes with those of suspected criminals.) Both methods are remarkably sensitive, with a lower detection limit below 5 mg of peanut protein per kg of foodstuff.

10.6 Toxic Agricultural Residues

Modern agricultural methods make use of innumerable products from the modern chemical and pharmaceutical industries. Whether we approve of them or not, the high yields demanded of agriculture are dependent on these chemicals and the food scientist should therefore be familiar with them.

^xThe name "cupin" comes from the Latin for barrel and refers to the shape of the subunits that make up these complex proteins.

The most worrying agricultural chemicals are obviously the pesticides. It was found in the 1960s that the insecticide dichlorodiphenyltrichloroethane (DDT) (10.20) had penetrated virtually every food chain studied. This was the result of a combination of its extreme stability in the environment and the vast scale on which it was used from the time of the Second World War until the early 1960s. Not only was DDT cheap to manufacture, it was also extremely effective in controlling both the insect plagues of mosquitoes carrying malaria and yellow fever and a wide range of agricultural pests. It was the accumulation of DDT in birds of prey and its catastrophic effects on their numbers that caught public attention, but it was the realisation that typical samples of human fat and milk were contaminated that led to restrictions in its use. In 1972, DDT levels of 2.5 ppm were found to be common in human fat in the UK. The mean levels of DDT in human milk in the UK have fallen sharply since the 1960s, from 0.12 mg kg⁻¹ to 0.01 mg kg⁻¹ in 1989–1991. There is no indication that DDT, at the levels normally encountered in our diet, contributes to the incidence of cancer.

In general, the levels of DDT in cow's milk were found to be around 1% of these figures. The insecticide had reached humans *via* the fat of beef and other meat, and through dairy products. Now that the use of DDT is either banned or greatly constrained in Europe, North America and many other parts of the world, the levels currently found in human tissues in these regions are much reduced. By contrast, its cost-effectiveness in the control of insect-borne diseases in Africa and other tropical regions makes its use a much more complex issue. A 1970 report from the National Academy of Sciences of the USA stated that DDT had prevented 500 million otherwise inevitable deaths from malaria; this fact had to be balanced against its impact on wildlife, particularly birds of prey. Since that time, the impact of banning DDT on the incidence of malaria has become only too apparent. After DDT was banned in the mid-1990s, the incidence of malaria rapidly increased to ten times the pre-ban levels. Limited spraying with DDT was resumed in 2001 and malaria rates have now begun to fall again.

It is now recognised that the key factor in protecting vulnerable human populations from malaria is to spray DDT indoors. Much lower levels of DDT are required than in outdoor spraying in the agricultural context. However, very high levels of DDT (up to 18 mg kg⁻¹ milk fat) have been found in human milk in studies of some South African villages where malaria-bearing mosquitoes have been controlled by indoor spraying.^{xi}

From the difficulties encountered in the use of DDT, the authorities learned the need for continual monitoring of pesticide levels in all types of human foodstuffs. The disturbing result is that we now realise just how many foods are contaminated with a wide range of pesticides and their breakdown products. Many countries, including the USA and those in the European Union, maintain intensive surveillance programmes to monitor the level of a large number of different pesticides in everyday food items. Some UK data for 2018, the most recent full year for which figures were available at the time of writing, is shown in Table 10.2. The surveillance programme conducted by the authorities in the UK analyses pesticide levels in foods purchased around the country throughout the year. Some foods, especially the most widely consumed or most

^{xi}H. Bouwman, H. Kylin, B. Sereda and R. Bornman, High levels of DDT in breast milk: Intake, risk, lactation duration, and involvement of gender, *Environ. Pollut.*, 2012, **170**, 63.

Table 10.2 Summary of results of the determination of pesticide residue in foods sold in the UK during 2018, including imported foodstuffs. The Maximum Residue Level (MRL) is explained in the text. The italicised data are examples selected from the major sections. In this study, 3385 samples, purchased at retail outlets throughout the UK, were tested for residues of up to 371 different pesticides^a.

Commodity	Total number of samples analysed		With no detectable residues (%)	With residues at or below the MRL (%)	With residues above the MRL (%)
Fruit and vegetables	1962		43.0	53.2	
<i>Apples</i>		96	37	59	0
<i>Potatoes</i>		157	80	76	1
<i>Broccoli</i>		96	68	27	1
<i>Grapes</i>		120	7	111	2
<i>Bananas</i>		71	30	41	0
<i>Peas (without pods)</i>		72	58	14	0
<i>Mushrooms (cultivated)</i>		71	58	12	1
Starchy foods and grains	288	14.6		85.4	0.0
<i>Bread</i>		216	29	187	0
Animal products	858	94.0		3.9	2.1
<i>Milk</i>		301	295	6	0
<i>Eggs</i>		107	107	0	0
<i>Beef</i>		90	73	11	6
Infant food (cereal based)	37	97.3		0.0	2.7

^aFrom: Expert Committee on Pesticide Residues in Food (PRiF) Annual Report 2018, Health and Safety Executive, UK. Last accessed in 2020, https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/824814/expert-committee-pesticide-residues-food-annual-report-2018.pdf.

commonly contaminated with pesticides, are sampled several times each year; others less often, at least on a rolling programme. Since 2008, annual surveys such as this have contributed to an ongoing European Union testing programme. In 2011, nearly 80 000 samples from European Union countries were tested. Of the 888 different pesticides that were sought, 381 were detected in measurable quantities.

The MRL is set as the largest amount of pesticide that would be expected in a crop when it has been treated in line with good agricultural practice and at a level below current safety limits. Since 2008, all the MRLs that apply in the UK are actually set on a European Union wide basis.^{xii} Individual MRL values for each combination of pesticide and food are available. However, any one particular pesticide will usually only be used on a limited range of crops, so the MRL values for other pesticide/crop combinations are set at, or close to, the lowest level that can be detected in the laboratory. The MRL values for the selection of pesticides given in Table 10.3 are typical of those that apply to foods where that particular pesticide may well have been used.

Acceptable Daily Intake (ADI) figures are established internationally by the combined efforts of the United Nations Food and Agriculture Organization and the World Health Organization (WHO). They represent the maximum daily exposure that offers no

^{xii}At the time of writing no changes in the relevant UK legislation resulting from withdrawal from the EU had been made.

Table 10.3 ADIs and MRLs for a selection of pesticides in use in the UK and Europe at the time of writing. The particular MRL values quoted here apply only to the crop shown in parentheses, but are presented as typical examples of the very large number of different fruits, vegetables, etc. listed in this database. Data derived from the database maintained by the European Food Standards Authority, <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN> (last accessed 2020).

	ADI (mg per kg body weight per day)	MRL (mg kg ⁻¹)
<i>Fungicides</i>		
Tebuconazole (10.20)	0.03	0.3 (apples)
Prothioconazole (10.21)	0.01	0.02 (almonds)
<i>Herbicides (weedkillers)</i>		
Glyphosate (10.22)	0.5	0.5 (potatoes)
Diiflufenican (10.23)	0.2	0.02 (barley)
Flufenacet (10.24)	0.005	0.05 (carrots)
Fluroxypyr (10.25)	0.8	0.01 (oranges)
<i>Insecticides</i>		
Lambda-cyhalothrin (10.26)	0.0025	0.2 (garlic)
Cypermethrin (10.27)	0.05	2.0 (wheat)
Esfenvalerate (10.28)	0.0175	0.2 (peaches)

appreciable risk. This is arrived at by dividing by 100 the highest level of exposure that has not resulted in any detectable toxicity in the most sensitive animal species tested, the NOEL.^{xiii} Determination of a NOEL is not simply based on a single test, but involves continuous exposure over the lifetime of the animal concerned. Large numbers of individual animals of several different species will be used in a single test.

NOELs are not readily established for carcinogens because our current understanding of carcinogenicity implies that even one molecule of a carcinogen has the potential to cause cancer. In such cases, risks are calculated on the basis of the number of *additional* cancers that lifetime exposure to a pesticide at a particular level are expected to cause. In the USA, the standard of “negligible risk” for a pesticide is one additional cancer arising per million of the population. This should be set against the overall incidence of all cancers of about one in four of the population, *i.e.* 250 000 per million. For suspected carcinogens, ADI values are sometimes replaced by TDI (Tolerable Daily Intake) values because the concept of NOEL cannot apply.

The authorities in each country of the European Union have their own policies and priorities regarding the choice of raw materials and commodities to be tested. In the UK, the major contributors to the national diet in which any residues might be expected to make a major contribution to a consumer’s total exposure – such as wheat and potatoes, apples, milk and margarine – are monitored very frequently. Among other fruit and vegetables, where there are large numbers of different species, varieties and sources that need to be monitored, two selection policies operate in parallel. Fruit and vegetables that are liable to present problems of pesticide residues on a regular basis, *e.g.*

^{xiii}These are sometimes reported as No Observable Adverse Effect Level (NOAEL) values.

celery and carrots, are subjected to continuous and intensive monitoring. By contrast, for more rarely consumed items, *e.g.* persimmons (*Diospyros kaki*) and pomegranates (*Punica granatum*), there is a rolling programme of intermittent tests. Food products for infants, including formula milks, are subjected to particular scrutiny. Depending on the food being examined, the total number of different residues determined in a single sample can range from as few as eight to >90. The sensitivity of modern instrumentation, especially gas chromatography, is such that the detection limit (*i.e.* the lowest level that can be detected) is rarely >50 mg kg⁻¹ (50 ppb) and is often one-tenth of this figure.

The degree of pesticide contamination varies from year to year and also from country to country, mostly due to differences in agricultural practices. For example, the number of bread samples in which malathion was detected by the UK authorities decreased from about 14% in previous years to zero between 1988 and 1990, a period of unusually hot dry summers. It has been suggested that the fine weather meant that a greater proportion of UK-grown wheat was suitable for bread-making, reducing the amount of wheat imported from countries that commonly treat wheat with malathion prior to shipping.

Despite the reassurance to consumers arising from data on the safety of pesticides and the regulatory efforts of the authorities, many consumers attempt to minimise their intake of pesticides, *etc.* by restricting their diet to “organic” foods. The exact details of what constitutes organic food vary from country to country and in the extent to which “organic” is defined in legislation. As a general rule, organic crops and livestock are assumed to be produced without the use of artificial fertilisers and pesticides, these being replaced by crop rotation, animal manure and traditional treatments to maintain soil fertility and control weeds, pests and diseases. In 2018, of the 3385 samples tested by the UK authorities, 351 were labelled as “organic”. Pesticide residues were detected in only 14 of these samples and in all but one sample the levels found were substantially below the relevant MRL value. The evaluation of the merits of organic foods is not necessarily straightforward. For example, the use of permitted levels of fungicides on peanut crops could be regarded as much less hazardous than the aflatoxins these fungicides prevent.

Readers of this chapter will recognise that its subject is the potentially harmful constituents of our food rather than undesirable aspects of the processes by which our food is produced. However, public opinion is becoming more concerned with the wider environmental impacts of modern agriculture, such as:

- (i) the effects of insecticides, particularly the neonicotinoids, on natural, and essential, pollinators such as bees – these can have adverse effects on the yields of particular crops, such as rapeseed, as well as the natural environment as a whole;
- (ii) the contribution of the methane generated by the rumen bacteria of cattle to global climate change; and
- (iii) the pollution of natural water courses by wastes from farm animals.

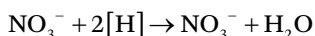
These are all issues for science to address, although they lie outside the expertise of food chemists.

Table 10.4 Typical nitrate levels in fresh vegetables. The nitrate content of individual samples of vegetables can vary between one-third and three times these values. Cooking in boiling water can reduce the figures by up to 75%.

	Nitrate as NaNO ₃ (mg per 100 g)
Beetroot	121
Celery	230
Spinach	163
Lettuce	105
Potatoes	16
Tomatoes	2

In Chapter 9 the problems posed by nitrosamines in cured meats were considered (see Section 9.2). However, the formation of nitrosamines in the human digestive tract from nitrates in our food is no less important. Of a typical daily intake of nitrates from food of around 90 mg, vegetables, including potatoes, contribute >75%. As shown in Table 10.4, some vegetables naturally contain high amounts of nitrates, but wide variations are found between different geographical regions, reflecting the local soil type, fertiliser usage and the levels present in the water supply. Solid food normally accounts for >80% of a human's total nitrate intake. However, if the nitrate levels in drinking water are high (>100 mg L⁻¹), then this becomes the major source of nitrates in our diet, increasing our total intake by two to three times. The average intake of nitrate from drinking water in the UK is about 13 mg day⁻¹, but the figure for SE England is nearly three times this and that for North Wales only one-twentieth. The UK limit for nitrate in drinking water is 80 mg L⁻¹ averaged over a period of three months.

It is now recognised that the reaction of nitrites and secondary amines to form nitrosamines can occur *in vivo*, *i.e.* after foods have been consumed, and is probably an important cause of gastric cancer. Nitrate entering the gastro-alimentary tract can be converted into nitrite:



at two points. Nitrate, absorbed from food and drink in the small intestine, is later secreted in saliva and encounters the nitrate-reducing bacteria present in the natural flora of the mouth. Once the saliva reaches the stomach, the acidity is usually sufficient to prevent this reduction from continuing. However, reduced hydrochloric acid secretion, achlorhydria, can lead to a sufficiently high pH in the stomach for bacterial nitrate reduction to occur. Like stomach cancer, achlorhydria is associated with both old age and poor levels of nutrition over a long period of time. The incidence of stomach cancer in elderly people is significantly higher in regions of the UK where high levels of nitrate in the water supply coincide with economic deprivation. The frequent apparent absence of an association between nitrate intake and stomach cancer in other parts of the world may well be the result of good nutrition, or a failure to include the incidence of stomach cancer in old age in medical statistics.

Another type of agricultural residue to be considered are the antibiotics. Antibiotics are used in agriculture for two distinct purposes. Their most obvious use is in the treatment of bacterial disease in farm animals. Good agricultural practice demands that milk from a cow treated for mastitis (inflammation of the udder or teats) is discarded and there are also regulations governing the maximum permitted levels of antibiotics such as penicillin in milk. An occasional lapse will have the potential to cause allergic reactions in sensitive people, but the major problem is for the manufacturers of dairy products such as yoghurt and cheese. These require lactobacilli to generate their lactic acid content and even very low levels of antibiotics will impair the growth of this unusually sensitive group of bacteria. By 2020, the total levels of antibiotics used for veterinary purposes in the UK had roughly halved from the levels used in the earlier years of this century.^{xiv}

Less obvious, and more of a problem, is the practice of including antibiotics in animal feedstuffs to act as growth promoters. A number of different antibiotics have been used for this purpose over the last 40 years. Antibiotics are added to feed at a rate of 5–50 g ton⁻¹, depending on the antibiotic and the animal species. Although the mechanism by which they can give an increase in growth rate of up to 50% and in feed conversion of up to 15% is obscure, the benefits are clear. The carry-over of antibiotic residues from the animal's diet into either meat or dairy products is insignificant, but this does not imply that the practice is without risk.

The problem is that an animal receiving an antibiotic over a prolonged period of time becomes a breeding ground for strains of bacteria that are resistant to the antibiotic. This is a particular problem with enteric bacteria such as *Escherichia coli* and the salmonellae. It is the ability of these bacteria to exchange genetic material, including the R-factors that carry antibiotic resistance, that creates havoc. Resistance developed within a harmless, or even beneficial, bacterial strain may be transferred to a dangerous pathogen, with serious long-term implications for animal or human health. Some years ago it was realised that the appearance of strains of disease-causing bacteria resistant to a wide range of antibiotics could be blamed, at least in part, on the use of antibiotics in agriculture. As a consequence, the use of antibiotics as growth promotion is now heavily restricted in many parts of the world. Within the EU, seven antibiotics are permitted, none of which are used in the treatment of human disease. Current UK regulations list the maximum levels of residues of a long list of antibiotics, sulfonamides and other medicinal products allowed in meat and meat products.

10.7 Toxic Metal Residues

Consideration of metallic residues requires us to depart from the classification of undesirables by origin. Toxic metals may reach our food from various sources. The more important of these are:

^{xiv}UK: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/909987/_1587146-v23-VARSS_2018_Report__2019_-accessible.pdf; USA: <https://www.cdc.gov/drugresistance/food.html>

Table 10.5 Undesirable metals in the total UK diet. The daily safety guidelines shown are the Provisional Tolerable Weekly Intakes (PTWIs) set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), divided by a factor of 7.

Metal	Mean estimated dietary exposure (µg per kg body weight per day)				
	Children (1.5–4.5 years)	Young people (4–18 years)	Adults	Elderly people ^a	Safety guidelines
Aluminium	187	123	71	58	1000
Arsenic ^b	0.161	0.107	0.061	0.049	2.1
Cadmium	0.41	0.29	0.16	0.12	1.0
Lead	0.23	0.14	0.10	0.07	3.6
Mercury	0.08	0.06	0.04	0.03	0.23 ^c
Tin	89.6	48.4	23.4	13.2	220

^aLiving in institutions.

^bInorganic, see Section 10.7.3.

^cMethyl mercury.

- the soil in which human or animal feedstuffs are grown;
- sewage sludge, fertilisers and other chemicals applied to agricultural land;
- the water used in food processing or cooking;
- contamination from dirt, *e.g.* soil on unwashed vegetables;
- equipment, containers and utensils used for food processing, storage or cooking.

Notable examples of the presence of particular undesirable metals in foods will be mentioned in the sections that follow. The UK authorities, like other authorities around the world, gather data on the incidence of undesirable metals in a wide range of different foods. An estimation of the average total exposure of sections of the population can be obtained when these data are combined with information on the contribution of different types of food to the total diet. A representative selection of the data from the UK 2006 survey^{xv} is shown in Table 10.5.

10.7.1 Lead

Lead is undoubtedly the metal that springs to mind when the issue of undesirable metals in food is raised. The Ancient Greeks were well aware of the health hazards it posed to miners and metal-workers and the need for legislation to protect both lead workers and the general public has been recognised ever since, although not necessarily provided. At least five million tons of lead are used annually worldwide, so it is hardly surprising that a certain amount of this finds its way into our lives. At one time petrol containing tetraethyllead was a major source of the lead we were exposed to, but the use of lead in petrol decreased through the 1990s and its use was banned in the European Union in 2000, four years after a similar ban in the USA. Somewhat surprisingly, at the time of the maximum

^{xv}Full details of this survey, covering the levels of 24 different metals in 20 different food groups, can be found in M. Rose, M. Baxter, N. Brereton and C. Baskaran, Dietary exposure to metals and other elements in the 2006 UK Total Diet Study and some trends over the last 30 years, *Food Addit. Contam.*, 2010, 27, 1380.

use of leaded petrol in the UK, neither crops from fields close to busy roads nor milk from cows grazing near busy roads showed the excess levels of lead that might be expected. Until the 1970s, lead oxide was commonly used in paint, especially for pipework and external woodwork. Fortunately, lead from this source is unlikely to find its way into food.

The level of lead in untreated water supplies varies widely and depends on the lead content of the rock the water has encountered; most supplies contain no more than about 5 ppb. The WHO recommends a safe maximum level of lead in drinking water of 50 ppb. The use of lead piping and lead-lined tanks for domestic water supplies can result in much higher levels if the water is particularly soft, such as the acidic moorland water still used in many parts of the uplands of the UK. The problem of acidic water dissolving lead from household pipes is exacerbated if the water has remained in contact with the household pipework overnight, as it may contain in excess of 100 ppb lead. The use of lead pipes and tanks in breweries and cider factories often gave rise to incidences of lead poisoning in the past. It was blamed in 1767 for what was, at the time, described as the “endemic colic of Devonshire”, and now recognised as a consequence of this region’s notably soft water. Lead contamination of beverages is now only found in illicitly distilled spirits, such as “moonshine” whisky and “bathtub” gin in the USA, and “poteen” in Ireland. Lead levels in excess of 1 mg L^{-1} are still occasionally reported in American moonshine. Home-made stills often use lead piping or scrapped car radiators with the metal joints sealed with lead-rich solder.

If earthenware pottery is glazed at temperatures $<120^\circ\text{C}$, the lead compounds in the glaze are not rendered sufficiently insoluble. There is then a serious risk that the lead salts will be leached into any acidic material stored in the pottery. There are numerous reported cases of fatal or near-fatal poisoning resulting from the storage of pickles, fruit juices, wine, cider and vinegar in the products of amateur potters. In one fatal case, the lead content of apple juice was reported to have reached 130 mg L^{-1} after three days’ storage in an inadequately glazed earthenware jar.

Canning was previously a potential source of lead contamination of food. Steel coated with tin (tinplate) was the original canning material, first used about 160 years ago, and is still used today. Lead-based solder was used to seal the joins in the cans and the relative positions of tin and lead in the electrochemical series ensured that the tin component was the first to dissolve. Unless solder was splashed into the can during manufacture, only a small surface area would normally be exposed to leaching by the contents of the can. An excellent illustration of this effect is the analysis of the contents of a can of veal a century after it was prepared for use in the Arctic expedition of 1837. The veal was found to contain 71 mg kg^{-1} iron and 783 mg kg^{-1} tin, but only 3 mg kg^{-1} lead.

Most of the tinplate used for canning today is lacquered. The lacquers are based on thermosetting resins that are polymerised onto the surface of the tinplate at high temperatures before the can is fabricated. Unfortunately, lacquering is not always as successful as might be expected. Blocking the reaction between the tin and the contents of the can apparently enhances the loss of lead from the solder. The lead content of canned foods is also affected by the time and temperature of storage as well as the acidity of the food. It is therefore difficult to be precise about the typical lead contents of canned food, but most analyses indicate figures between 100 and $1000 \text{ }\mu\text{g kg}^{-1}$.

Another source of lead in the diet, admittedly affecting only a small section of the population, is the lead shot present in game birds^{xvi} such as pheasants and wild ducks, and in rabbits, hares and venison (*i.e.* deer). An entire lead pellet lodging in a roast pheasant breast is unlikely to be missed, but lead pellets hitting bone frequently shatter to unnoticed fragments. Some lead may also dissolve in the mildly acidic muscle fluids during the long periods game carcasses are often hung before being cooked and eaten. This can give rise to levels approaching 10 mg kg⁻¹ in the meat and in products such as game pâté. The consumption of game is unlikely to be banned completely (not a likely prospect when one considers the lifestyles of many of our legislators), but there are now pressures to use non-toxic alternatives to lead, following the lead of Denmark in 1996. Some British supermarket chains have undertaken to only sell game shot with lead-free ammunition. Another valid objection to game consumption is that, in its pursuit, a vast amount of lead is fired off into the environment that never actually hits anything edible.

Most unprocessed food, and foods in which the processing has not added to the lead content, have lead levels in the range 50–500 µg kg⁻¹. In the UK, most foods are subject to regulations restricting their lead content to 1.0 mg kg⁻¹. Food products specifically sold for infants must contain <0.2 mg kg⁻¹ lead. In recognition of their frequently high lead content, and their relatively small share of most people's diets, both game birds^{xv} and shellfish are subject to a higher limit of 10 mg kg⁻¹. The low water solubility of most lead compounds is a major factor in its poor absorption from the gastrointestinal tract, indeed about 90% of the intake remains unabsorbed and is excreted in the faeces. Unfortunately, young children may absorb a much higher percentage than this.

The inevitable result of all this lead in our diet is that the human body contains a measurable amount of lead, even before birth. The lead content of our body builds up as we get older and adults typically contain between 100 and 400 mg, all but about 10% bound tightly, but not irreversibly, in the bones. The remaining lead is held in equilibrium in our soft tissues and blood. Three-quarters of the lead absorbed by our body is excreted in urine and also *via* sweat, hair and nails. It is widely believed that serum lead levels <40 µg per 100 mL are not deleterious to health. Between 40 and 80 µg per 100 mL, there are biochemical indications that the body is failing to cope with the excessive lead burden. Levels >80 µg per 100 mL indicate chronic lead poisoning. Clinical studies suggest that an adult man needs to assimilate >100 mg daily before his serum lead level rises above 40 µg per 100 mL. This corresponds to a dietary intake of about 1 mg day⁻¹. Data from the UK 2006 Total Diet Study (TDS) indicate an average dietary exposure of 6 µg day⁻¹, similar to the figure reported in the year 2000 TDS, but only one-twentieth of the 1980 figure.

Ordinary consumption in the diet will not lead to acute lead poisoning. Furthermore, the obvious symptoms of chronic lead poisoning, such as anaemia, are rarely associated with intakes from food or drink. More worrying are the effects on young children of serum levels approaching the "safe" level quoted here. There are indications that a number of neuropsychological indicators, including IQ test performance and aspects

^{xvi}Defined in UK law since 1860 as pheasant (*Phasianus colchicus*), partridge (*Perdix perdix*), grouse (*Tetraoninae* sp.), woodcock (*Scolopax* sp.) and snipe (*Gallinago gallinago*).

of social and learning skills, show definite negative correlations with serum lead levels once all other variables have been allowed for. At the present time, it is difficult to assess the contribution of dietary lead to the damage suffered by some children. There appears to be a particular problem for children growing up in inner city environments and it may well be that tetraethyllead in vehicle exhaust fumes has been the prime cause rather than food.

10.7.2 Mercury

Mercury presents food scientists with problems similar to those of lead, but on a different scale. Like lead, it occurs in three different forms: the free metal (Hg^0), inorganic mercuric (Hg^{2+}) salts and alkyl mercury compounds. Liquid mercury has occasionally found its way into food – for example, from broken thermometers – but mercury cannot be regarded as particularly toxic in this form. There are reports of people drinking 500 g and suffering no more than occasional diarrhoea. Mercuric salts are more hazardous (the lethal dose of Hg^{2+} is about 1 g), although still very rare as food contaminants. It is, however, much harder to dismiss the problem posed by organic mercury compounds.

Alkyl mercury compounds reach our food from two sources. There have been some serious outbreaks of mercury poisoning caused by the consumption of cereal grains intended for planting that had been dressed with antifungal mercurial compounds. The practice of dyeing treated seeds lurid colours has not prevented this happening and many countries now ban the use of mercury in seed dressings. The most important source of alkyl mercury compounds is industrial pollution of coastal waters, leading to contamination of fish and other seafood. The problem of marine pollution by mercury compounds first came to the world's attention in Japan in the mid-1950s when a number of cases of mercury poisoning were traced to the contamination of Minamata Bay by methylmercury (CH_3Hg^+). Despite the nature and source of the poisoning being identified, the pollution continued until the 1970s, by which time there had been >700 cases of poisoning, including 46 deaths. Fish and shellfish in the bay had mercury levels of up to 29 mg kg⁻¹, which pushed the average daily intake in the locality to >300 mg per person. A more normal daily intake in Japan would be unlikely to exceed 10 mg. Even higher levels were subsequently found in fish from some polluted Swedish and North American lakes, but local fishing bans have prevented further tragedies. In the UK, the 2006 TDS indicated a population exposure to mercury in the range 1–3 mg per person per day. For UK adults, the daily exposure to mercury was 0.05 mg per kg body weight per day.

Dangerous levels of methylmercury accumulate in the environment even if the pollutant is an inorganic mercury salt or the free metal. Various species of anaerobic methane-producing bacteria that occur in muddy sediments carry out the reactions:



The methyl groups are donated by methylcobalamin.

Pollution is not always to blame for high mercury levels in seafood. Some deep-sea fish such as tuna appear to accumulate mercury, as the methyl compound CH_3Hg^+ , to around 0.5 mg kg^{-1} . Fish from the most polluted of the UK's coastal waters have similar levels. The widespread occurrence of mercury in fish leads inevitably to its accumulation in the tissues of marine mammals. However, these animals can detoxify the mercury by converting it into an uncharacterised complex involving mercury and selenium. The formation of a similar complex in humans is believed to provide a degree of protection to those exposed to mercury over long periods of time.

It is generally recognised that the clinical signs of mercury poisoning are seen in adults when the intake of organic mercury exceeds 300 mg per day; a safe limit for adults has therefore been adopted at about one-tenth of this. There is every reason to suppose that children may be more sensitive. The symptoms of mercury poisoning are variable, but all point to damage to the central nervous system. In the case of the Minamata poisonings, many mothers who themselves showed no clinical sign of poisoning gave birth to children who showed symptoms resembling cerebral palsy.

10.7.3 Arsenic

As a consequence of its widespread distribution in nature, arsenic occurs in most types of food, but at low levels. Ordinary drinking water in most parts of the world contains around 0.5 ppb , but some thermal spring and spa waters may have as much as 1 ppm , *i.e.* 1 mg L^{-1} . Groundwater levels in some parts of the USA exceed 10 ppm . With the exception of seafood, the amount of arsenic in food rarely exceeds 1 mg kg^{-1} . The tendency of fish and crustaceans to accumulate toxic metals is difficult to explain, but, as we have seen in the case of mercury, it can cause problems. Prawns with arsenic levels as high as 170 mg kg^{-1} have been caught in UK coastal waters. Data from the UK 2000 TDS indicated an average dietary exposure of $61\text{--}64 \text{ mg}$ per day, of which fish accounted for $>80\%$. An important factor in the consideration of the elevated levels of arsenic in fish is that most of it is present as the organic derivative arsenobetaine, $(\text{CH}_3)_3\text{As}^+\text{CH}_2\text{COO}^-$, a compound which is not metabolised by humans and is therefore not regarded as hazardous. Published safety guidelines, such as the PTWIs set by JECFA, refer only to inorganic forms of arsenic.

Most cases of arsenic poisoning *via* food have occurred following large-scale accidental contamination. A classic incident occurred in 1900 when 6000 cases of arsenic poisoning, including 70 deaths, occurred among beer-drinkers in the Manchester area. The cause was the “brewing sugar” used by less scrupulous brewers as a cheap replacement for malted barley. This was a glucose-rich syrup produced from starchy materials such as potatoes by hydrolysis with crude sulfuric acid, which, in this instance, was contaminated with arsenic. In Japan, in 1955, 12 000 infants were poisoned by the dried milk in a bottle-feeding formula; at least 120 died. Sodium phosphate had been used as a stabiliser in the milk powder, but was contaminated with arsenic trioxide.

It is unclear at what level arsenic in food becomes hazardous. At present, UK legislation sets a limit of 1 ppm for most foods, apart from seafood, for which no limit is specified.

10.7.4 Cadmium

Had it not been for one outbreak in Japan in the late 1960s, cadmium would hardly warrant a mention as a serious toxic metal contaminant in food. The amount in different foodstuffs varies widely, but it averages around 50 ppb in most diets. Data from the UK 2000 TDS indicated an average dietary exposure of 9 μg per day. The WHO suggests that 50 μg daily should be the maximum for an adult and normal diets do not therefore present any difficulty. The Japanese outbreak occurred over a period of 12 years, during which rice paddies were irrigated with water contaminated with cadmium from a mining operation. The contamination of the rice resulted in daily intakes of cadmium between 100 and 1000 mg. The cadmium caused an extremely painful demineralisation of the skeleton, especially in post-menopausal women. The disease, known as *itai-itai* from the Japanese equivalent of “ouch-ouch”, was exacerbated by dietary calcium deficiency.

Two other sources of cadmium should be noted. One is the use of cadmium-plated components in food processing machinery. Although specialist manufacturers of such machinery can be expected to avoid the use of cadmium plating, there can be a risk if non-food machinery is adapted for foodstuff use. Another possible source of cadmium is from zinc plating or galvanising. Zinc usually contains some cadmium. If galvanised containers are used for storing acidic foods, some zinc, in itself harmless, may dissolve and carry traces of cadmium with it into solution.

10.7.5 Tin and Aluminium

The occurrence of tin in canned foods has already been mentioned in connection with lead. Most studies of tin toxicity have concluded that even quite high intakes are harmless; the minimum toxic dose is around 400 mg for an adult.

Aluminium is another important food packaging metal. There is no substantial evidence that ingested aluminium is harmful in any way. We consume it in considerable quantities from a number of sources, including toothpaste, baking powder and alkaline indigestion remedies. Several aluminium compounds are used as food additives. For example, sodium aluminium phosphate is a slow-acting leavening agent used in combination with sodium bicarbonate, and sodium and calcium aluminium silicates are used as anti-caking agents. The general level in fresh unprocessed food averages $<10 \text{ mg kg}^{-1}$ (many foods contain $<1 \text{ mg kg}^{-1}$), but when levels above this are encountered in some processed foods it is almost invariably due to the use of aluminium-based additives such as those mentioned. Tea is often quoted as an exception, but in spite of levels of about 500 mg kg^{-1} present in the leaves, the aluminium remains undissolved and tea infusions contain only about 3 mg L^{-1} . The level of aluminium in beer supplied in aluminium casks and cans is no higher than that from wooden casks or glass bottles.

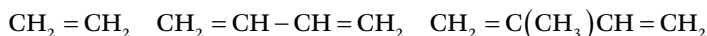
Concern was expressed about the higher levels of aluminium in infant formula based on soya protein, compared with formula based on cow's milk, averaging 0.98 and 0.11 mg L^{-1} , respectively. Reformulation of the soya products has halved this figure, but the natural aluminium content of soya beans limits what is achievable. The

amount of aluminium leached from aluminium cooking utensils is low. Eliminating the use of aluminium cooking utensils and foil typically reduces the total daily intake by only 2 mg.

The amounts of aluminium consumed as the hydroxide in indigestion remedies and buffered aspirin preparations can be much higher than the intake from food, as much as 1 g per day. The only question mark over the safety record of aluminium is the suggested link to Alzheimer's disease. Although several epidemiological studies have hinted at an association between the incidence of Alzheimer's disease and the concentration of aluminium in local drinking water, the small contribution of water to the total intake makes these results difficult to interpret. We need to know much more about the absorption of the metal from the gut before useful conclusions can be drawn. At the present time, there is every reason to believe that this accumulation is a secondary and unimportant effect of this distressing condition rather than a cause.

10.8 Toxins Generated During Heat Treatment of Food

A substantial proportion of the food we eat is heated before consumption. The benefits of this are clearly considerable. In addition to the physical effect of tenderisation, for example, cooking provides a measure of protection against food-borne microorganisms and many of their toxins. Unfortunately, we cannot automatically assume that the application of heat to food is in itself an entirely blameless procedure. Polyaromatic (or polynuclear) hydrocarbons (PAHs) were mentioned in the previous chapter as components of smoke. It is now becoming clear that this type of carcinogen may be present in other types of food. When carbohydrates or, more significantly, fatty acids are heated to $>500^{\circ}\text{C}$ they can give rise to unsaturated fragments such as:

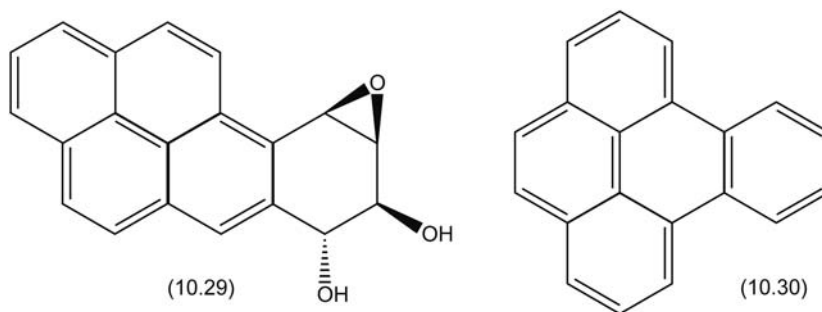


and their radicals. These condense to form progressively larger polyaromatic systems until compounds such as 3,4-benzpyrene (9.6) are formed. Although smoked fish, bacon and sausages typically have levels of 3,4-benzpyrene <5 ppb, barbecued meats, hamburgers, chops, steaks, *etc.* often have much higher levels, up to around 100 ppb. These elevated levels appear to be caused by fat dripping from the meat on to the burning charcoal of the barbecue and decomposing in the familiar puff of smoke and flames. Designs for barbecues and grills that prevent fat dripping into the fire will clearly be of benefit. "Clean" fuels such as charcoal are also to be preferred to wood or coal.

There is little doubt as to the carcinogenicity of the PAHs, at least in laboratory animals. There is considerable argument, however, as to whether the levels actually ingested from barbecued or grilled meats are a significant cause of tumours in humans. Much higher levels occur in the tar of tobacco smoke and in diesel exhaust fumes, and eliminating these would do much more for human health than outlawing barbecues.

Studies published by the UK Food Standards Agency in 2002 indicated that estimated average levels of benzo[a]pyrene consumption from food were 1.6 ng per kg body weight per day, approximately one-fifth of the level found in 1979.

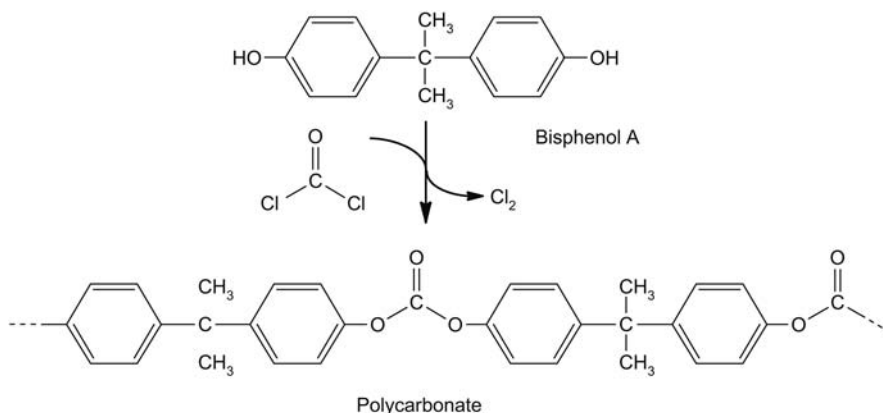
Strictly speaking 3,4-benzo[a]pyrene should be described as a procarcinogen. Enzyme activity in target tissues converts it into an epoxide (10.29) and it is this substance that actually binds to DNA. The specificity of these enzymes is such that some isomers of 3,4-benzo[a]pyrene, notably 4,5-benzo[a]pyrene (benzo[e]pyrene, 10.30) are not substrates of these enzymes and do not appear to be carcinogenic.



The formation of potentially carcinogenic heterocyclic aromatic amines during high-temperature cooking of meat and poultry has been recognised for several years. As the Maillard reaction has been implicated, these compounds were dealt with in Chapter 2 (see Chapter 2, Section 2.6). Similarly, the unexpected appearance of benzene, another carcinogen, as a contaminant in some types of soft drinks was dealt with in Chapter 9 (see Chapter 9, Section 9.5).

10.9 Packaging Residues

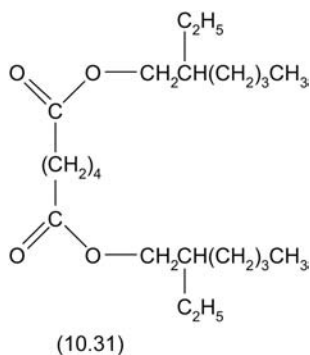
The plastics used to package our food can give rise to two types of contaminants. The plastics themselves are polymers of extremely high molecular weight and there is therefore little possibility of traces of the actual polymer contaminating food products. However, polymeric materials may contain trace amounts of the corresponding low molecular weight monomers (or oligomers) from which the polymer was synthesised. Inevitably, these may migrate into food in contact with them for prolonged periods of time. For example, the vinyl chloride monomer (VCM), $\text{H}_2\text{C}=\text{CHCl}$, can be leached out of polyvinyl chloride (PVC) and this is a potential issue for the long-term storage of food in PVC containers. Although there have been occasional incidents when much higher concentrations have been detected, UK legislation requires that food that has been packaged in PVC contains $<10 \text{ mg kg}^{-1}$ VCM. Materials such as cooking oils are potentially more vulnerable to contamination with VCM than non-fatty materials such as mineral water, but fortunately PVC bottles are rarely used in this role. In recent years another plastic, polycarbonate, has attracted particular attention. It is synthesised from bisphenol A (BPA):



Polycarbonate is strong, easily moulded and is well suited to the manufacture of clear bottles for storing drinks. Unfortunately, small amounts of BPA have been found to leach into the bottle contents during long-term storage. BPA also has several non-food uses that can contribute to our total exposure. BPA's status as an "endocrine active substance" (EAS)^{xvii} has made it a cause for concern and a considerable amount of work has been carried out, in Europe and elsewhere, to obtain both accurate data on actual exposure levels and to establish values for the TDI.

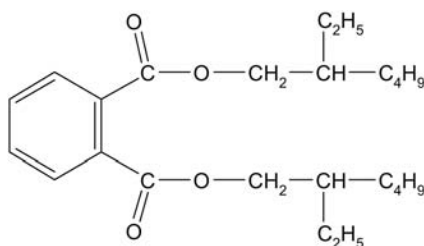
In 2023 the European Food Safety Authority (EFSA) reduced the Tolerable Daily Intake (see page 363) 0.2 ng (*i.e.* 0.2 billionths of a gram) per kg bodyweight per day, a 20 000 fold reduction of the TDI figure published in 2015 (<https://www.efsa.europa.eu/en/topics/topic/bisphenol#latest>).

Also of concern are plasticisers. These are substances incorporated into plastics such as PVC to render them flexible. At one time, attention was focused on the cling films used for wrapping food. These were originally made of PVC, in films about 10 mm thick. Their clinging property made them ideal for wrapping irregular food items and covering glass vessels, and they also had an acceptable degree of permeability to oxygen and water vapour. The plasticiser most commonly used in PVC cling film, and the one that has received most attention from the authorities, is dioctyl adipate (di-2-ethylhexyl, DOA or DEHA, (10.31)). A wide variety of plasticisers has been used in PVC in the past, but we will concentrate here on DEHA.



^{xvii}Soya phytoestrogens have, in the past, been described as ESAs, but this is nowadays disputed. See ref. 1.

Extensive surveys conducted in the late 1980s showed that significant levels of DEHA were present in a wide range of the foods consumed in the UK. Based on the typical consumption of these foods, the maximum daily intake of DEHA in 1988 was calculated to be 8.2 mg per person. This figure was half that recorded in 1986. In response to the problems posed by PVC, manufacturers turned to the use of low-density polyethylene (LDPE) for cling films. These are undoubtedly inferior to PVC-based films in terms of both clinging ability and permeability, but they have helped to sustain a continued decrease in the intake of packaging residues. Phthalates, in particular di(2-diethylhexyl)phthalate (DEHP, (10.32)), were also widely used as plasticisers in food packaging materials. They have now been almost entirely eliminated from food packaging, but they remain an important issue of which food chemists should be fully aware. Although these do not appear to be carcinogenic, the possibility is now recognised that, in some cases, they may mimic mammalian female sex hormones. This could implicate them in the decrease in male fertility and increase in other disorders of the male reproductive system reported over recent decades, in both humans and other species. Regardless of the outcome, this is a valuable reminder that toxicologists need to maintain vigilance in areas beyond the current preoccupation with carcinogenicity.



(10.32)

The complex issue of food packaging is one that is largely outside the normal scope of food chemists and has therefore only received brief attention in this book. A major cause of the complexity is the enormous variety of different polymers now in use and their tendency to turn up unexpectedly. For example, an innocent-looking glass jar will probably have a lid that includes a plastic liner to ensure that it seals properly. All that can reasonably be expected of the readers of this book is an awareness that the problem exists; a real understanding of the situation demands specialised expertise beyond that of the food scientist. Recent research has demonstrated that there is some future prospect of using microorganisms to depolymerise polyethylene terephthalate (PET), widely used for food packaging.^{xvii} If successful, this would constitute a major step forward in dealing with the environmental impacts of modern food processing and marketing systems.

^{xvii}B. C. Knott *et al.* Characterization and engineering of a two-enzyme system for plastics depolymerisation, *Proc. Natl. Acad. Sci. U. S. A.* Latest Articles, 2020 at www.pnas.org/cgi/doi/10.1073/pnas.2006753117, accessed 2012.

10.10 Environmental Pollutants

Some environmental pollutants require the attention of food chemists despite their origins having little to do with food. The dioxins, the closely related dibenzofurans (often referred to in this context as “furans”) and the dioxin-like polychlorinated biphenyls (PCBs) are the most important. Their structures are shown in Figure 10.6. In view of the wide differences between the toxicities of the numerous variants, analytical data on the dioxins, furans and PCBs are reported as Toxic Equivalents (TEQs), *i.e.* 2,3,7,8-TCDD Toxic Equivalents. By analogy with the retinol equivalents used for vitamin E measurements (see Chapter 8, Section 8.11), 1 g of TEQs consists of a quantity of dioxins *etc.* whose toxicity is equivalent to that of 1 g of 2,3,7,8-TCDD. The values to be applied to UK survey data on the relative toxicities of dioxins *etc.* (the Toxic Equivalency Factors, or TEFs) are periodically reviewed in the light of current research by the WHO, most recently in 2005. Where appropriate, the data on dietary intakes are then adjusted. TEFs range from 1 to as low as 0.00003.

PCBs were originally manufactured for use as the dielectric fluid in transformers and capacitors. In the light of their toxicological problems and persistence in the environment, their manufacture had ceased by 1980 and their use is now banned. Existing electrical equipment containing PCBs is being gradually replaced and its PCB content destroyed. Dioxins and dibenzofurans arise from numerous sources. By-products of

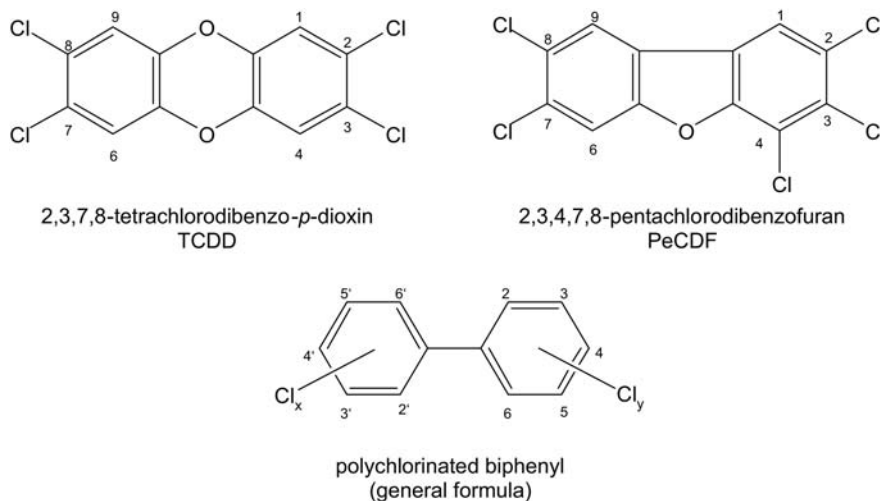


Figure 10.6 Structures of dioxins, dibenzofurans and polychlorinated biphenyls. The dioxin shown is just one of 210 possible congeners, which differ only in their patterns of chlorine substitution. A similar abundance of dibenzofuran congeners is possible. PCBs are not usually referred to specifically in terms of the numbers or location of the substituent chlorine atoms. When commercial PCBs were supplied to the electrical industry, they were simply identified by the average number of carbon atoms and chlorine atoms per molecule present in a batch.

the industrial production of chlorinated chemicals account for about one-third of the total. The bleaching of wood pulp with chlorine can also generate them, but the major source is the combustion of materials containing chlorinated compounds. This occurs in waste incinerators, metal refining, domestic and industrial coal-burning, and in petrol and diesel engines. Replacing the dumping of sewage sludge at sea by incineration or spreading it on the land may be an important future source. There are also suggestions that natural events, such as forest fires, also contribute to the levels of dioxins in the environment.

With the exception of workers in the chemical industry, particularly those affected by accidents such as the one at Seveso in Italy in 1973, human exposure to dioxins is almost entirely through food. However, their ubiquitous character, and their persistence in the environment, make it impossible to identify particular routes by which they reach our food. Once in the food chain, their solubility in fats, and their total insolubility in water, cause them to accumulate in the fatty tissues of animals, fish and humans. When high levels of dioxins and related substances have been detected in food, the source has proved to be contaminated animal feed. For example, in 1999 pork, poultry and eggs were found to have high levels of PCBs from animal feed contaminated with illegally disposed waste industrial oil.

Although levels of dioxins and PCBs in our diets have decreased steadily over recent decades, the incidence of these compounds in human milk remains considerably higher than in cow's milk. This reflects the differences between the diets of cows and humans, but the advantages of feeding infants with human milk far outweigh this isolated disadvantage. Total diet studies of dioxins and PCBs, similar to those mentioned earlier in this chapter with reference to pesticide residues, have been carried out by the UK authorities. The results from the 2001 survey are shown in Table 10.6. The UK authorities currently accept a value for the TDI of 2 pg TEQ per kg of body weight per day. Table 10.6 also includes details of the proportion of the UK population estimated at the time of the survey to have a dioxin and PCB intake in excess of the TDI. These data showed that an uncomfortably high proportion of young children exceed the TDI. However, the TDI relates to exposure over the full natural lifespan.

Table 10.6 Total Diet Studies of dioxins and PCBs in the diet of adults and children in the UK in 2001. Estimated levels of dioxins plus dibenzofurans (dioxins) and dioxin-like PCBs are expressed as pg of Toxic Equivalents (TEQs) using 2005 TEFs, as discussed in the text. (Based on data published by the Food Standards Agency in *Food Surveillance Information Sheet 4/00*, 2000.)

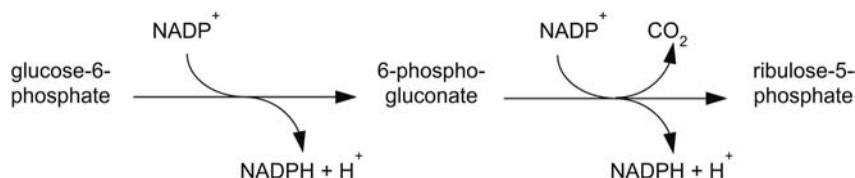
	Average dietary intake (pg TEQ per kg body weight per day)		Percentage estimated to exceed the TDI
	Dioxins	PCBs	
Children (1.5–2.5 years)	1.0	0.9	34.0
Children (4–6 years)	0.7	0.7	14.0
Children (11–14 years)	0.4	0.3	0.0
Adults	0.3	0.2	0.03

The implications of exceeding the TDI in childhood are not clear. What is clear, however, is that there is a great incentive for authorities worldwide to maintain the policies that have resulted in the sharp decrease in the level of these pollutants over recent decades. Reducing the level of dioxins in the diet is regarded as a priority for governments in Europe and elsewhere, but it is more likely to be achieved through controls on environmental pollution rather than by the action of farmers and food processors. More recent studies do not appear to have changed official views of dioxins in food or prompted any specific regulatory action.

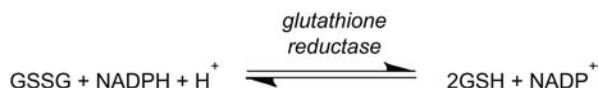
10.11 Special Topics

10.11.1 Favism

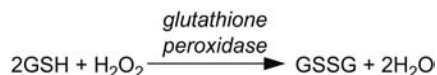
A full appreciation of the actual mechanism by which the *Vicia faba* toxins damage red blood cells requires specialised knowledge. The following additional details may be helpful. Red blood cells are unusual in lacking the mitochondria that, in other mammalian cells, provide reducing power. As a consequence, they rely on the pentose phosphate pathway (also known as the hexose monophosphate shunt) that provides reducing power in the form of NADPH (see Chapter 8). This is generated in two steps in the initial, oxidative phase of the pathway, catalysed, in turn, by glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase:



The NADPH is required for a number of processes, including the synthesis of the haem component of haemoglobin. However, we are most concerned with its reaction with glutathione (5.13), which we first encountered in the context of the sulfhydryl reactions in dough (see Chapter 5, Section 5.5.5), a reaction catalysed by the enzyme glutathione reductase:



The ratio of the reduced form (the dimer GSSG) to the oxidised form (the monomer GSH) is usually about 500 to 1. The reduced glutathione has a number of crucial roles in erythrocytes. One of these is maintaining the iron atom of haemoglobin in the ferrous (Fe^{II}) state in which it carries out its normal function of reversibly binding oxygen. It is also responsible for disposing of hydrogen peroxide, an inevitable, but dangerous, by-product of oxygen metabolism:



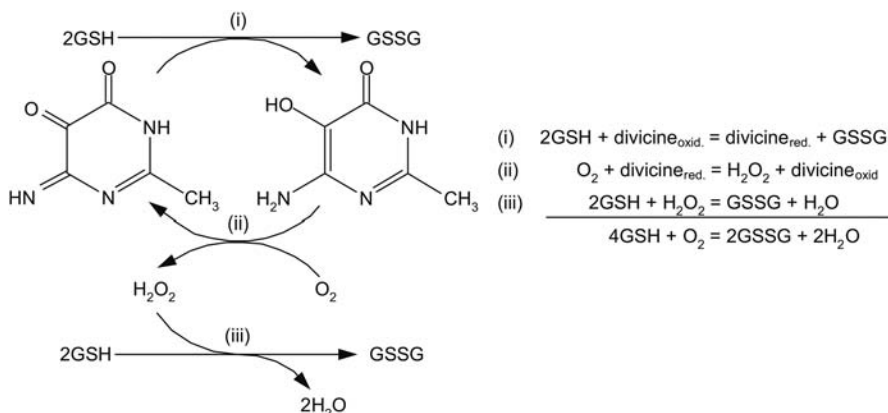


Figure 10.7 Removal of reduced glutathione by the spontaneous futile cycle reactions involving divicine. The other fava bean pyrimidine, isouramil, participates in the same reactions. The “futility” of this sequence of reactions is best demonstrated by arranging the equations for all three in a sum, as here on the right. In the best algebraic tradition, any term appearing on both sides of the equals sign has been “cancelled”.

Reducing conditions and a plentiful supply of reduced glutathione are essential for the development of malarial parasites in the erythrocytes. This means that mutations reducing the activity of G6PD in erythrocytes makes them an inhospitable environment for the parasites and could confer a degree of resistance to the disease.

The impact of the fava bean toxins on this situation is now well understood. As shown in Figure 10.7, their aglycone forms react spontaneously with oxygen, with the concomitant production of hydrogen peroxide. The oxidised product is then reduced in a spontaneous reaction with reduced glutathione. The reaction scheme in Figure 10.7 shows how these reactions become what is known as a “futile cycle”, in which the erythrocytes’ normal balance between the reduced and oxidised forms of glutathione is disrupted. The result is a similarly inhospitable environment for the malarial parasite as that which results from reduced levels of G6PD.

The problem of favism arises when a person whose supply of GSH is already rendered marginal by an inherited low level of erythrocyte G6PD activity consumes fava beans. An attack of haemolytic anaemia is the inevitable outcome of the twin assaults on the GSH economy of the erythrocytes.

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11 Minerals

Chapter 10 showed us that many undesirable inorganic elements are present in our diet. The food chemist has a special responsibility to ensure that these elements are not consumed in harmful amounts and therefore needs to understand how they reach our diet– for example, from the environment, raw materials or processing operations. The food chemist's task is simpler when we consider the nutritionally desirable elements and can be examined under three headings:

- The analysis of foodstuffs to determine how much of the various inorganic nutrients they contain in relation to our nutritional needs. There are a number of laboratory techniques available for each mineral element depending on the analytical objectives and the resources available.ⁱ
- Studies of the form in which certain inorganic elements occur in foodstuffs where this affects our ability to absorb them from the gastrointestinal tract.
- Studies of the actual behaviour of inorganic elements in food when this has an influence on other aspects of food quality, including nutritional value.

The list of bulk and trace elements required in the human diet is long but, as Figure 11.1 shows, it is largely restricted to elements of low atomic number. As we have observed previously, the fact that our diet consists almost exclusively of materials that were once living organisms, coupled with the broad similarities of the biochemistry of all forms of animal and plant life, means that we can expect any reasonably mixed diet to supply our mineral needs in about the right proportions.

11.1 Major Minerals

11.1.1 Sodium

The alkali metal sodium, as the sodium cation Na^+ , has a well-established role in animal physiology and is abundant in both human tissues and human diets. It occurs at levels between 50 and 100 mg per 100 g in most foods of animal origin, including milk, meat

ⁱAn aspect of food chemistry that is largely outside the scope of this book.

Group \ Period	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	H																
2	Li	Be											B	N	C	O	F
3	Na	Mg											Al	P	Si	S	Cl
4	K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	As	Ge	Se	Br
5	Rb	Sr	Sr	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	Sn	Sb	Sn	Te	I

Figure 11.1 The elements essential for animal life. The section of the periodic table shown here includes, in bold, all the elements regarded as nutritionally essential. The groups are numbered here as recommended by the International Union of Pure and Applied Chemistry, replacing the two different systems originally used in Europe and the USA.

and fish, although eggs contain around 150 mg per 100 g. In their raw state, vegetables and cereals have much less sodium, between 1 and 10 mg per 100 g, but the practice of adding sodium chloride during cooking or at the table to enhance flavour brings their sodium content, as eaten, up to similar levels to those in animal foods.

It is difficult to state with any certainty a minimum sodium intake necessary for good health. The special low-sodium diets prescribed for patients with heart disease and certain other conditions can bring the daily intake of salt down to about 2 g, but this requires a considerable distortion of their normal eating habits. A strict vegetarian diet without added salt could approach this figure, but at the cost of low palatability. One should not overlook the fact that our taste buds are able to distinguish sodium chloride from other salts (see Chapter 7) and that there is a sound physiological basis for the other responses provided by our sense of taste.

The importance of sodium chloride as a preservative was discussed in Chapter 9. In foods in which it has been used in this role, *e.g.* bacon, kippers and butter, levels ten times those quoted here are common. There is considerable pressure from the medical profession for us to reduce the salt content of our diet. For many years the emphasis has been on the use of low-sodium diets in the management of patients with hypertension, *i.e.* high blood pressure. Hypertension is widely accepted as a major risk factor in the development of cardiovascular disease.ⁱⁱ

Two major investigations in the USA, the Intersalt study published in 1988 and the Dietary Approaches to Stop Hypertension (DASH) study that reported in 2003, confirmed that the relative risk of cardiovascular disease increases as blood pressure rises, even within the normal, presumably healthy, range of blood pressure values. Another important point which emerged from the DASH study was that individuals vary widely in the response of their blood pressure to a reduced intake of sodium. Identifying people as sodium-responders, *i.e.* those who will respond favourably to a low-sodium diet, is not easy, supporting the view that the current recommendations for a lower salt intake are more usefully directed at populations as a whole rather than attempting to identify susceptible individuals.

ⁱⁱAlthough the first suggestions by the modern medical profession were made in the 1940s, Chinese physicians were warning their patients as early as 2500 BC that too much salt in their food would “harden their pulse”.

In 2013 the World Health Organization published similar conclusions regarding European diets. At that time, most adult Europeans consumed about 8–11 g of sodium daily, much more than the 5 g that could be expected to reduce the risk of cerebrovascular disease (stroke) by 23% and the general rates of cardiovascular disease by 17%. In 2019 the European Food Safety Authority's Panel on Nutrition, Novel Foods and Food Allergens set the Dietary Reference Value for sodium at 2 g per day for adults (corresponding to 5 g of sodium chloride). The corresponding figures for children are 1.3, 1.7 and 2.0 g per day of children aged 1–3, 4–6, 7–10 and 11–17 years, respectively.ⁱⁱⁱ

The appropriate authorities in most European countries, and elsewhere in the world, have attempted to address this gulf between desirable and actual salt consumption by encouraging consumers to consume less salty food and food manufacturers to incorporate less salt in their products. Concern is often expressed about the quantities of “discretionary” salt used in European kitchens. In fact, most of the salt added to the water in which vegetables are cooked is discarded when the vegetables are drained before serving. Potassium chloride, either alone or in mixtures with sodium chloride, is marketed as an alternative to salt to be added to food by individual consumers at the table. However, the taste of potassium chloride has a distinctive bitter element that reduces its appeal. Although extra potassium in the diet can be regarded as beneficial in healthy people (it tends to lower blood pressure), daily intakes above 4.7 g may present a risk to anyone with impaired kidney function. The only other plausible alternative is monosodium glutamate (see Chapter 7, Section 7.1.7). Monosodium glutamate can replace some of the salt in soups and meat dishes, such as lasagne, with a net reduction in the sodium content.

Bread is a significant source of salt in the diets of people in the industrialised countries. British bakers^{iv} have achieved considerable reductions in salt use in recent years. Prior to the UK Food Standards Agency's initiative in 2012, commercial bread recipes contained 1.8–2.2% w/w salt (based on the weight of flour), giving 1.2–1.4% w/w in the finished loaf, *i.e.* 470–550 mg sodium per 100 g bread. By 2019 the baking industry had brought the average level of salt in British bread down to 0.97 g (equivalent to 388 mg of sodium) per 100 g bread. When the reduction was staged progressively over many months, the small, but real, changes in the flavour of the bread tended to be unnoticed by consumers. There are a number of small changes in the rheological properties of the dough with lower salt levels, but mixing times *etc.* can readily be adjusted to ensure that no loss of bread quality occurs. A survey covering the years 2003–2008 revealed that pizza was the largest single source of salt (accounting for 10.3% of total salt intake) in the diet of young people in the USA.^v

Snack foods such as potato crisps (chips in the USA) or roasted peanuts are hardly dietary essentials, but can represent a significant source of dietary salt. The salt coating on these products is an essential element of their appeal to consumers, but it has proved possible to reduce the salt content without an adverse response. The

ⁱⁱⁱEuropean Food Safety Authority, Dietary reference values for sodium, *EFSA J.*, 2019, 17, 5778.

^{iv}Both the large manufacturers of the wrapped sliced bread sold in supermarkets and the small local artisan or craft bakeries.

^vA. Drewnowski and C. D. Rehm, Sodium intakes of US children and adults from foods and beverages by location of origin and by specific food source, *Nutrients*, 2013, 5, 1840.

technique is to reduce the salt crystal size, which accelerates the rate at which the crystals dissolve in the saliva on the tongue, giving a more intense, though shorter-lived, burst of flavour.

Where salt has a key technological role in the manufacture of foods such as hard cheese (where salting is essential for curd formation) and cured meats such as bacon and ham, then reducing the levels used is more of a problem. Advances in the technology of refrigeration and packaging have made the antimicrobial role of salt less crucial and recent decades have seen a steady reduction in the amounts used. For example, tomato ketchup sold in the UK used to contain around 2.8 g of salt per 100 g, but in 2014 the figure had been reduced to 1.75 g of salt per 100 g. Tomato ketchup promoted on the basis of a particularly low salt content (0.5 g per 100 g) is now on sale in the UK. Its flavour seems to be unimpaired, but where once an opened bottle could be stored for an indeterminate period at room temperature, this product, once opened, must be discarded after two months in the refrigerator.

11.1.2 Potassium

Like sodium, the alkali metal potassium also has an important role in animal physiology. It occurs abundantly in both human tissues and human diets, in foodstuffs of both animal and plant origin. With the exception of the oils and fats, which have virtually no mineral content, and seeds and nuts, with 0.5–1.0%, the potassium contents of most foods lie within the range 100–350 mg per 100 g. The character of one's diet therefore has little effect on the total potassium intake. The only food in which the potassium level is of particular interest is jam. This has nothing to do with the nutritional role of potassium (or jam), but it does provide an insight into that most elusive quality parameter of jam, its fruit content. The essential similarity of the sugars, the organic acids, pectins, pigments, and many of the more easily determined flavour compounds, makes analytical confirmation of the jam-maker's assertions about the ingredients almost impossible. It is routine for acids and pectins to be included from sources other than the fruit named on the label. The level of potassium in a jam will provide a rough measure of the total content of fruit (and vegetable) tissues, provided potassium metabisulfite has not been added as a preservative. The weakness of this entire area of food analysis becomes apparent when one considers that identifying and counting the pips is still sometimes used in the quality assessment of many types of jam.

11.1.3 Magnesium

Like potassium, magnesium is widely distributed in foodstuffs of all types (at 10–40 mg per 100 g), although foods derived from plant seeds (such as wholemeal flour, nuts and legumes) often contain more than 100 mg per 100 g. It is difficult to envisage an otherwise nutritionally adequate diet that is deficient in either potassium or magnesium. Beer brewed in many parts of the UK (notably in Yorkshire) owes some of its bitterness, and perhaps some of its cathartic effect, to the naturally high levels of magnesium sulfate in the water used.

11.1.4 Calcium

When we turn to calcium, we find a much more complex situation. The calcium content of different foodstuffs covers a very wide range. At the lower end are some fruits and vegetables, such as apples, peas and potatoes, with <10 mg per 100 g. Other leafy green vegetables contain much more, *e.g.* broccoli and spinach with about 100 and 600 mg per 100 g, respectively. Meat and fish are generally low in calcium, about 10 mg per 100 g. Cereal grains are also relatively low, leading to wholemeal flour having about 35 mg per 100 g. White flours are fortified with additional calcium carbonate. Current UK regulations require that white flour for retail sale and commercial baking^{vi} must contain between 235 and 390 mg of calcium carbonate per 100 g (equivalent to between 94 and 156 mg of calcium). Self-raising flour always contains some additional calcium carbonate as the source of carbon dioxide. There is evidence from some parts of the UK that, even in today's enlightened and affluent world, many children's diets would contain dangerously low levels of calcium without this fortification. The question of adult calcium requirements is complex and largely beyond the scope of this book. Mild calcium deficiency is difficult to diagnose because the body maintains serum levels of calcium at the expense of skeletal reserves. Similarly, the level of calcium in milk during lactation is maintained by the depletion of the mother's reserves. The uptake of calcium from the intestine and its movement between the blood, skeleton and other tissues are under hormonal control. When deficiencies in calcium levels do occur, resulting in rickets and other disorders, they are most often found to be caused by impaired alimentary absorption resulting from a deficiency in vitamin D.

Dairy products are traditionally regarded as our primary source of calcium. The calcium content of cow's milk varies, but the average is about 120 mg per 100 g. In hard cheeses, such as cheddar, the calcium from milk is concentrated to levels of about 800 mg per 100 g, but in soft cheeses levels in the region of 450 mg per 100 g are more typical. The biological reason for the presence of large quantities of calcium in milk is obvious: the new born mammal has a skeleton to build. The skeleton's primary raw material is calcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$, which presents a problem. One of the features of calcium phosphate that commends it for skeleton construction is that it is insoluble at the neutral pH values of animal tissues. However, the phosphate must also be delivered in milk and it is not easy to visualise how both calcium and phosphate ions could share the same delivery medium without forming insoluble crystals. Nature's answer lies in the subtle interactions of these two incompatible ions with the caseins discussed in Chapter 5.

Cow's milk contains, on average:

0.065 mol L⁻³ inorganic phosphate (0.62 g per 100 cm³, as PO_4^{3-});

0.024 mol L⁻³ organic phosphate (0.23 g per 100 cm³, as PO_4^{3-}), essentially the phosphoserine of α - and β -caseins;

0.009 mol L⁻³ citrate;

0.030 mol L⁻³ calcium (0.12 g per 100 cm³ as Ca^{2+}).

^{vi}Flours for making communion wafers and matzos are exempt.

At the normal pH of fresh milk, about 6.6, the inorganic phosphate is present almost entirely as HPO_4^{2-} and H_2PO_4^- in the ratio 1 : 3. The virtual absence of PO_4^{3-} , the fact that a significant proportion of the HPO_4^{2-} and H_2PO_4^- are protein-bound, and the intervention of the citrate ion (largely citrate^{3-} at pH 6.6) rule out the formation of the highly insoluble calcium phosphate $\text{Ca}_3(\text{PO}_4)_2$. Instead, we have a loose network of anions and cations that helps to maintain the structure of the casein micelles, as discussed in Chapter 5. The interaction of calcium with phytic acid (3.18) and polysaccharides such as pectins and alginates was discussed in Chapter 3.

11.1.5 Phosphorus

Phosphorus is a component of all living organisms and is found in the form of both inorganic phosphates and organic esters. In mammals it is, of course, concentrated in the skeleton, but it should not be forgotten that the intermediary metabolism of sugars takes place in the form of their phosphate esters. Sugar phosphates are also essential components of the nucleotide coenzymes and the nucleic acids, and all biological membranes contain phospholipids. The metabolic role of phosphate is reflected in its distribution in foodstuffs. For example, lean meat (muscle) has around 180 mg of phosphorus per 100 g, whereas the corresponding figure for liver is 370 mg per 100 g. Foodstuffs of plant origin derived from seeds, such as legumes, cereals and nuts, also have high levels of phosphorus, between 100 and 400 mg per 100 g. Other vegetables, including potatoes, spinach and broccoli, and fruit such as apples and grapes, have levels of phosphorus <100 mg per 100 g. The phosphorus in the wheat grain is concentrated in the bran fraction, which means that white flour has only about one-third of the level in wholemeal flour, 130 mg compared with 340 mg per 100 g. Most of the phosphorus in bran is in the form of phytic acid (see Chapter 3, Section 3.4), a compact phosphate reserve for the germinating seed. Nuts are similar, in that 70–85% of their phosphorus content is in the form of phytic acid. The human digestive system usually has low levels of the enzyme phytase, which liberates some of the phosphate from the phytic acid, but it is likely that <50% of the phosphate from plant sources is absorbed.

As shown in Table 11.1, a considerable numbers of food additives contain phosphate (many of which are dealt with in this chapter or elsewhere in this book) and it has been estimated that around half the daily intake of phosphate in industrialised countries is derived from such additives. Patients with chronic kidney disease, especially those in the later stages of the disease who are receiving dialysis treatment, may need to restrict their intake of phosphates. However, the phosphorus content of food (whether processed or not) is not usually listed on packaging, making it difficult for patients to control their intake. The high phosphorus content of milk has already been mentioned and the presence of phosphorus in most types of cheese at about 350–550 mg per 100 g is therefore to be expected. Eggs are another good source of phosphorus, with around 220 mg per 100 g. The widespread distribution of phosphorus in foodstuffs means that phosphorus deficiency is not encountered in otherwise satisfactory diets.

Not all the phosphorus in our diet is accounted for by the raw materials of our diet – additives and other processing ingredients also make a contribution. Phosphoric

Table 11.1 Phosphates, including nucleotides and polyphosphates, that are permitted additives in Europe.

E No.		E No.		E No.	
E101	(i) Riboflavin	E450	Diphosphates:	E630	Lnosinic acid
	(ii) Riboflavin-5'-P		(iv) Tetrapotassium	E631	Disodium inosinate
E322	Lecithins		(v) Dicalcium	E541	Sodium aluminium phosphate
E338	Phosphoric acid		(vi) Calcium dihydrogen	E626	Guanylic acid
E339	Sodium phosphates	E451	Triphosphates:	E627	Disodium guanylate
E340	Potassium phosphates		(i) Pentasodium	E632	Dipotassium inosinate
E341	(i) Monocalcium phosphate		(ii) Pentapotassium	E633	Calcium inosinate
	(ii) Dicalcium phosphate	E452	Polyphosphates	E634	Calcium 5'-ribonucleotides
	(iii) Tricalcium phosphate		(i) Sodium	E635	Disodium 5'-ribonucleotides
E343	(i) Monomagnesium phosphate		(ii) Potassium	E1410	Monostarch phosphate
	(ii) Dimagnesium phosphate		(iii) Sodium calcium	E1412	Distarch phosphate
E442	Ammonium phosphatide		(iv) Calcium	E1413	Phosphated distarch phosphate
E450	(i) to (viii) diphosphates of sodium, potassium or calcium	E628	Dipotassium guanylate	E1414	Acetylated distarch phosphate
		E629	Calcium guanylate	E1442	Hydroxy propyl distarch phosphate

acid (E338) and sodium, potassium and calcium phosphates (E339, E340 and E341, respectively) have a variety of uses. Phosphoric acid is used as an acidulant, *i.e.* to lower the pH, notably in cola drinks, where its sharp flavour is considered valuable. When sodium and potassium phosphates are dissolved in water, the liberated phosphate ions will sequester any excess calcium ions that may be present – for example, when hard water has been used – helping to stabilise milk proteins in dessert products and control the rate of gel formation in some types of instant desserts. The calcium phosphates listed here have various food uses, notably as the acid components of raising agents in cake batters:

E341(a) monocalcium phosphate, $\text{Ca}(\text{H}_2\text{PO}_4)_2$

E341(b) dicalcium phosphate, $\text{CaH}(\text{PO}_4)_2$

E341(c) tricalcium phosphate $\text{Ca}_3(\text{PO}_4)_2$.

The widely used group of food additives collectively known as polyphosphates are di-, tri- and polyphosphates of sodium, potassium and calcium (Figure 11.2). The term “polyphosphate” can be used to describe both the group as a whole and, more specifically, the salts with three or more (up to about 20) phosphate groups. They are manufactured from phosphate ores by controlled heating at very high temperatures that lead to dehydration and condensation (11.1).

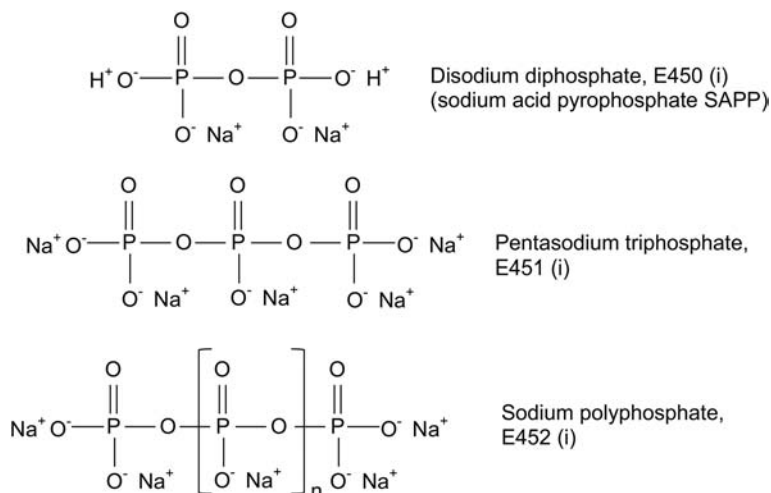
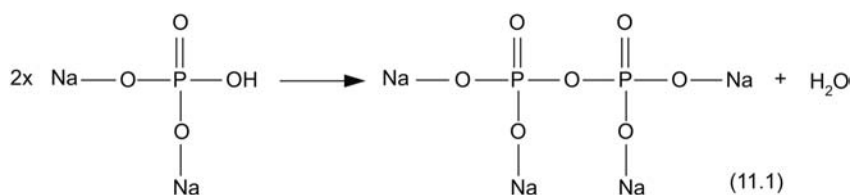


Figure 11.2 Polyphosphates. Those currently permitted in the EU are listed in Table 11.1.



Polyphosphates are popular additives in many meat products, especially ready-sliced ham, luncheon meat and pre-packed frozen poultry. Polyphosphates enhance the water-binding properties of muscle proteins. Apart from the obvious advantage (to the processor) of improving the apparent yield of product from a given weight of raw meat, the greater retention of muscle water and its solutes during cooking enhances the juiciness and flavour of products such as burgers, ham and bacon. Despite the addition of water entailed by the use of polyphosphates, and the apparent wetness of many pre-packaged ham-type products, their final water content can be lower than that of the original unprocessed meat. This results in the curious statement occasionally seen on labels of cured items that the product contains 110% meat. This is the unavoidable consequence of the meat content being calculated on the basis of the Kjeldahl nitrogen protein determination and the factors used to convert the protein content into meat content being based on the protein content of fresh, *i.e.* uncured, meat. An incidental advantage of polyphosphates is that they appear to delay the onset of rancidity in some products. It is assumed that this effect arises from their sequestration of iron and copper, which would otherwise promote the autoxidation of meat lipids (see Chapter 4). They are normally used at levels of about 0.1–0.3%. Polyphosphates at these levels pose no health problems because they appear to be hydrolysed to orthophosphates by the pyrophosphatase enzymes present in most animal (and plant) tissues. However, their contribution to the total phosphate intake may not be without health implications for some consumers, as mentioned earlier in the case of patients with chronic kidney disease.

The 5'-nucleotides used to enhance the umami flavour of many foods (see Chapter 7, Section 7.1.7) also contain phosphate, but the amounts used are too small to make a significant contribution to the total intake of phosphate from the diet.

11.2 Trace Minerals

11.2.1 Iron

Of all the metals, iron is probably the one which lay people are most aware of as a nutrient, and also the one which is perceived to be potentially in short supply in the diet. One reason for this is that most people are familiar with the need for iron in their blood, in the myoglobin of muscle and in respiratory enzyme systems generally. Iron is the most important transition metal in the body of animals, where it occurs almost entirely in elaborate coordination compounds based on the porphyrin nucleus, notably the haem pigments (see Figure 5.6), which are responsible for carrying oxygen. Alternation between its two oxidation states, Fe^{II} (ferrous) and Fe^{III} (ferric) is an essential feature of its contribution to the oxidation–reduction reactions involving the other iron porphyrin proteins active in respiration, the cytochromes. A deficiency of iron is manifested as anaemia, *i.e.* an abnormally low blood haemoglobin level.

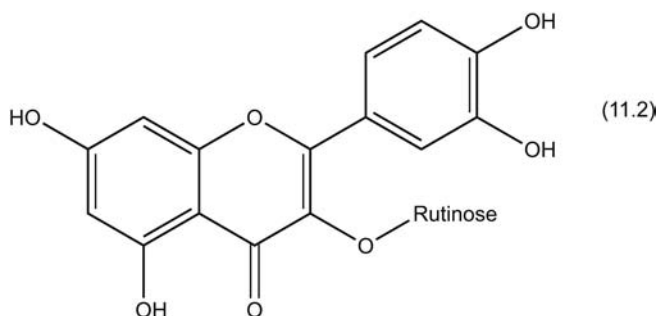
Iron is present in most foodstuffs of plant as well as animal origin. Lean meat contains between 2 and 4 mg per 100 g, mostly as myoglobin, which means that the relative redness of different cuts of meat is a guide to the relative abundance of the metal. Chicken, lamb and ox livers contain more iron, about 9 mg per 100 g, and pig's liver about twice as much. The iron in liver is not present as myoglobin or haemoglobin, but is bound by specialist iron-binding storage proteins, notably ferritin. The core of a single ferritin molecule is able to contain up to about 4000 iron atoms in a crystalline structure resembling the mineral ferrihydrate, which has the overall formula $[\text{FeO}(\text{OH})]_8[\text{FeO}(\text{H}_2\text{PO}_4)]$. Leafy green vegetables, legumes, nuts and whole cereal grains contain between 2 and 4 mg of iron per 100 g. Most fruit, potatoes and white fish such as cod have between 0.3 and 1.2 mg per 100 g. Although spinach is one of the vegetables at the upper end of the range for iron content (about 4 mg per 100 g), there is no scientific justification for the special enthusiasm shown by the food pundits of the popular media. The mild astringency of cooked spinach is often attributed to its iron content, but, in fact, it is the combination of oxalic acid (7.16) (0.5–1.2 g per 100 g) and various polyphenolics (see Chapter 6) that cause this. Much more remarkable is the figure of 26 mg per 100 g reported for the humble cockle (*Cardium* sp.), but this observation is unlikely to be relevant to anyone's regular diet. The iron content of cow's milk is remarkably low at 0.1–0.4 mg per 100 g, but the iron in dairy products such as butter and cheese is concentrated to about 0.16 and 0.4 mg per 100 g, respectively. Eggs contain around 2 mg per 100 g.

It is tempting to deduce from these data that iron is abundant in the diet and that iron deficiency should be rare. It is true that most people's diet contains between 10 and 14 mg per day and the absence of any specific mechanism for iron secretion from the body would appear to support this. Losses during menstruation can reach 15–20 mg

of iron per month, but other losses are much smaller. A total of up to 1 mg daily may be lost *via* urine, sweat and the epithelial cells shed from the skin and lining of the intestines. The difficulty is that iron is poorly absorbed from the intestine and the degree of absorption is highly dependent on the nature of the iron compounds present in the diet. Only about 10% is absorbed from typical European diets. The different behaviour of ferric and ferrous ions in aqueous solution is a key factor.

In the acidic environment of the stomach, ferrous and ferric ions occur as the soluble hydrated ions $\text{Fe}(\text{H}_2\text{O})_6^{2+}$ and $\text{Fe}(\text{H}_2\text{O})_6^{3+}$, respectively, but as the pH increases to 8 in the duodenum, the much less soluble hydroxides $\text{Fe}(\text{OH})_2$ and $\text{Fe}(\text{OH})_3$ are formed. Ferrous hydroxide is soluble up to 10 mmol dm^{-3} , but ferric hydroxide is essentially insoluble, the maximum concentration in solution being $10^{-18} \text{ mol dm}^{-3}$. Clearly, there is little prospect of absorbing useful quantities of ferric iron at pH 8. Studies with radioactive isotopes of iron, ^{55}Fe or ^{59}Fe , have shown that the presence of ascorbic acid in the duodenum greatly enhances iron uptake. This is partly due to its action in reducing Fe^{III} to Fe^{II} . The acidic conditions in the stomach will also help to ensure that any insoluble ferric iron in the food is solubilised. Ascorbic acid is also an effective chelating agent (a role it shares with many other substances, notably sugars, citric acid and the amino acids), forming soluble complexes with iron that make it available for absorption. Home-made beer, with a significant residual sugar level and brewed in iron vessels, has been blamed for the excessive iron intake that is one cause of the cirrhosis of the liver common among the urban African population of South Africa. There is a likelihood that iron overload will occur whenever iron vessels are extensively used for food preparation.

Although there is no suggestion that toxicity has resulted, it is worth noting that some canned fruits and vegetables may have high levels of dissolved iron if the cans are inadequately tinned. An instance has been recorded in which canned blackcurrants were found to contain 130 mg iron per 100 g. The high nitrate level in the tropical pawpaw (*Carica papaya*) solubilises so much iron that this fruit is not regarded as suitable for canning. It is sometimes observed that a discoloration of canned foods appears if the contents are kept in the can after opening. When air is admitted to the can, any ferrous ions dissolved from the inner surface may be oxidised to ferric iron. Ferric iron forms a black coordination compound with rutin (11.2), a flavonoid commonly found in fruit and vegetables and a glycoside of the flavonol quercetin.



Some natural drinking water contains high levels of iron. If water is stored in iron tanks or transported through cast-iron pipes, high concentrations of iron may result. Iron gives a bitter or astringent taste to drinking water and beverages.

The substances complexing iron in our diet have a considerable influence on the proportion absorbed by the body. When haem iron is the dietary source, *e.g.* the myoglobin of meat or fish skeletal muscle tissue, 15–25% is absorbed. By contrast, no more than 8% of the iron in plant foods, including cereals, legumes and leafy vegetables, is absorbed. The figure for spinach has been reported to be around 2.5%. The iron in plant foods is mainly present as insoluble complexes of ferrous iron with phytic acid, oxalates, phosphates and carbonates. One reassuring aspect of iron absorption from the intestine is that its efficiency increases when the body's iron reserves are low, and *vice versa*. As there is no mechanism for the excretion of excess iron, such regulation of intake seems essential, even though it may not always be wholly effective.

The low level of iron in both human and cow's milk has already been mentioned. The iron in milk is carried by a specialised protein, lactoferrin. This is a similar protein to transferrin, the non-haem iron protein that transports iron in the bloodstream. The most curious aspect of this protein is that its full iron-binding capacity is not taken up in milk, even when there is no suggestion of iron deficiency in the lactating animal. For their first few months of life, humans, and many other mammals, have to rely on the reserves built up in the fetus before birth. The most likely explanation for this phenomenon is that having low levels of free iron in milk has other benefits. Lactoferrin unsaturated with iron maintains a very low concentration of free iron in the milk and, as a consequence, in the upper reaches of the gastrointestinal tract. This is believed to provide new born mammals with protection against undesirable bacteria that have a strict requirement for iron. Among these are members of the Enterobacteriaceae, including pathogenic strains of *Escherichia coli*, which can cause severe diarrhoea in infants. More welcome bacteria, such as the lactobacilli, do not have the same demand for iron. Notably, the concentration of lactoferrin in human milk is about 100 times greater than that in cow's milk, even though the total iron content in human milk is only marginally greater than that in cow's milk.

11.2.2 Copper

Copper rarely causes problems today. It is widely distributed, as a component of a variety of enzymes in foodstuffs of all kinds, at levels between 0.1 and 0.5 mg per 100 g. Milk is notably low in copper, at about 0.2 mg per 100 g, and mammalian liver is exceptionally high, at around 8 mg per 100 g. The daily intake from normal adult diets is between 1 and 3 mg, which is roughly the level recommended by most authorities. As with iron, it appears that nature expects new born infants to utilise the reserves built up before birth until they are weaned. The occasional cases of copper deficiency in infants, seen as a form of anaemia, are invariably associated with other physiological or nutritional disorders.

Copper is not as toxic as one might imagine. Large doses of copper sulfate are emetic, and a dose of 100 g is reported to have resulted in liver and kidney damage. Long-term intakes of moderately high amounts of copper can arise from the regular drinking of water from hot taps in houses with copper plumbing, but the only likely outcome is no more than a gastrointestinal upset. This fairly optimistic attitude to the toxicity of copper salts has not always been the rule. Elizabeth Raffald, an eighteenth century cookery

writer and forerunner of the more famous Mrs Beeton, warned against the practice of using copper salts when pickling vegetables:

... for nothing is more common than to green pickles in a brass pan for the sake of having them a good green, when at the same time they will green as well by heating the liquor without the help of brass, or verdegrease of any kind, for it is a poison to a great degree ...^{vii}

It was not until 1855 that public pressure began to force commercial manufacturers of pickles to abandon the use of copper, but for a time sales actually went down until consumers got used to the more natural brown colour. It is ironic that caramel is now added to enhance the brown colour of pickles that was once looked upon as undesirable.

11.2.3 Zinc

The distribution of zinc in foodstuffs has much in common with copper. It is an essential component of the active sites of many enzymes and it is therefore not surprising to find it at high levels in animal tissues, such as lean meat and liver, at around 4 mg per 100 g. Wholemeal flour, dried peas and nuts contain similar levels, although white flour has only one-third as much. Other fruit and vegetables contain about 0.5 mg per 100 g or less. Of the dairy products, only eggs, with 1.5 mg per 100 g, can be regarded as a good source of zinc. Cow's milk has around 0.35 mg per 100 ml, but human milk has less than a third of this. As with some other minerals, new born infants have to rely on reserves laid down in the later stages of fetal development. Typical diets have been calculated to supply between 7 and 17 mg per day, of which about 20% is absorbed.

The potential for zinc absorption to be reduced by the presence of large quantities of fibre, containing phytic acid (see Chapter 3, Section 3.4 and Section 11.1.5), in the diet does not seem to be borne out in ordinary European diets. Although the occurrence of zinc deficiency and its symptoms are well recognised in veterinary practice, there is little to indicate that it is a significant problem in humans. There is some evidence that zinc deficiency is common in some poor rural communities in the Middle East, where a major part of the diet is unleavened bread made from wholemeal flour. When this poor diet is supplemented with zinc over a long period of time, a number of symptoms, including delayed puberty and reduced stature, tend to disappear.

11.2.4 Selenium

Selenium^{viii} was regarded as toxic decades before it was recognised as an essential nutrient in the 1950s. The toxic effects of selenium were identified in farm animals in parts of the world where there are unusually high levels of in soils. A few species of

^{vii}E. Raffald, *The Experienced English Housekeeper*, Baldwin, London, 1782; 8th edn, published in facsimile by E. W. Books, London, 1970.

^{viii}Chemically, selenium is defined as a metalloid, *i.e.* a substance having the appearance and other physical properties of a metal, but the chemical properties of a non-metal.

pasture plants accumulate extraordinary amounts of selenium, >1 g per kg (dry weight) when grown on selenium-rich soil. Wide variations are found in the selenium content of human diets. At one extreme is New Zealand, where much of the agricultural land is deficient in this element and the average per capita daily intake has been calculated to be only 25 mg. The corresponding figure for the UK, taken from the 1995 Total Diet Survey, is 34 mg. This is also low by international standards and when compared with the Reference Nutrient Intake (see Appendix I) of 60 and 75 mg per day for adult females and males, respectively. In different regions of the North American continent, dietary intakes of selenium range from 60 to 220 mg per day. Even within the UK, a wide range of selenium contents can be found in the same commodity produced in different regions. In the UK, the selenium content of cereals lies in the range 0.03–0.23 mg kg⁻¹, meat and fish 0.09–0.38 mg kg⁻¹, and fruit, vegetables and milk about 0.01 mg kg⁻¹.

Nuts are generally very rich in selenium; some samples of Brazil nuts have been found with 53 mg kg⁻¹. Just one of these nuts would supply the daily intake of selenium recommended by some authorities. Care must always be exercised in the pursuit of a healthy selenium intake because it is believed that a prolonged intake of in excess of 3 mg daily can give symptoms of selenium poisoning.

The biological function of selenium was mentioned in Chapter 8 as part of the discussion of the antioxidant role of vitamin E. Evidence is beginning to accumulate that selenium intake towards the top of the normal range is beneficial – for example, the incidence of certain cancers in the USA is being tentatively linked to the low selenium levels of the local diets. Human diseases that can definitely be attributed to selenium deficiency are rare, although there are indications of thyroid function disorders associated with selenium deficiency.

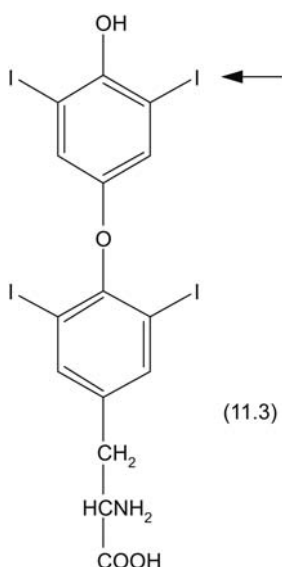
The relatively narrow safety margin between beneficial and harmful intake makes wide-scale dietary supplementation difficult and self-medication decidedly hazardous. However, in regions of China where selenium deficiency is endemic, salt has been fortified with 15 mg kg⁻¹ sodium selenite (Na₂SeO₄) since 1983. This has greatly reduced the prevalence of Keshan disease (a type of cardiomyopathy) in children in these regions as daily selenium intakes have increased from 11 to 80 mg. Infant formulas in the USA have been fortified with selenium (as sodium selenite) since 1989 and in 2013 the US Food and Drug Administration proposed minimum and maximum selenium levels of 2.0 and 7.0 mg per 100 kcal, respectively. An alternative approach has been adopted in Finland since the early 1980s, where supplementation of agricultural fertilisers has increased selenium intakes from 30–40 to 85 mg per day.

11.2.5 Iodine

Iodine is another nutrient whose concentration in the diet can vary dramatically from one locality to another. Unlike selenium, however, the variation is sufficient to result in regions of iodine deficiency being widely distributed throughout the world. Some years ago, the World Health Organization estimated that at least 200 million people were affected by iodine deficiency.

Iodine has only one role in the body, as a component of the pair of thyroid hormones thyroxine (11.3) and triiodothyronine (which differs from thyroxine only in lacking the

arrowed iodine atom). The release of these two hormones enables the thyroid gland in the neck to regulate metabolic activity and to promote growth and development. Inadequate levels of the hormones are associated with a lowered metabolic rate and what might be regarded as the corresponding physical symptoms: listlessness, constipation, a slow pulse and a tendency to “feel cold”. The visible manifestation of iodine deficiency is goitre, a massive enlargement of the thyroid gland as the body attempts to synthesise more thyroxine and triiodothyronine. More severe symptoms occur if the iodine intake is very low during critical stages of growth. The mental and physical abnormalities described collectively as cretinism are found in the children of mothers who experienced severe iodine deficiency during or immediately before pregnancy. Children with a low iodine intake during their growing years are affected by a related disorder known as myxoedema.



The incidence of goitre is closely related to the levels of iodine in the local soil and water supply and is most prevalent in mountainous regions, including the Alps, Pyrenees, Himalaya, parts of the Andes and the Rocky Mountains. In the UK, where goitre was once known as “Derbyshire neck”, it is particularly associated with areas in the Pennines. Among the flatter regions of the world that are also potentially vulnerable to endemic goitre are the Great Lakes of North America and the Thames Valley in southern England.

Different samples of the same foodstuff can differ considerably in their iodine content (always as iodide), which means that lists of typical values are relatively meaningless. For the record, the 1995 UK Total Diet Study found the average intake to be in the range 151–209 mg per day. What is certain is that most foods are poor sources of iodine. Fruit and vegetables, meat, freshwater fish, cereals and dairy products usually contain between 20 and 50 mg kg⁻¹. Seafood is the only consistently useful source, some popular fish species being recorded as containing several thousand mg kg⁻¹. Goitre is almost unknown in Japan, where seaweed, containing 4–5 mg of iodine per 100 g,

is regularly consumed. Some countries have regions free from goitre and others where it is endemic, and studies have clearly shown the influence of local food supplies. For example, a study of two such regions in Greece showed differences in iodine content across a wide range of foodstuffs ranging from 1.66 times higher for cow's milk to 7.05 times higher in eggs. Superimposed on these factors was the proximity to the sea of the low goitre region.

Several different methods have been used to supply iodine to populations in iodine-deficient areas. The only consistently successful technique has been to add iodide to the salt sold for domestic use. A good example of what can be achieved is provided by data from Michigan, USA. Before iodised salt became available (at 0.02% w/w) in 1924, nearly 40% of Michigan schoolchildren showed signs of goitre. By 1928 the figure had fallen to 9% and by 1951 to 1.4%. Sadly, by 1970 the incidence was back up to 6% due to complacency on the part of the public and less intensive health education. That non-iodised salt should ever be on sale at all in such regions is hard to understand. In 2014, the World Health Organization stated that iodine deficiency is the most common preventable cause of brain damage globally. Members of iodine-deficient communities are observed to have average IQs up to 13.5 points lower than similar iodine-sufficient communities.

In the UK, it was previously generally agreed that the average diet contained adequate levels of iodine. One reason for this was the popularity of sea fish. The increasing degree of industrialisation of our food supply also means that we are much less dependent on the produce of our immediate neighbourhoods than in the past. There is no official encouragement in the UK for the sale of iodised salt and little is on sale for domestic use, either in cooking or for discretionary use at the table. However, since 2008 the general complacency regarding dietary iodine levels has begun to be questioned, with particular concern being expressed about the iodine status of young women. Studies in the UK^{ix} and elsewhere in Europe have shown that organically produced milk has levels of iodine around half that of the conventional product. This appears to be the direct result of the strict regulation of the use of mineral supplements in the feed of organically reared dairy cattle and is of particular concern because milk and dairy products provide nearly half the iodine in conventional UK diets.

11.3 Other Trace Minerals

There are a number of other essential trace elements, including boron, silicon, vanadium, chromium, manganese, cobalt, nickel and molybdenum. Our need for these has been inferred from their occurrence in various enzymes isolated from animal sources, rather than by identifying human diseases caused by their deficiency.

Strontium appears to be of little interest to food chemists. It is common in nature, occurring in small amounts in most agricultural soils and constituting between 0.01 and 0.001% of the dry weight of animal and plant tissues. It has no known biological

^{ix}S. C. Bath, S. Button and M. P. Rayman, Iodine concentration of organic and conventional milk: implications for iodine intake, *Br. J. Nutr.*, 2012, **107**, 935.

role and is not regarded as toxic. Being chemically similar to calcium (see Figure 11.1), it can mimic its way into our bodies, ending up in our bones. Natural strontium is a mixture of four stable isotopes, ^{84}Sr , ^{86}Sr , ^{87}Sr and ^{88}Sr , which normally occur in proportions of about 0.56, 9.86, 7.00 and 82.58%, respectively. The values of the ratio $^{87}\text{Sr}/^{86}\text{Sr}$ range from about 0.716 in granites to about 0.703 in basalts. The ratios found in soils, and the plants growing in them, reflect those of the underlying rocks. It has been shown to be possible to discriminate between otherwise similar samples of food crops grown in different parts of the world using multi-collector inductively coupled plasma mass spectrometry. The theory and details of this technique are outside the scope of this book, but the results are impressive and relevant.^x

Examples include distinguishing between:

Japanese brown rice grown in Japan from cheaper rice grown elsewhere (Australia, China, Vietnam and California)

Asparagus grown in a particular area of Austria from that grown elsewhere in Austria and other parts of Europe

Paprika from particular varieties of peppers grown in one region of Hungary from those grown elsewhere.

This technique has also been used to verify the geographical origin of samples of wines, dairy products, orange juice, and meat and fish products. Although these results have no obvious health implications for consumers, they can have considerable economic and commercial implications.

Further Reading

Books

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12 Enzymes

The preceding ten chapters of this book, and the next, are devoted to the different chemical classes of substances found in the food we eat. They are all important to us, whether or not we regard them as nutritionally valuable, aesthetically desirable, or potentially damaging to our health or well-being. Of course, we are not content to simply record their presence and comment on their nutritional properties. We are also interested in how they are affected by food preservation and processing strategies and the way in which they interact with each other. For the most part, we concentrate on the chemistry of these effects; two examples are the oxidation of fats and oils during storage (Chapter 4) and the physical effects of heat on the proteins of meat (Chapter 5). However, many of the chemical changes that take place in food materials would not take place at significant rates without the presence of enzymes, the remarkable catalysts that are characteristic of all living systems, animal, plant or microbial. The contribution that enzymes make to the properties and behaviour of food materials is the theme of this chapter.

12.1 General Principles

The examples given in the preceding paragraph, and many other chemical reactions that loom large in the study of food chemistry, have the common feature that they proceed slowly at ambient temperatures, *e.g.* oils and fats can be stored for many weeks in the presence of air before rancidity becomes obvious. High temperatures, in excess of 100 °C, are required for the decomposition of sugars to caramels within a reasonable timeframe. By contrast, the organic reactions that characterise “life” in animal, plant and microbial cells, some of which we consume, directly or indirectly, as food are capable of far greater rapidity. For example, if we add a trace of sugar to a suspension of yeast cells, bubbles will appear moments later, even though the conversion of sucrose into carbon dioxide requires a sequence of 12 chemical steps. The crucial feature of the reactions typical of living systems is that they are subject to catalysis. The catalysts involved are known as enzymes, a name first suggested in 1878 by Wilhelm Kühne.

Enzymes consist of globular proteins and the enzyme catalysing a particular reaction will have a unique structure in terms of both the amino acid sequence of its polypeptide chain (its primary structure) and, in consequence, the pattern of folding into its globular structure (its secondary and tertiary levels of structural organisation). Of course, the version of an enzyme from one species tends not to differ greatly from the corresponding enzyme from a closely related species. These aspects of protein structure were considered in more detail in Chapter 5. Enzyme proteins bind specifically to particular substrate molecules and, in doing so, are highly effective catalysts of chemical reactions. The substrate molecules bind spontaneously to a specific region of the enzyme molecule, known as the active site, to form an enzyme–substrate complex. Originally, in the early years of the twentieth century, the existence of the enzyme–substrate complex was inferred from the kinetics of a small number of enzyme-catalysed reactions, studied *in vitro*, in particular the relationship between the velocity of the reaction and concentration of the substrate. The expression



where E, S, ES and P indicate the enzyme, substrate, enzyme–substrate complex and product, respectively, in which the initial formation of the complex is relatively rapid, but the slower breakdown to form the product is rate-limiting, has stood the test of time. The other assumption is that, in molar terms, the concentration of the substrate(s) will far exceed the concentration of enzyme. The Michaelis constant (K_M), expressed in units of substrate concentration, is a measure of the affinity of the enzyme for the substrate and can also be regarded as the concentration of substrate that half-saturates the enzyme. The determination of Michaelis constants has been a regular laboratory exercise for generations of biochemistry students.

12.1.1 Enzyme Active Sites

Modern methods, such as X-ray crystallography and electron microscopy, have confirmed the original concepts of enzymes. The substrate binds to the enzyme at a specific site on the surface of the enzyme, usually a cleft or crevice, known as the active site. The amino acids that line the active site are located in different regions of the amino acid sequence of the protein, brought together by folding of the polypeptide chain: its secondary and tertiary structures. The characteristics of the amino acid side-chains – including their physical dimensions and orientation, ionisation and opportunities to form hydrogen bonds – all affect the binding of the substrate molecule to the active site. The binding will be the result of multiple weak non-covalent attractive forces rather than a single strong covalent bond. The selectivity, *i.e.* specificity, shown by enzymes towards their substrates is the inevitable consequence of the formation of an intimate enzyme–substrate complex.ⁱ

Many enzymes carry small non-protein molecules, or metal cations, permanently attached in the region of the active site that participate in the reaction sequence.

ⁱThe details of the mechanisms of many types of enzymes are now well established, but are outside the scope of this book. Readers wishing to explore this aspect of enzymes will find it well covered in most modern biochemistry textbooks.

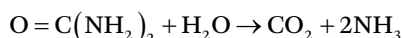
In chemical terms, most of the molecules, known as prosthetic groups, having this role are derived from the B-vitamins: thiamin pyrophosphate (TPP); flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD); pyridoxal phosphate; nicotinamide adenine dinucleotide (NAD) and its phosphate derivative NADP; biotin; and pantothenic acid.

The contributions of these substances to the activity of enzymes involved in metabolism were dealt with in Chapter 8. Some of these substances also have separate roles as metabolic intermediates in the cytoplasm of cells. For example, NAD and NADP collect (or their reduced forms donate) hydrogen atoms and electrons in numerous oxidation or reduction reactions. The haem groups attached to carrier proteins such as haemoglobin and myoglobin are also regarded as prosthetic groups.

The active sites of many enzymes include metal cations, usually held *via* coordinate bonds to amino acid side-chains. As Table 12.1 shows, most are transition metals facilitating their attachment by coordinate bonding and with variable valency, enabling a role for some in oxidation–reduction reactions.

12.1.2 Enzyme Specificity and Classification

With a few exceptions, particular enzymes catalyse only one type of reaction, but the degree of specificity shown towards the substrates of particular enzymes can vary considerably. Some enzymes are notable for their extreme specificity, *e.g.* urea is the only known substrate of the enzyme urease (found in the endosperm of legume seeds), which catalyses the hydrolysis of urea to carbon dioxide and ammonia:



Another example of extreme specificity is carbonic anhydrase, which is involved in the transfer of carbon dioxide from tissues into blood cells. One molecule of this enzyme can theoretically catalyse the conversion of 10^5 molecules of carbon dioxide (already dissolved in water) into bicarbonate ions per second, which is more than a million times faster than the uncatalysed rate:ⁱⁱ

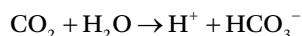
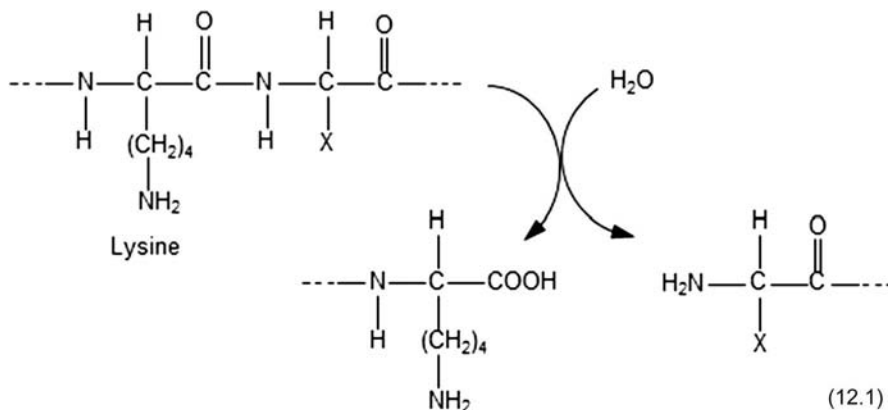


Table 12.1 Metals involved in the active site of enzymes. When incorporated into the enzyme active site, the metals will occur as cations. Otherwise similar enzymes from different sources, *e.g.* plants rather than animals, often differ in the identity of the metal cation involved.

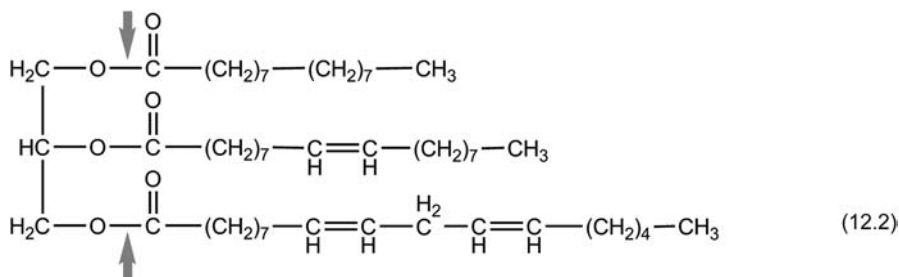
Metal	Example enzyme	Metal	Example enzyme
Vanadium	Nitrate reductase	Molybdenum	Xanthine oxidase
Manganese	Superoxide dismutase	Iron	Cytochrome oxidase
Cobalt	Glutamate mutase	Nickel	Urease
Copper	Ascorbic acid oxidase	Zinc	Carbonic anhydrase

ⁱⁱThis enzyme also occurs in saliva where, in catalysing the reverse reaction, it is said to be responsible for the fizz when the dissolved bicarbonate ions in carbonated drinks are “instantly” converted into bubbles of the much less soluble carbon dioxide gas.

By contrast, proteases, which catalyse the hydrolysis of the peptide bonds of proteins, vary considerably in their degree of specificity. Subtilisin, a protease secreted by several bacteria, is fairly indifferent to the identity of the two amino acids involved in the peptide bond (see Figure 5.3). However, trypsin, one of the enzymes active in the digestion of proteins in the mammalian small intestine, will only hydrolyse peptide bonds with a lysine or arginine residue on the carboxyl side (12.1).



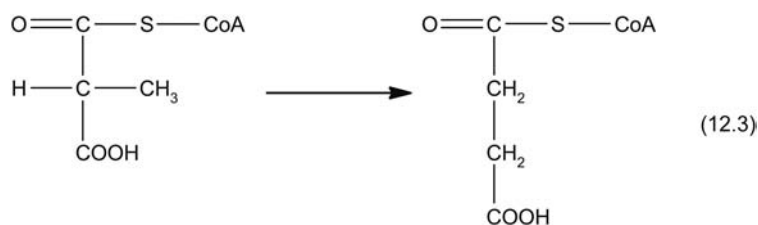
When the substrate has an asymmetrical centre (see Chapter 2) that is either involved in the reaction or structurally very close by, then enzymes are invariably, and totally, specific for one or the other isomer. Thus enzymes catalysing reactions involving D-sugars or L-amino acids do not catalyse the corresponding reactions with L-sugars or D-amino acids. Lipases (see Chapter 4), the enzymes that catalyse the hydrolysis of triglycerides, provide another illustration of the importance of the three-dimensional characteristics of an enzyme's substrate. Pancreatic lipase, involved in the digestion of fats in the small intestine, will liberate fatty acids from a triglyceride, but only those attached to the 1- and 3-positions of glycerol (12.2). The presence of double bonds some distance from the sites of hydrolysis (arrowed) makes no difference to the activity of the enzyme.



Regardless of the degree of substrate specificity shown by a particular enzyme, a single enzyme, almost without exception,ⁱⁱⁱ only catalyses a single type of reaction. As a result, enzymes are classified on the basis of the reactions they catalyse rather than features of their protein structure or origin. The reactions are divided into a number of classes.

ⁱⁱⁱThe best-known exception is the enzyme phenolase, discussed in Chapter 6, Section 6.5.

1. *Oxidoreductases* – catalyse oxidation/reduction reactions. These often involve a coenzyme such as NAD (see Chapter 8, Section 8.4) or flavin adenine dinucleotide (see Chapter 8, Section 8.2) as electron donors or acceptors and are particularly associated with the key metabolic pathways of intermediary metabolism, such as the Krebs (tricarboxylic acid) cycle. Molecular oxygen is directly involved in the reactions catalysed by oxygenases, such as lipoxygenase (see Chapter 7, Section 7.2.3), phenolase (see Chapter 6, Section 6.5) and ascorbic acid oxidase (see Chapter 5, Section 5.5.5).
2. *Transferases* – catalyse the transfer of a functional group from one molecule to another. The groups transferred include phosphate (often involving adenosine triphosphate, *e.g.* see Chapter 5, Section 5.5.4), one-carbon units (often involving tetrahydrofolate see Chapter 8, Section 8.6), acyl or glycosyl groups.
3. *Hydrolases* – catalyse the hydrolysis of a wide variety of compounds, including esters (*e.g.* pectin methyl esterase, see Chapter 3, section 3.2), glycosides (*e.g.* invertase, see Chapter 2, section 2.3) and peptides (*e.g.* calpains, see Chapter 5, section 5.5.4).
4. *Lyases* – mostly catalyse the cleavage of C–O, C–C or C–N bonds in elimination reactions that leave behind a double bond (*e.g.* pectin lyase, see Chapter 3, Section 3.2).
5. *Isomerases* – catalyse intramolecular rearrangements, including keto/enol isomerisation (glucose isomerase, see Chapter 3, Section 3.6.2), *cis-trans* isomerisation and the interconversion of D- and L-isomers (see Chapter 2, section 2.1). Some isomerases are capable of impressive molecular dexterity. One example is the enzyme that catalyses the isomerisation of methylmalonyl coenzyme A to succinyl coenzyme A (12.3). Coenzyme A is a nucleotide carrier of acyl groups, the structure of which is shown on in Figure 9.3, best known for its role in the metabolism of fatty acids.



6. *Ligases* – catalyse the formation of C–O, C–C, C–S or C–N bonds in reactions that also involve ATP as a source of energy. When the reaction involves the addition of CO₂ to form a carboxyl group (as in the conversion of pyruvate into oxaloacetate), the vitamin biotin (see Chapter 8, section 8.7) is involved in the reaction.

This classification is the basis for the formal naming and numbering system adopted by the International Union of Biochemistry and Molecular Biology (IUBMB) since the 1960s. The traditional naming system has always been based on the addition of the suffix “-ase” to the name of the substrate, *e.g.* protease and amylase, enzymes that catalyse the hydrolysis of proteins and starch, respectively, supplemented when required by an indication of the type of reaction catalysed, *e.g.* glucose oxidase (see Figure 2.8). However, the formal name for this enzyme, numbered EC 1.1.3.4, is β-D-glucose:oxygen

1-oxidoreductase.^{iv} These formal names are most often encountered in the initial paragraphs of research papers, but the traditional, much shorter, names always take over thereafter.

12.1.3 Effects of pH and Temperature

The protein nature of enzymes accounts for their sensitivity to their environment. Extremes of pH not normally encountered in biological systems will lead to the breakdown of enzymes and other proteins. Ionisable amino acid side-chains, *e.g.* aspartate and histidine, often contribute to the active site and, of course, the structures of many substrates will include ionisable groups such as -NH_2 or -COOH . Restriction of the optimum activity of a particular enzyme to a fairly narrow pH range, *e.g.* 6.5 ± 0.5 , is therefore inevitable. Of course, the evolution of an enzyme's structure over time will have ensured that the optimum pH we now observe in the laboratory is closely matched to the natural pH of the environment in which the enzyme is naturally active. For example, the optimum pH ranges of the human digestive enzymes α -amylase (EC 3.2.1.1), in the mouth, pepsin (EC 3.4.23.1), in the stomach, and trypsin (EC 3.4.21.4), in the duodenum, reflect the usual pH values of saliva (6.4–7.0), gastric juices (1.5–2.0) and the duodenum contents (5–7), respectively. Extremes of pH, especially at high temperatures, will lead to the irreversible inactivation of enzymes. Peptide bonds are liable to hydrolysis at low pH values, with catastrophic consequences for the three-dimensional structure of the protein. The side-chains of several amino acids, including cysteine, arginine, glutamine, asparagine and lysine, are vulnerable to alkaline pH values, especially at high temperatures. The mechanisms involved were set out in Figure 5.8.

The denaturation of proteins at elevated temperatures, briefly described in Chapter 5, disrupts the numerous non-covalent interactions that normally maintain the catalytically active conformation. This will inevitably lead to the irreversible disruption of the active site of enzymes and the irreversible elimination of catalytic activity, *i.e.* inactivation. An enzyme will often be described as being inactivated at a particular temperature. What this statement actually means is that the velocity of the inactivation process is highly dependent on temperature, so that inactivation only proceeds extremely slowly at temperatures a few degrees below the observed “inactivation temperature”, but the rate of inactivation becomes very fast with only a modest increase in temperature. A few degrees higher still and the rate of inactivation is sufficiently rapid to appear almost instantaneous. It is a general observation that the inactivation temperature of an enzyme, as observed in the laboratory, is related to the temperature at which the organism that was the source of the enzyme normally thrives. Thus most enzymes occurring in mammals are inactivated at temperatures between 40 and 50 °C. Enzymes obtained from thermophilic bacteria, some species of which thrive at temperatures approaching 100 °C,^v are normally stable at remarkably high temperatures, comparable with the

^{iv}EC – Enzyme Commission. A full searchable index of enzymes, including EC numbers, may be found at www.brenda-enzymes.info (last accessed in 2020).

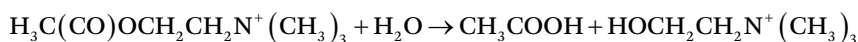
^vThe natural habitats of the most extreme thermophiles are the thermal springs of volcanic regions. Others, which grow at temperatures in the range 50–65 °C, are often associated with compost heaps, *etc.*

natural habitats of the particular source species. When the remarkable stability of the enzymes from thermophilic bacteria began to be studied, it was often assumed that their thermal stability was the result of some unique feature of their protein structure. However, it is now recognised that thermostable enzymes simply have more of the intramolecular structural features, such as sulfur bridges, hydrogen bonds *etc.* (described in Chapter 5) that maintain the tertiary structure of protein molecules than their less thermostable counterparts in mesophilic or psychrophilic organisms.

12.1.4 Inhibition

The catalysts used in industrial chemistry are well known for losing their activity over time. For example, the efficiency of the catalysts used in the hydrogenation of oils for margarine manufacture decreases over time as traces of sulfur compounds from the oil accumulate on the surface of the catalyst. Enzymes are also prone to a number of types of inhibition, broadly divided into *competitive* or *non-competitive*.^{vi} Competitive inhibitors are molecules that resemble the substrate sufficiently to bind to the active site, in competition with the substrate, but are released again without undergoing any chemical changes induced by the enzyme. In kinetic terms they increase the concentration of the substrate that half-saturates the enzyme. Examples of competitive inhibition relevant to food chemistry or biochemistry are rare, but one enzyme, glucose oxidase (EC 1.1.3.4), which has a valued role in food analysis (see Chapter 2, Section 2.1), is competitively inhibited by 2-deoxy- α -D-glucose and D-arabinose, shown in Figure 12.1. Fortunately, neither of these two sugars is likely to occur in significant concentrations in the practical food or clinical situations in which glucose oxidase is used.

Several non-competitive enzyme inhibitors are of considerable interest to food chemists because they are used as agricultural insecticides and are found in trace, but not insignificant, amounts in food products. Many of the residues that concern food chemists are inhibitors of the enzyme acetylcholinesterase (EC 3.1.1.7), *e.g.* malathion, parathion and chlorpyrifos. The reaction that is normally catalysed by this enzyme



has an essential role in the transmission of nerve impulses across synapses. As shown in Figure 12.2, the insecticide permanently blocks the active site of the enzyme, with catastrophic effects on the insect's nervous system.

The ions of many metals, notably silver, copper, mercury and lead, are also potent enzyme inhibitors. The term “heavy metals” is often used collectively for these metals, together with several others, but there is no clear distinction between heavy and light metals. Most of the heavy metals are ‘transition metals, in Groups 3 to 11 of the Periodic Table (see Figure 12.1). Inhibition results from irreversible reactions between the

^{vi}The terms *reversible* and *irreversible* inhibition are also used by specialist enzymologists as most, but not all, cases of competitive inhibition are reversible and most, but not all, cases of irreversible inhibition are non-competitive. These academic distinctions need not trouble us here.

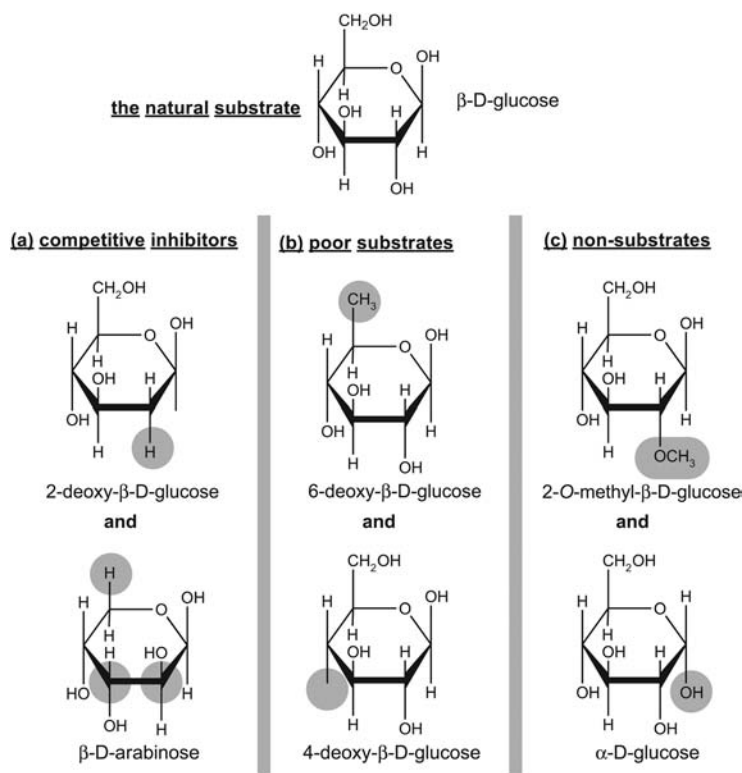
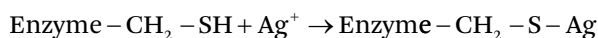
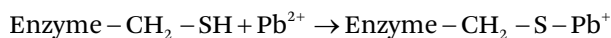


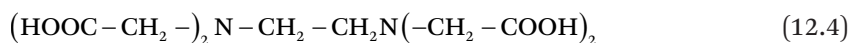
Figure 12.1 Structure of the natural substrate of glucose oxidase, β -D-glucose, compared with (a) competitive inhibitors, (b) alternative substrates with which the enzyme shows greatly reduced activity and (c) potential substrates with which the enzyme is inactive. The sites of differences between structures (a), (b) and (c) and β -D-glucose are shaded.

metal ion and sulfhydryl groups, either in the active site or a site otherwise critical to the structure of the enzyme, *e.g.*



The carboxyl groups of amino acids, such as aspartic acid, may also form chelates with these metals.

Enzymes located inside cells are largely protected from this problem by the cell membrane, but once extracted from cells for applications in food processing operations or food analysis (to be considered later in this chapter), they are potentially vulnerable. It is common for the buffer solutions used in the extraction and purification of enzymes to include low concentrations of chelating agents, such as ethylenediaminetetraacetic acid (EDTA) (12.4) to scavenge any undesirable heavy metal ions.



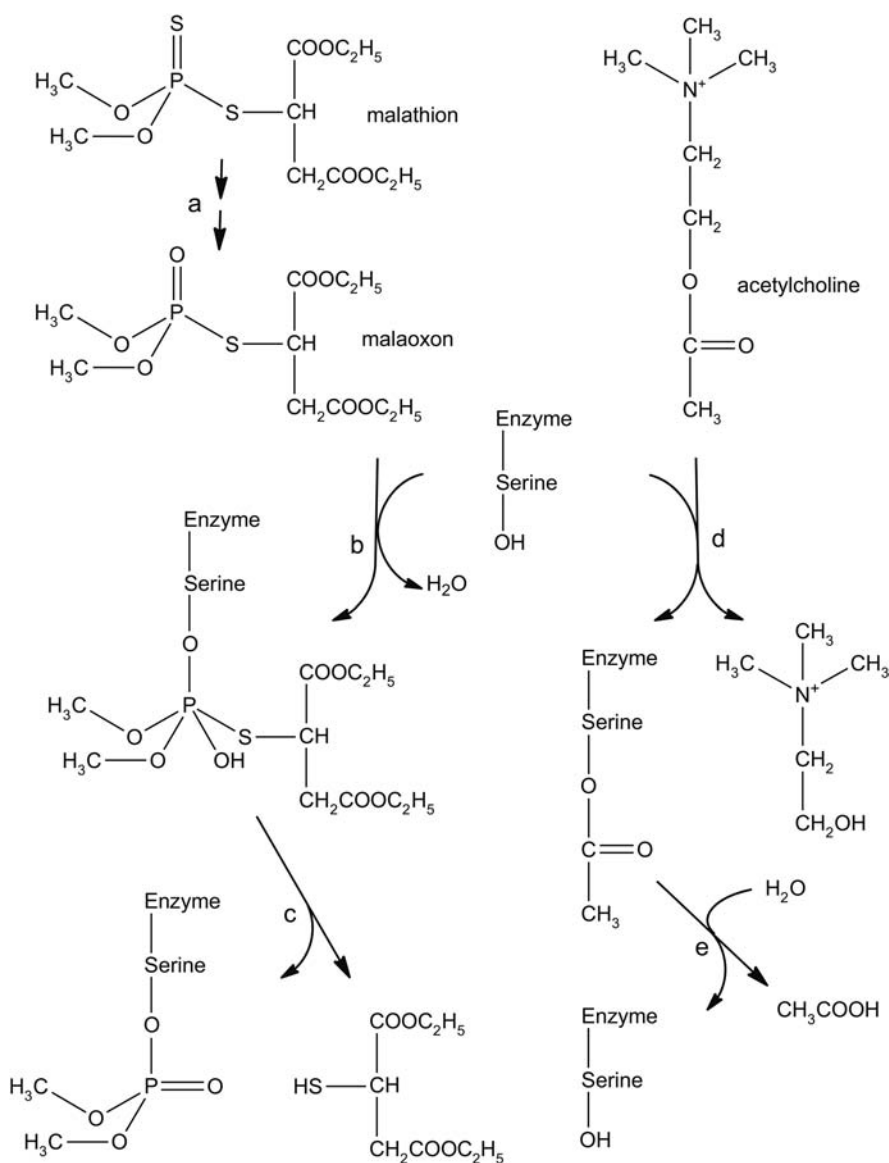


Figure 12.2 Inhibition of acetylcholinesterase by malathion. On entering the cell, enzymes convert malathion to malaoxon, replacing a sulfur atom with oxygen (a). The P=O group then binds to the serine hydroxyl of the acetylcholinesterase active site (b) in an analogous reaction to that of the C=O group of the normal substrate acetylcholine (d). The hydrolysis of the P-S bond (c) allows part of the malaoxon molecule to leave, but the remainder is now permanently attached to the serine residue, preventing any further acetylcholinesterase activity. By contrast, the hydrolysis of the attachment of the acetyl group (e) regenerates the active enzyme.

Enzymes whose activity depends on the presence of a transition metal cation are vulnerable to inhibitors that, in effect, withdraw the cation. For food chemists, the most relevant example is that of the enzyme phenolase. This enzyme is readily inhibited by substances that react strongly with the copper ion, (assumed to be Cu^{I} , cuprous copper), which participates in the reaction. Unfortunately, none of these inhibitors is in any way suitable for use in food situations.

12.2 Natural Enzyme Activity in Food Materials

Most of the proteins considered so far in this book, particularly in Chapter 5, have either a nutritional role, *e.g.* the gluten proteins of wheat grains or the caseins of milk, or a physical role, *e.g.* the actin, myosin and collagen of muscle. However, all of these food materials also continue to contain the enzymes that facilitated the metabolism of the original tissues. The death of an animal or the harvest of a plant in no way switches off these enzymes and they will remain potentially active. Their activity will be limited by a declining supply of the substrate, energy, *etc.* by natural regulatory mechanisms based on feedback inhibition and hormones, as well as the natural deterioration of the tissues.

In many raw materials of foodstuffs, there are enzymes whose potential for catalytic activity only becomes obvious as the raw material is stored or processed. Many of these have already been considered in some detail in other chapters, but it may be valuable to bring them together here.

12.2.1 Plant Enzymes

Plant tissues often contain enzymes that only become active when they are physically damaged, usually when a fruit or vegetable is chopped or sliced as part of its preparation in the kitchen or processing plant, *e.g.* lipoxygenase (EC 1.13.11.12), phenolase (EC 1.10.3.1), myrosinase (EC 3.2.1.147) and alliinase (EC 4.4.1.4). The first of these, lipoxygenase, catalyses the oxidation of polyunsaturated fatty acids (see Chapter 7, Section 6.2.3) released from cell membranes, leading to the formation of a variety of low molecular weight compounds. The ripening of fruit can lead to membrane breakdown, which makes the fatty acids vulnerable to oxidation and the generation of desirable flavour compounds. However, the tissue damage that can accompany the harvesting of peas will lead to fatty acid breakdown products that cause off-flavours. In the case of fruit, a balance must be struck between the maximisation of flavour production and the softening that occurs at the same time. The other three enzymes only become active when the membrane separating the vacuole of the plant cell from the cytoplasm (the tonoplast) is ruptured and they gain access to their substrates in solution in the vacuole.

Alliinase (notably in onions and garlic) and myrosinase (in brassicas) only catalyse the reactions (dealt with in detail in Chapter 7) that lead to the characteristic flavour compounds of these vegetables when culinary activities lead to the rupture of the tonoplast. Phenolases similarly become active when the breakdown of cell structures allows access of the enzyme to phenolic compounds dissolved in the vacuole. From the plant's point of view the phenolase activity that accompanies bruising is defensive, the

oxidation products having a recognised antifungal action. This bruising is, of course, unwelcome to the consumer. However, the bruising and browning that accompanies the maceration of tea leaves during the manufacture of black and oolong tea is an essential part of the process (discussed in detail in Chapter 6).

Traditional beer-making using barley, and sometimes a proportion of other cereal grains, is a process that has always depended on the enzymes naturally present in the raw materials. The endosperm of the grain contains a high proportion of starch that must be converted to sugars before fermentation starts. The first stage of the process is to soak the grains in water to initiate germination. One starch-degrading enzyme, β -amylase (EC 3.2.1.2), is already present in the ungerminated grain, but the other, α -amylase (EC 3.2.1.1), is produced during germination.^{vii}

After four to six days, germination is halted by heating (kilning) the grain to 45–50 °C. This reduces the water content to about 5% and halts the germination process, but does not denature the amylases. The product is known as malt, or malted barley. The malt is then crushed and mixed with any other supplementary carbohydrate sources before being mixed with warm water for several hours, a stage known as “mashing”. The exact details of the temperature regime during mashing vary with the type of beer being produced (*e.g.* ale or lager) and regional traditions, but most often the initial temperature is about 45 °C, increasing steadily to about 75 °C. While the temperature is still <55 °C, β -glucanases (EC 3.2.1.6) in the malt attack the cell wall β -glucans (see Chapter 3, Section 3.4), which improves the accessibility of the starch in the malt to the α - and β -amylases. Fungal β -glucanases are also used to supplement the activity of the malt enzymes. At the same time, proteases in the malt break down proteins to provide amino acids for the yeast in the subsequent fermentation process and also eliminate soluble proteins that can contribute to hazes in the finished beer. Between 60 and 75 °C the amylases reach their maximum activity, producing fermentable sugars. At the end of this stage the sugars are washed off the insoluble residues of the malt and filtered, giving the liquid known as wort (or sweet wort) that goes on to be boiled with hops (see Chapter 7, Section 7.1.2) before being fermented. As we shall see in the next section, modern brewers often supplement the malt’s own enzymes with enzymes from microbial sources. These enable brewers to use cheaper sources of starch that are not automatically accompanied by starch-degrading enzymes.

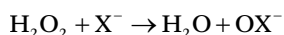
12.2.2 Animal Enzymes

When an animal dies, or more specifically in the context of this book, is slaughtered for meat, the enzymes in the muscle cells remain fully functional, their activity only being limited by declining concentrations of substrates and by the absence of stimuli from the nervous system. The sequence of *post mortem* events is described in detail in

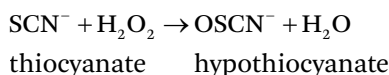
^{vii} α -Amylase catalyses the hydrolysis of starch (amylose and amylopectin) at α 1–4 links (see Chapter 3, Section 3.1) randomly along the length of the polymer chains, generating oligosaccharide fragments down to two or three glucose units in size, but does not attack the α 1–6 branch points characteristic of amylopectin. β -Amylase catalyses the hydrolysis of alternate α 1–4 links from the non-reducing ends of the polymer chains (from intact amylose or amylopectin molecules or fragments resulting from α -amylase activity), liberating successive maltose molecules. In the case of amylopectin, the process is brought to a halt when α 1–6 branch points are encountered.

Chapter 5, but the activity of one particular group of enzymes, the proteases known as calpains (EC 3.4.22.52 and EC 3.4.22.53) (see Chapter 5, section 5.5.4) are worthy of note in the present context. These enzymes, present in the sarcoplasm (*i.e.* cytoplasm of the contractile muscle cells) appear to be inactive in the live animal. They require the low pH and high concentration of Ca^{2+} ions that only occur as *rigor mortis* sets in before they are able to attack the links between the myofibrillar proteins and initiate tenderisation.

Peroxidases (EC 1.11.1.7) catalyse a range of reactions:



where X represents a wide variety of organic compounds, particularly phenolic compounds. The classic source of the enzyme used in biochemistry laboratories is horseradish (*Armoracia lapathifolia*), but peroxidases occur widely in plant tissues. They are also known to occur in the milk, colostrum, tears and saliva of many mammals, including humans and cattle. The peroxidase naturally present in milk, known as lactoperoxidase, can give rise to hypothiocyanate from the low, natural levels of thiocyanate present in cow's milk:



Hypothiocyanate prevents the growth of gram-positive bacteria and kills gram-negative bacteria. The lactoperoxidase system is therefore believed to be a part of the natural protection of suckling animals against enteric infection. Lactic acid bacteria in the milk can produce hydrogen peroxide, but the amount usually produced in milk is insufficient to have a useful effect. Extensive studies, both in the laboratory and in the field, have shown that the supplementation of both the thiocyanate and peroxide levels in raw milk can provide a useful enhancement of its keeping properties (adding five to six days at 4 °C and 4–7 h at 31–35 °C) and some antibacterial effects on typical milk-borne pathogens. To achieve these effects, sodium thiocyanate and sodium percarbonate^{viii} are added in tablet form to give 10–15 mg L⁻¹ and 30 mg L⁻¹, respectively. This level of thiocyanate is well below that which might cause problems for iodine metabolism (see Chapter 11, section 11.2.5 and Chapter 10, section 10.1). This approach to milk processing offers real benefits in parts of the world where modern pasteurisation and bottling techniques are impracticable, but where milk, bovine or otherwise, is an important part of the human diet.

12.3 Food Processing Applications of Isolated Enzymes

The sensitivity of enzymes to extremes of temperature, pH, *etc.* may suggest that they have limited usefulness as catalysts to be brought to bear on reactions of industrial importance. However, in the context of food, the opposite is true. This sensitivity, and

^{viii}Sodium percarbonate, an adduct of sodium carbonate and hydrogen peroxide ($2\text{Na}_2\text{CO}_3 \cdot 3\text{H}_2\text{O}_2$), is a far more convenient, and safer, source of peroxide ions than hydrogen peroxide diluted in water.

the specificity that accompanies it, actually means that enzymic catalysis can be used to facilitate reactions in food materials that share the same sensitivities as enzymes without any risk of adverse effects on the desirable components of the food. One example of this, encountered in Chapter 2, is the manufacture of glucose syrups. These can be manufactured from starch by hydrolysis with sulfuric acid, but the conditions required for the reaction, prolonged high temperatures and extreme acidity, inevitably result in incomplete hydrolysis and the formation of undesirable by-products. By contrast, the conditions required for enzymic hydrolysis are comparatively mild and can give very high yields requiring little purification. The specificity shown by most enzymes can be valuable in food processing, but comes into its own in food analysis.

12.3.1 Enzyme Production

In theory, any type of organism could be used as a source of enzymes for use in food processing, but, in fact, the choices are fairly limited. Enzymes normally found in the company of hundreds of other enzymes in the cytoplasm of cells (animal, plant or microbial) are routinely isolated from cell extracts on a laboratory scale. However, the techniques required, although established for decades by biochemists, do not lend themselves to use on an industrial scale.

The solution is to take advantage of the *extracellular* enzymes that are secreted by bacteria or unicellular fungi to break down macromolecules, lipids, proteins or polysaccharides, providing molecules small enough to then be absorbed as a source of energy or raw materials for growth. These processes mirror the enzymic digestion of food materials in the alimentary tracts of animals. Obtaining digestive juices, rich in enzymes, from animals on a large scale is usually regarded as impractical, although the traditional manufacture of rennet (containing the enzyme chymosin, EC 3.4.23.4) used in cheese manufacture (see Chapter 5, page 5.5.2) is an exception. Rennet was traditionally obtained from slaughtered calves by macerating the walls of the abomasum (the fourth stomach) in weak brine to extract the inactive proenzyme^{ix} form of the enzyme. The crude extract was then briefly acidified to convert the proenzyme to its active form and then filtered. This preparation, which also contained small proportions of other proteolytic enzymes, was then simply refrigerated for storage before use in cheese-making.

The recovery of enzymes from the growth medium of microorganisms is far more straightforward. Microorganisms can be grown in large-scale liquid media that will contain, when growth is complete, few, if any, other proteins. The preferred microorganisms are either *Bacillus* species or filamentous fungi. Many species in these groups have the necessary properties for enzyme production, including:

- A well-established safety record in not secreting toxins – the problems posed by production of mycotoxins were raised in Chapter 10.

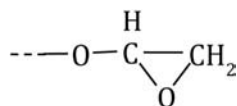
^{ix}The digestive enzymes of animals are initially synthesised in the pancreas or gastric mucosa as inactive proenzymes (sometimes known as zymogens). Their active sites are protected by a short region of the polypeptide chain so that they do not attack the proteins in the mucosal cells. Only when they are safely in the lumen of the stomach or intestine do the acid conditions or other specific enzymes remove the protective section of the polypeptide chain to activate the enzymes.

- Ease of genetic manipulation/modification so that an enzyme associated with a difficult source can be utilised by transferring the relevant gene(s) to a more manageable microorganism. The source need not be another microorganism. For example, the enzyme chymosin, used in cheese-making, was traditionally obtained as a crude extract of the lining of the fourth stomach of slaughtered calves, but an identical enzyme is now obtained using genetically modified microorganisms.
- Suitability for cultivation in large-scale fermenters.

Detailed considerations of these aspects of industrial-scale biochemistry and microbiology lie outside the scope of this book, but are provided by texts listed in the Further Reading section at the end of this chapter.

There is a general assumption that the reactions that are the subject of this chapter involve substrates and enzymes in solution. This need not be the case and, in fact, reaction systems in which the enzyme is immobilised, *i.e.* permanently attached to a solid matrix, can have considerable advantages. Instead of the enzyme becoming irretrievably mixed with the reaction products and lost, immobilisation *may* make it possible to reuse the enzyme for subsequent batches of substrates. Immobilised enzymes are particularly appropriate for continuous rather than batch processes. The enzymic manufacture of high-fructose syrup described in Chapter 3 (see Section 3.6.2) is an important application of an immobilised enzyme in a continuous process.

The essential feature of an immobilised enzyme system is a particulate, insoluble, porous and chemically stable material to form a carrier for the enzyme. Ideally, the carrier particles should be around 0.2–1 mm in diameter with pores large enough (20–40 nm diameter) to accommodate the enzyme molecules. The total surface area of the pores in which the enzyme is absorbed may be 50–150 m² per gram of the carrier. A wide variety of different substances are used as carriers. Among the fairly natural substances are inorganic materials, such as porous silica (SiO₂, silica gel), and organic polymers, such as cellulose derivatives and agarose. Derivatives of synthetic polymers, such as polystyrene and polyacrylic ester, are widely used in the form of ion-exchange resins that are effective binders of protein molecules. Covalent chemical bonds between free amino groups (–NH₂), on the surface of enzyme proteins, and functional groups such as epoxide or aldehyde (–CHO), located on the surface of the polymer beads, provide a permanent attachment for the enzyme. An alternative approach is to immobilise entire microbial cells that contain the desired enzyme (possibly as products of genetic modification). The cells can be immobilised by chemically cross-linking them to each other. Substrate molecules diffuse into the cells, interact with trapped enzyme and then diffuse out again.



These continuous processes, based on pumping substrate solutions through a reactor packed with catalytic particles, are best suited to reactions in which small molecules, such as sugars, are to be modified, *e.g.* the isomerisation of glucose to fructose by glucose isomerase. The high viscosity of strong solutions of polymers such as starch

rules out immobilised enzyme systems for their hydrolysis, as does the presence of particulate material in beer-making, *etc.*

12.3.2 Enzyme Applications in Food Manufacture

The number of different enzymes currently in use by food and drink manufacturers is now considerable (see Table 12.2) and is likely to lengthen in the future. Most of them have already been considered in earlier chapters of this book, where their substrates were the focus of attention. Many of these enzymes are used to break down polysaccharides, but proteins, the other major class of food polymers, are less often enzyme targets. The enzyme chymosin is the obvious exception, but two others, papain and transglutaminase, are considered here. The specificity of enzymes towards particular optical isomers of asymmetrical substrates has already been mentioned and this has been exploited in the synthesis of two food additives that, being optically active, would pose particular difficulties for ordinary organic chemistry.

12.3.2.1 *L*-ACT-Hydrolase in *L*-Cysteine Synthesis

L-Cysteine is one of the 20 amino acids that occur in the structure of almost all proteins, but only in trace amounts as a free amino acid in microbial cells or animal and plant tissues. Its optical isomer, *D*-cysteine, is virtually unknown outside chemical laboratories and would definitely qualify for the epithet “unnatural”. In recent years, *L*-cysteine has become a valued additive in industrial-scale bread-making, making the dough more extensible and easier to handle mechanically. These properties are particularly important with products such as burger buns.

Unfortunately, there is no easily extractable natural source of *L*-cysteine. Most proteins contain 1–2% at most, although there is one exception, keratin, the principal component of birds’ feathers and animal hair, including wool. For many years, digests of hair and feathers provided the major source of commercial *L*-cysteine. In spite of denials from importers, it is widely believed (perhaps an “urban myth”) that human hair, supposedly collected from hairdressing establishments, was a favoured feedstock. Whether or not human keratin really is a commercial source, these sources of *L*-cysteine

Table 12.2 Enzymes currently in use by food and drink manufacturers. Most of these applications have already been considered in appropriate detail in earlier chapters, but some, asterisked, receive particular attention in this chapter.

Enzyme	EC number	Enzyme	EC number
Glucose oxidase	1.1.3.4	Lipase	3.1.1.3
β -Galactosidase	3.2.1.23	Chymosin	3.4.23.4
Invertase	3.2.1.26	Papain*	3.4.22.2
Asparaginase	3.5.1.1	β -Amylase*	3.2.1.2
Pectin methyl esterase	3.2.1.11	β -Glucanase*	3.2.1.6
Pectinases	3.2.1.15 <i>etc.</i>	<i>L</i> -ACT-Hydrolase*	3.5.2.-
α -Amylase	3.2.1.1	Transglutaminase*	2.3.2.13
Amyloglucosidase	3.2.1.3	Lysozyme	3.2.1.17
Glucose isomerase	5.3.1.18	Thermolysin*	3.4.24.27
Pullulanase	3.2.1.41		

present vegans, vegetarians and adherents of many religious faiths with major problems. Unfortunately, any purely chemical process inevitably leads to the synthesis of an equal parts mixture of the two optical isomers D- and L-cysteine, the first of which could never be described as “natural”. Enzymes, with their optical specificity, have provided an answer in the hybrid chemical/microbiological process shown in Figure 12.3.

12.3.2.2 Thermolysin in Aspartame Synthesis

The properties and uses of the artificial sweetener aspartame were introduced in Chapter 7 (see Section 7.1.1), but not its manufacture. One of the most important methods for its industrial synthesis (a variety of different methods have been proposed at different times) provides a useful illustration of the exploitation and manipulation of the special characteristics of enzymic catalysis. An outline of the reactions, both chemical and enzyme catalysed, is shown Figure 12.4.

Aspartame is a dipeptide, *i.e.* two amino acid units linked by a peptide bond (see Chapter 5, Section 5.2), but obviously the usual mechanisms of peptide bond formation in protein synthesis, involving ribosomes, RNA, ATP, *etc.* are completely impracticable on this scale. There are a large number of different enzymes, the proteases and peptidases, that naturally catalyse the reverse reaction, *i.e.* the hydrolysis of peptide bonds. However, the equilibrium position for their reactions always lies strongly in the direction of hydrolysis. In the case of aspartame synthesis, this equilibrium is drawn over towards synthesis because the reaction product is continually removed as it precipitates out.^x The commercial reaction uses the enzyme thermolysin. This is an extracellular bacterial protease produced by *Bacillus thermoproteolyticus*, whose thermal

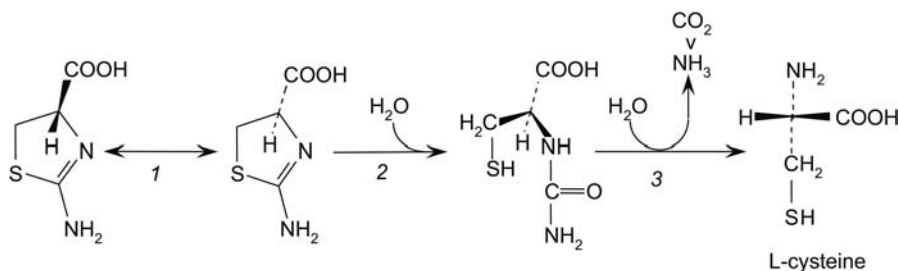


Figure 12.3 The Ajinomoto process for the synthesis of L-cysteine. In the initial chemical stages, methyl acrylate is first chlorinated and then condensed with thiourea [$\text{S}=\text{C}(\text{NH}_2)_2$] to give a mixture of the substrate for the next stage of the reaction, L-2-amino- Δ^2 -thiazoline-4-carboxylic acid (L-ACT) and its enantiomer D-ACT. This racemic mixture is then incubated with a preparation of the cells of any of several bacterial species, notably *Pseudomonas thiazolinophilum*, which contain all three of the enzymes required. A racemase (1) maintains the supply of the substrate for the L-ACT hydrolase (2), whose specificity for only one of the two isomers of ACT is the key to the whole process. The final step, which gives the desired end-product, is catalysed by S-carbomoyl-L-cysteine hydrolase (3).

^xThis is an example of Le Chatelier's principle, often stated as: "If a chemical system at equilibrium experiences a change in concentration, temperature, volume, or pressure, then the equilibrium shifts to counteract the imposed change and a new equilibrium is established".

stability and specificity for peptide bonds involving hydrophobic amino acids lends itself to this application (see Figure 12.4). The enzyme is potentially capable of linking the two amino acids in a variety of different ways, but this is prevented by blocking the amino group of the L-aspartic acid with benzyl chloroformate in a preliminary reaction, thus ensuring that only the correct amino and carboxyl groups are joined. The optical specificity of the enzyme ensures that the final product contains only the L-isomer of phenylalanine.

12.3.2.3 Papain in Meat Tenderisation

Toughness is an important characteristic that consumers try to avoid in meat. A number of proteolytic enzymes derived from plant sources have been used for meat

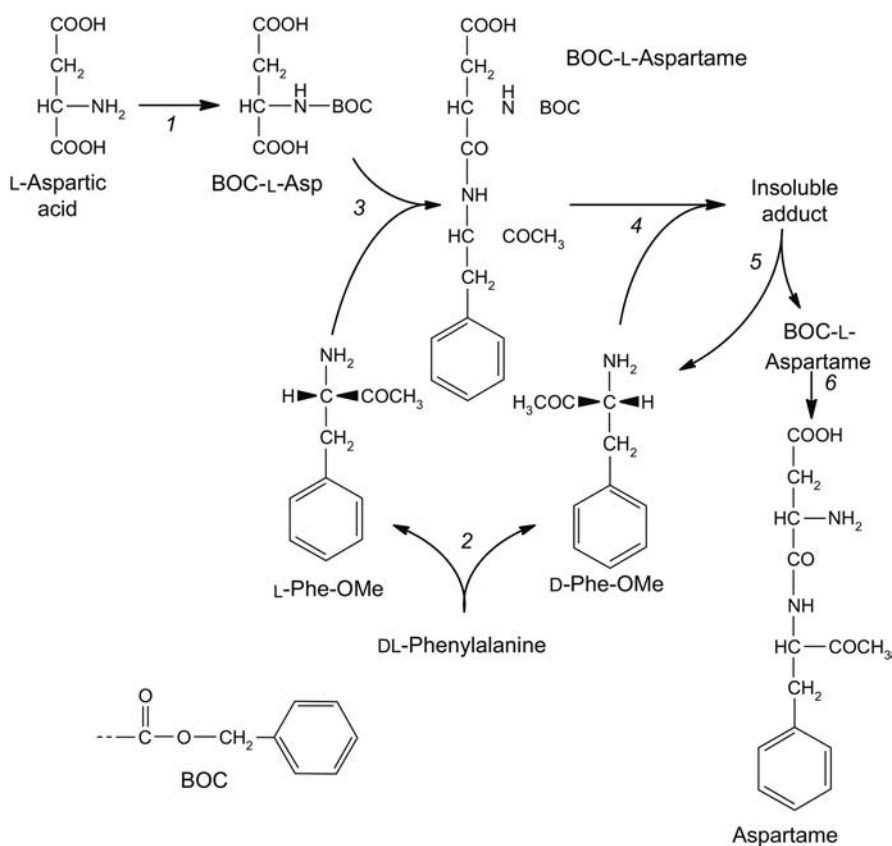


Figure 12.4 Industrial synthesis of aspartame. In preliminary reactions, L-aspartic acid is converted to *N*-benzyloxycarbonyl-L-aspartic acid (BOC-L-asp) to block its amino group (1), and DL-phenylalanine is esterified with methanol (2). The reaction (3) between BOC-L-asp and L-phenylalanine methyl ester is catalysed by immobilised thermolysin. The other product of reaction 2, D-phenylalanine methyl ester, immediately forms (4) an insoluble adduct with the BOC-L-aspartame, which precipitates out and can be recovered. A change in pH releases (5) the BOC-L-aspartame from the adduct and, after chemical removal (6) of the blocking reagent, aspartame, is obtained.

tenderisation. Papain, from papaya (*Carica papaya*) latex, bromelain (EC 3.4.22.32) from pineapple (*Ananas comosus*) and ficin (EC 3.4.22.3) from fig tree (*Ficus carica*) latex are well known, but, of the three, only papain has been used extensively on a commercial basis. Papain has a broad substrate specificity that includes the muscle proteins actin and myosin. However, it will not attack collagen, the connective tissue that makes the greatest contribution to meat toughness, in its native state, *i.e.* in raw meat. Before the papain can gain access to collagen peptide bonds, it first must be denatured by heat, loosening the individual polypeptide chains from the tight tropocollagen triple helix (see Figure 5.17). Little of the enzyme, whether applied as a dry powder or in solution, will penetrate below the surface of a piece of raw meat. For this reason, most suggested applications concern meat products such as burgers and sausages, where the enzyme can be included with the salt and other flavourings when the raw meat is minced or chopped. As papain is unusually heat-stable and active up to temperatures approaching 90 °C, some collagen breakdown can be expected when the product is cooked. The accompanying breakdown of actin and myosin will have little or no effect on the toughness of the product, but the release of short polypeptide fragments may contribute water-binding and enhance the moistness of the product.

The presence of trace amounts of the protease bromelain in raw pineapple flesh may have an unexpected effect. It is not uncommon (in the UK) to serve dessert jellies (“jello” in the USA) containing pieces of fruit at children’s parties. However, using pieces of raw, rather than canned, pineapple may have the unexpected effect of liquefying the jelly as bromelain diffuses out of the fruit.

12.3.2.4 Transglutaminase in Fish Product Manufacture

Over two million tons of fish are processed annually into the product for human consumption known by the Japanese name *surimi*.^{xi} The flesh of a wide range of both marine and freshwater fish species is used, as well as crustaceans, such as lobster and crab. Lobster and crab meat is particularly important as a flavouring in the manufacture of so-called “crab sticks”. The lean flesh is minced or pulverised, washed to remove unwanted “fishy” flavours and then mixed with a variety of other ingredients. These can include egg white, starch or soy protein to enhance texture, vegetable oil and flavourings, such as salt and monosodium glutamate. The final glutinous paste is extruded or moulded as required and then cooked. In the traditional product, the firm texture is attributed to the protein myosin (see Chapter 5, section 5.5.4), which leaches out of the muscle tissue and then cross-links as it is denatured by the heat of cooking.

Fish muscle naturally contains low levels of the enzyme transglutaminase, which can contribute to the setting process. Transglutaminase catalyses the formation of cross-links between the side-chains of the amino acids glutamate and lysine in the polypeptide chains of muscle proteins such as myosin (Figure 12.5). In recent years it has become routine to supplement the natural transglutaminase activity of the fish with the transglutaminase obtained from bacteria. Enzyme preparations containing the enzyme from various species of the bacterial genus *Streptoverticillium* are routinely

^{xi}In some parts of the Far East, *surimi* products based on meat such as beef, pork or poultry are popular.

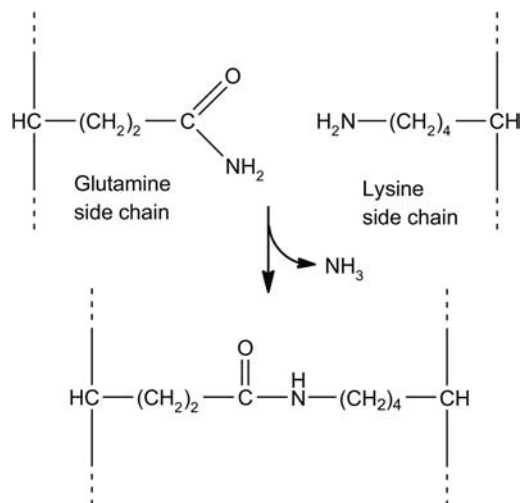


Figure 12.5 Formation cross-links between proteins catalysed by transglutaminase.

used as “texture enhancers”. This enzyme has the virtue of being stable up to 50 °C and is therefore able to catalyse cross-linking between the proteins that leak out of the muscle tissue at the onset of cooking.

12.3.2.5 Lysozyme in Cheese-making

This enzyme shares with the lactoperoxidase described earlier (see section 12.2.2) an antibacterial role in certain food processing operations. Lysozyme, also known as muramidase, has been well known to biochemists for many years,^{xiii} although its application in food processing dates from the 1990s. It occurs widely in animal secretions, notably milk, saliva and tears, and also in egg albumin, and in all these situations its biological role is antibacterial. It catalyses the hydrolysis of the peptidoglycan component of the cell walls of gram-positive genera of bacteria such as *Bacillus*, *Streptococcus* and *Clostridium* (Figure 12.6), causing the death of these potentially dangerous bacteria. Gram-negative bacteria, such as *Salmonella* spp., are not usually susceptible to lysozyme as they have a protective layer of lipopolysaccharide outside the peptidoglycan layer of their cell wall.

Lysozyme, isolated from egg albumin, is now used to control a defect, known as “late blowing” that can occur in a number of traditional types of hard and semi-hard cheeses manufactured in Europe, notably Gouda (the Netherlands), Grand Padano (Italy), Emmental (Switzerland) and Danbo (Denmark). Anaerobic bacteria of the species *Clostridium tyrobutyricum* in raw milk produces gas in the later stages of cheese-making that leads to irregular holes in the cheese. Traditionally, the growth of clostridia was inhibited by adding potassium nitrate to the raw milk, but this practice is now discouraged (see p. 364) and lysozyme is added instead. This kills vegetative cells and prevents the

^{xiii}In 1965, lysozyme was the first enzyme to have its three-dimensional molecular structure revealed in detail by X-ray crystallography.

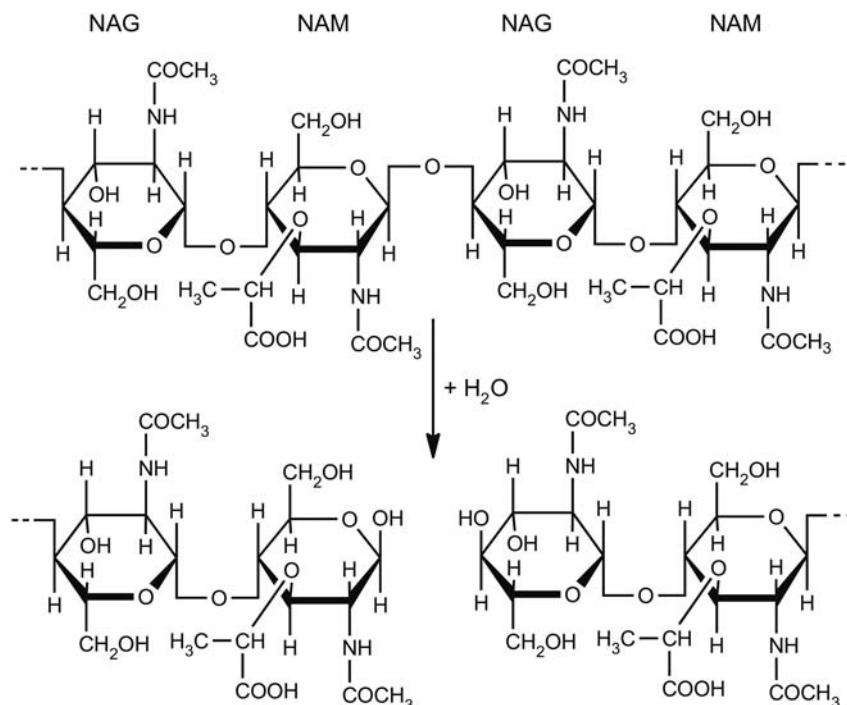


Figure 12.6 Hydrolysis of the cell wall peptidoglycan of bacteria by the enzyme lysozyme. The chains of alternating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) are cross-linked by short peptides attached to the NAM residues to form a multi-layered network.

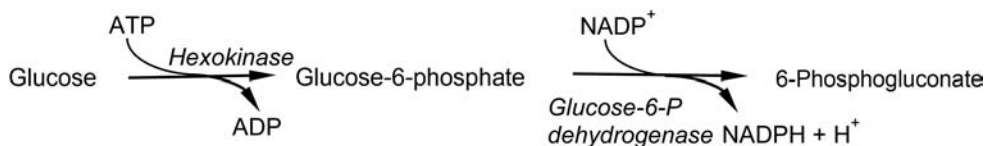
germination of clostridial spores. Fortunately, the lactobacilli essential to cheese-making are less sensitive to lysozyme than clostridia, but careful control of the level of lysozyme used is essential. European regulations also permit the use of lysozyme as an antibacterial agent in wine-making.

12.4 Food Analysis

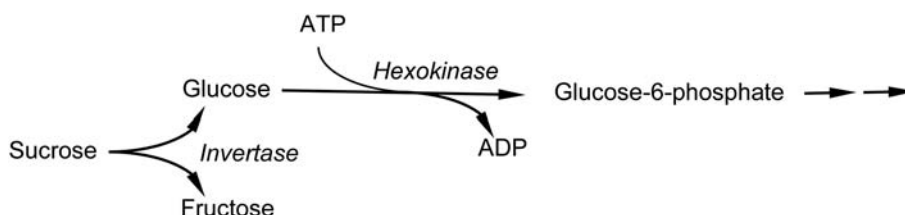
The details of food analysis techniques are outside the scope of this book, but it is appropriate to give readers, especially those with biochemical leanings, a brief insight into the application of enzymes in food analysis. Earlier in this chapter, the remarkable degrees of specificity that enzymes can show for different substrates was described and this is often exploited by food analysts. The following brief account of some enzyme-based methods used for the determination of particular food sugars should provide some insight into the possibilities.

In these techniques, small samples (often as little as 0.2 mL) of aqueous extracts of food materials are added to mixtures of the appropriate enzymes, buffer solutions and other reagents, often supplied commercially as complete kits. The resulting change in the absorption of ultraviolet or visible light is measured in a spectrophotometer. For the determination of glucose in the presence of other sugars, the reaction requires two

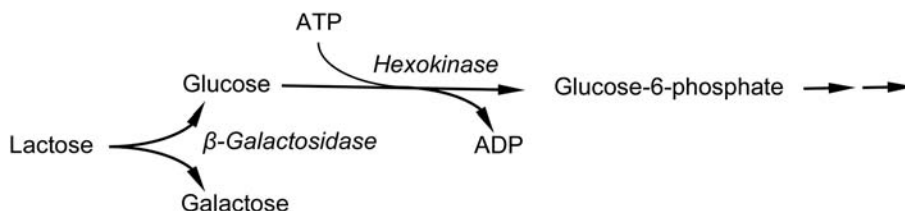
enzymes, hexokinase (*HK*) and glucose-6-phosphate dehydrogenase (*G6PDH*), and the reactants, ATP (adenosine triphosphate, see p. 202) and NADP^+ (nicotinamide adenine dinucleotide phosphate, see p. 368). NADPH, like NADH, has a very high molar extinction coefficient at 340 nm so that the change in absorbance at this wavelength can provide a sensitive and accurate measure of the glucose concentration:



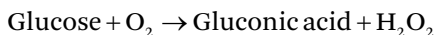
Other enzymes can be added to this basic system to determine other sugars. For example, a comparison of the change in absorbance with and without the addition of the enzyme invertase will provide a measure of the sucrose content of a sample in the presence of other sugars:



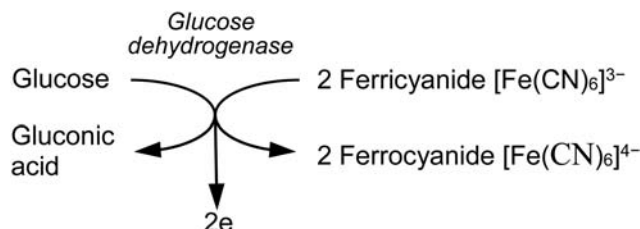
The lactose content of a food extract can similarly be achieved by the use of β -galactosidase, which catalyses the hydrolysis of lactose to glucose plus galactose:



Enzymes are also the basis of the automated devices that are widely used by patients with diabetes to determine the glucose concentration in their blood. The original devices used test strips containing the enzyme glucose oxidase, which catalyses the oxidation of glucose to gluconic acid.



The test strips also contained a benzidine derivative that reacted with hydrogen peroxide to give a blue-coloured product. The intensity of the colour that developed in a defined period provided a reasonably precise measure of the glucose content of the sample. The glucose meters in use today utilize test strips with a capillary that draws a small sample of blood (about 2 μL) onto an electrode coated with glucose dehydrogenase. The enzyme catalyses the oxidation of the glucose, transferring electrons to ferrocyanide, which, in turn, transfers the electrons to the electrode. The current then gives an accurate measurement of the glucose concentration in the blood sample.



12.5 Regulation and Legislation

The regulation of enzyme use in food processing is not a high priority for regulatory authorities around the world for a number of reasons.

- (i) Most enzymes used in food processing are inactivated by the subsequent heating stages of processing and are therefore undetectable in the final product.
- (ii) Having been denatured during processing and/or cooking, there is no reason to suspect that an added enzyme is likely to have any adverse effect on consumers.
- (iii) Many of the enzymes used – for example, in brewing – have a very long history of use without adverse effects on consumers.
- (iv) Many of the enzymes used are extracted from materials that are themselves widely acceptable to consumers as wholesome food ingredients, or are derived from the culture of microorganisms that are similarly recognised as safe.

This is reflected in the diversity of approaches to regulation and legislation that have been adopted. At one extreme of the legislative spectrum, the authorities in Canada (in 2014) included a considerable number of enzymes in the list of permitted food additives, implying that other unlisted enzymes were not permitted. At the other extreme, several countries, *e.g.* Japan and Russia, do not name any particular enzymes in their food legislation. In Europe (*i.e.* the EU and also the UK), only two enzymes are currently treated as food additives (invertase E1103 and lysozyme E1105), although other unspecified enzymes are allowed, subject to the more general regulations regarding food safety. In 2023 there was no formalised list of food enzymes authorised for use in the EU or UK. An additive enzyme is allowed if it satisfies the following conditions: (a) It does not pose a health concern to the consumer, (b) there is a technological need for it, and (c) its use does not mislead the consumer.

Further Reading

Books

Enzymes in Food Technology, Improvements and Innovations, ed. M. Kuddus, Springer Singapore, 2018.

Enzymes in Food Biotechnology Production, Applications, and Future Prospects, ed. M. Kuddus, Academic Press, 2018.

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13 Water

The final chapter of this book is devoted to the most abundant, and most frequently overlooked, of all the components of food: water. Although we are quick to recognise our biological need for drinking water, we tend to overlook the vast quantities of water that our solid foods contain. Table 13.1 lists the water content of a range of foodstuffs and a few drinks; it is not difficult to see from these data the contribution that solid foods can make to one's total water intake.

13.1 Water Structure

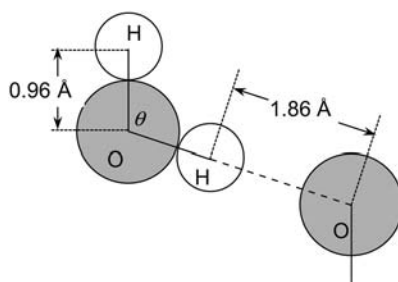
The chemical structure of water, H_2O , seems at first sight exceedingly simple, but all is not as it seems. The first point is that water cannot be automatically regarded as a single pure compound. Present in all natural water samples are a large number of variants caused by the natural occurrence of isotopes, such as ^{17}O , ^{18}O , ^2H and ^3H . Fortunately, for food chemists, their quantitative and qualitative contributions are small enough to be ignored. Furthermore, the ionisation of water to hydroxyl (OH^-) and hydrogen ions (as H_3O^+), while obviously important to the behaviour of other ionising molecules, does not change the composition of water very far from 100% H_2O , except at extremes of pH irrelevant to food materials.

The problems arise from the structure and behaviour of H_2O itself. Figure 13.1 shows the dimensions of the water molecule. If one visualises the oxygen atom being at the centre of a tetrahedron, the σ -bonds point towards two of its corners. The orbitals of the non-bonding electron pairs of the oxygen atom point towards the other two corners. As a result of the hydrogen ions moving between molecules, individual H_2O entities have an average lifetime of only about a millisecond.

By comparison with similar molecules involving neighbouring atoms in the Periodic Table (*e.g.* H_2S), one would expect water to melt at about $-100\text{ }^\circ\text{C}$ and boil at about $-50\text{ }^\circ\text{C}$. As these are clearly not the temperatures observed, we must conclude that, in both the liquid and solid states, water molecules in some way associate together so that their effective molecular weight is, at least as far as some aspects of their behaviour are

Table 13.1 Typical water content of food and drinks.

Food	Water (% w/w)	Food	Water (% w/w)
Lettuce, tomatoes	95	Dried fruit (currants, sultanas, <i>etc.</i>)	18
Cabbage, broccoli	92	Butter, margarine	16
Carrots, potatoes	90	Low-fat spread	50
Citrus fruit	87	Wheat flour	12
Apples, cherries	85	Dried pasta	12
Raw fish, <i>e.g.</i> cod	84	Milk powder	4
Raw poultry	72		
Raw lean meat	60	<i>Drink</i>	<i>Water (% v/v)</i>
Egg white (yolk)	88 (51)	Beer	90
Cheese	37	Fruit juices	87
White bread	35	Milk	87
Salami	30	Scotch whisky	60
Jam (and other preserves)	28		
Honey	20		

**Figure 13.1** Dimensions of the water molecule and its hydrogen bonds ($1 \text{ \AA} = 0.1 \text{ nm}$). The angle θ formed by the H-O-H σ -bonds depends on the state of the water:

in the gaseous state, $\theta = 104^\circ 52'$;

in the liquid state, $\theta = 105^\circ 0'$;

in the solid state (ice) $\theta = 109^\circ 47'$;

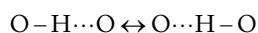
in a perfect tetrahedron $\theta = 109^\circ 28'$.

The other preferred angles are:

O-H...O ffi 180° (*i.e.* a straight line);

H...O...H and H...O-H ffi 109° .

concerned, much higher. The large forces of association between water molecules can be traced to the polarised nature of the O-H bond (40% partial ionic character) and the V-shape of the molecule. These facilitate hydrogen bonding between the oxygen atoms, which carry partial negative charges, and the corresponding positively charged hydrogen atoms of two other water molecules. It is this ability of water molecules to form links with four others that allows the formation of three-dimensional structures. An extended three-dimensional lattice occurs in ice (Figure 13.2). Arrays of oxygen atoms are arranged in layers of chair structures resembling those of pyranose sugars. The links between the layers create rings in the boat conformation. There is continual oscillation of the hydrogen atoms between their two possible positions, bound covalently to neighbouring oxygen atoms:



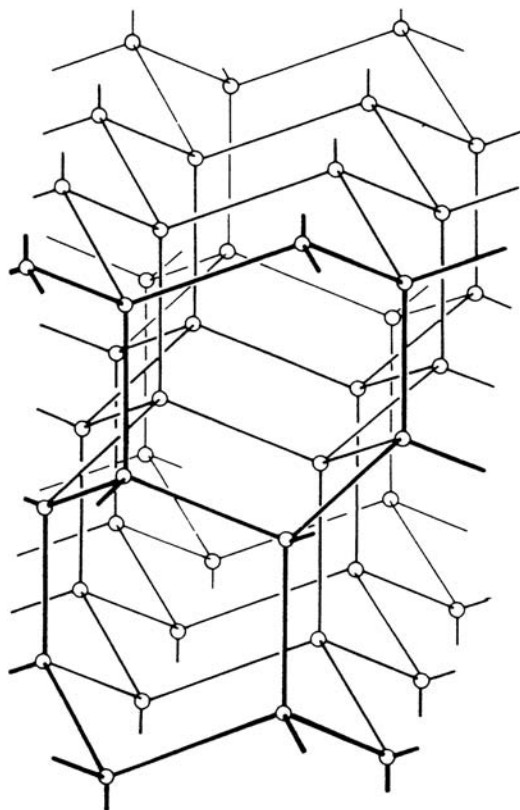


Figure 13.2 Lattice structure of ice. For clarity, only the positions of the oxygen atoms at the junctions of the tetrahedrally arranged bonds are shown.

The structure of ice is by no means static. Even at the lowest temperatures typically used to preserve food (about -20°C), the hydrogen atoms oscillate as shown in Figure 13.2 and a considerable fraction of the water molecules will not be perfectly integrated into the lattice structure. There is plenty of evidence to show that water molecules can diffuse slowly through ice. The OH^- and H_3O^+ ions may also be joined by entrapped solute molecules to disrupt the lattice. As the temperature is increased, the proportion of hydroxyl groups not involved in hydrogen bonding near the ice's surface slowly increases. When liquid at 0°C , water has $>90\%$ of its hydroxyl groups still involved in hydrogen bonding. This figure decreases to only a little below 80% when the normal boiling point is reached. There is considerable evidence to suggest that the hydroxyl groups not involved in hydrogen bonding are not evenly, nor randomly, distributed. Liquid water appears instead to consist of irregular clusters or aggregates of molecules arranged in unique lattice structures. The surfaces of these clusters are marked by the orientation defects caused by non-hydrogen-bonded hydroxyl groups. At room temperature, the average number of molecules in a cluster is estimated to be several hundred.

The structure of the liquid water clusters has attracted considerable attention in recent years. Although attractive, the idea that the clusters have the same lattice structure as ice seems unlikely. It is now generally accepted that liquid water consists of two types of dynamically coexisting and exchanging clusters. One type is a low-density,

three-dimensional, fully hydrogen-bonded cooperative cluster. These clusters contain many rings of water molecules, such as hexamers in both chair and boat forms and more or less planar pentamers. The other type of water cluster consists mostly of chains of water molecules that fold into higher density structures with many closely spaced water molecules missing their full complement of hydrogen bonds. The H–O–H angle in a planar five-membered ring is 108° , even closer to the supposed ideal (*i.e.* in the gaseous state) of 104.52° than that in ice, which is 109.47° . Figure 13.3 shows 14 water molecules linked together forming, in the example shown, two planar pentamers and three hexamers in the boat conformation.

It should never be overlooked that the cluster structure of liquid water is essentially dynamic. The lifetime of an individual hydrogen bond is measured in picoseconds (one picosecond = 10^{-12} seconds) because hydrogen atoms oscillate from one oxygen atom to another. Studies of the dielectric relaxation time suggest that a molecule of liquid water at 0°C changes its orientation with respect to its neighbours about 10^{11} times per second. In spite of these apparently brief periods of continuous stability for individual molecules, the lifetimes of individual clusters are much longer, up to 10^{-8} seconds. This is the result of the cooperativity between nearby hydrogen bonds in maintaining the cluster structure. Although the bonds holding one molecule in position may be broken, the structure as a whole will still be maintained by the bonding between surrounding molecules.

Although the details of the cluster structure of water are unresolved, there is general agreement that two types of clusters are always present in the liquid state. One

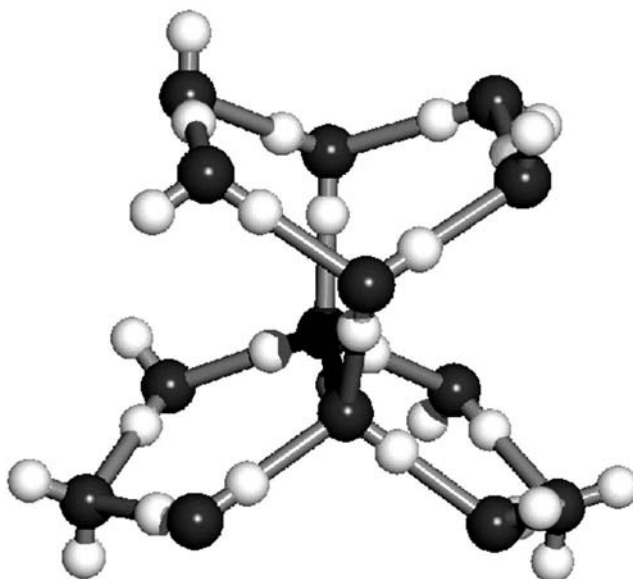


Figure 13.3 Clusters of hydrogen-bonded water molecules containing two pentamers and three hexamers in a boat conformation. Oxygen and hydrogen atoms are represented by black and white spheres, respectively. No attempt has been made to discriminate between covalent and hydrogen bonds, and the location of the hydrogen atoms closer to one or the other of the two oxygen atoms is arbitrary. (Based on a computer-generated drawing kindly provided by Professor M. F. Chaplin.)

type of cluster, typified by that just described, is referred to as expanded and low density, being very open and offering the possibility of small non-hydrogen-bonded entities penetrating into its pores. The other type of cluster is a collapsed, higher density structure in which the rigid geometry imposed by the hydrogen bonds is, to some extent, lost because they weaken at higher temperatures and other forces draw the non-hydrogen-bonded water molecules closer together. The six- and five-membered rings become distorted and broken, with values of θ closer to 90° .

As the temperature decreases, the proportion of water in the expanded state increases and the proportion in the collapsed state decreases. It is the large difference between the electronegativity of the oxygen atom and the electropositivity of each hydrogen atom that promotes the formation of hydrogen bonds between water molecules. This typically leads to a total of four molecules being associated with each individual water molecule (see Figures 13.2 and 13.3). However, a total of five water molecules can be attached (Figure 13.4). Also, a fifth non-hydrogen-bonded water molecule may approach almost as closely within the collapsed clusters. The average number of these nearest neighbours, referred to as the coordination number, increases from 4.0 in ice to 4.4 in water at 1.5°C and 4.9 at 83°C , but reduces again to about four in supercooled water below 0°C . As would be expected, temperature also affects the average distance between neighbouring water molecules. In ice at 0°C , and in water at 1.5 and 83°C , the $\text{O} \leftrightarrow \text{O}$ distances are 2.76, 2.9 and $3.05 \text{ \AA}$, respectively.

There are several significant consequences of this behaviour that influence the properties of food materials, but one of the most obvious is the change in density. Liquid water has a maximum density at 3.98°C . The difference in density between ice at 0°C (0.9167 g cm^{-3}) and liquid water at the same temperature (0.9998 g cm^{-3}) is readily accounted for by the more compact packing shown by the increase in coordination number. Up to 3.98°C the increase in coordination number is the dominating influence

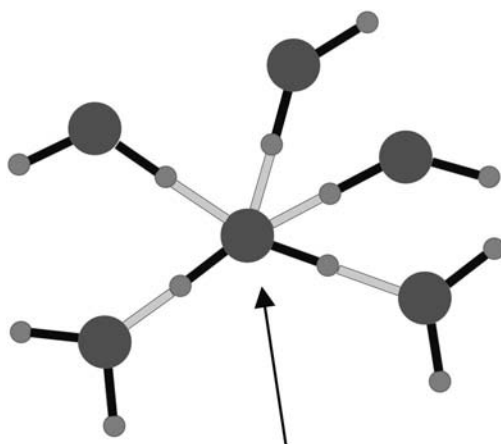


Figure 13.4 A simplified representation of a five-coordinated water molecule. No attempt has been made to show the three-dimensional relationship of the five-coordinated molecule (arrowed) to the others. The large and small circles represent oxygen and hydrogen atoms, respectively; covalent bonds are shown in black and the hydrogen bonds are shaded grey.

on the density, but above this temperature the nearest neighbour distance takes over. These observations correctly predict that water will increase in volume by 9% as it freezes. This change in density is, of course, of profound significance to the evolution of life in aquatic environments. In winter, ice floating on the surface of ponds *etc.*, tends to insulate deeper water and reduce the likelihood of the freezing and death of fish, *etc.* Food scientists have to be content with its more mundane influences – for example, on the design of moulds for ice lollies. The expansion taking place on freezing is also partially responsible for the destructive effects on the structure of soft fruit, such as strawberries, caused by freezing and thawing.

Other anomalous properties of liquid water are caused by changes in the proportion of the expanded and collapsed clusters between hot and cold water. Anomalous behaviour more accentuated at low temperatures when the properties of supercooled water often diverge from those of ice. In particular, several properties of water change at about 50 °C, just above the body temperature of mammals and above which many proteins denature. As (supercooled) cold liquid water is heated, individual molecules shrink and the bulk volume of water decreases, becoming less easy to compress. Atmospheric gases become less soluble. It is easier to heat the water and it conducts heat better. By contrast, warm liquid water expands as it is heated and becomes easier to compress. Atmospheric gases become more soluble in very hot water. It is harder to heat the water and it is a poorer conductor of heat. Individual molecules expand with increasing pressure. Molecules move faster in cold water, but slower in hot water. Hot water freezes faster than cold water. Ice melts when compressed; by contrast, liquid water freezes when compressed at very high pressures.

The interrelationships between water in its liquid, solid and gaseous states are summarised in the phase diagram shown in Figure 13.5. The diagram shows that, if the pressure is low enough, ice (*i.e.* water in the solid state) can change directly into a gaseous (vapour) state, *i.e.* sublime. This is the basis of the technique of food dehydration known as freeze drying. Delicate food extracts – for example, in the manufacture of instant coffee – are first frozen and then subjected to a high vacuum. The latent heat of sublimation of ice is 2837 J g^{-1} at -20°C . This high figure means that when the vacuum is first drawn on the frozen material, the sublimation of water abstracts so much heat that the temperature decreases to the point at which water is no longer removed at a satisfactory rate. In the usual commercial process, known as accelerated freeze drying (AFD), this is counteracted by applying radiant heat to the frozen material until almost all the water has been removed. In spite of this heating, the temperature remains well below 0°C throughout the process.

13.2 Interactions of Water With Food Components

The interactions between water and other food components are crucial to many of the properties of food systems. Interactions at the molecular level will be considered first, with particular attention to carbohydrates, lipids and proteins.

Some features of the interaction of sugars with water were discussed in Chapter 2. There it was pointed out that, in aqueous solutions, some water molecules are fairly

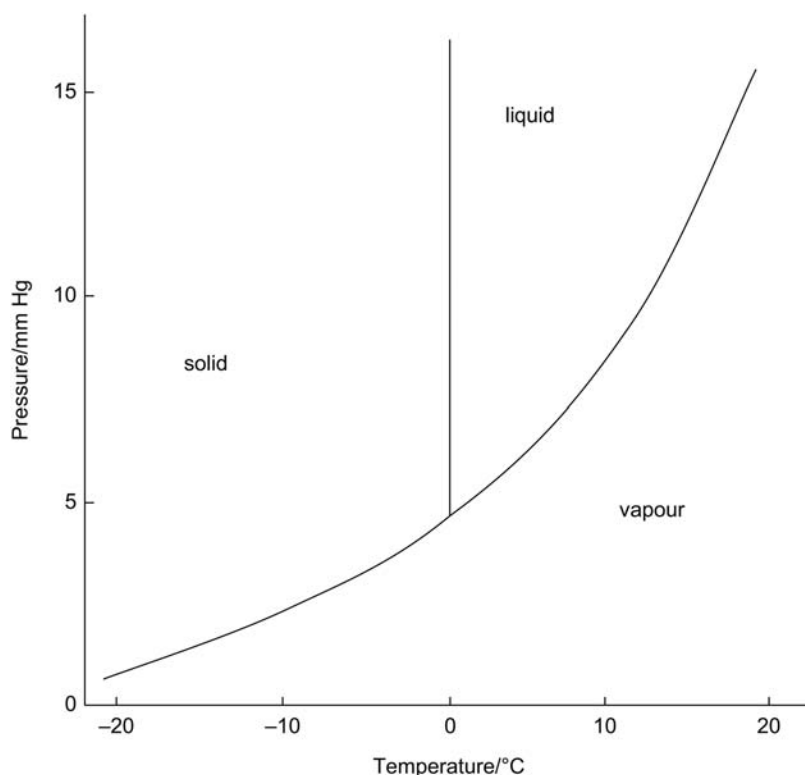


Figure 13.5 Phase diagram for water. At least 20 different ice crystal structures have been found to occur at particular combinations of pressure and temperature, but because the pressures required for most of these are very high, and the others are unstable or only occur at extremely low temperatures, they are irrelevant to food studies and have been ignored.

tightly bound to sugar molecules. The value of 3.7 for the number of water molecules bound by glucose is accounted for in the structural diagram in Figure 13.6. The distance between the pairs of hydroxyl oxygen atoms on the same face of the pyranose ring of β -D-glucose (*i.e.* those on carbons 1 and 3 and those on carbons 2 and 4) is 4.86 Å. This is remarkably close to the distance of 4.9 Å between the oxygen atoms in the same plane of the water lattice. Not only are the dimensions therefore correct, but for equatorially positioned hydroxyl groups, the geometry is also correct. The figure of 3.7 is close to the average number of equatorial hydroxyl groups of D-glucose in an aqueous solution (4 on the more abundant β -anomer and 3 on the α -anomer).

It has been suggested that the tendency of glucose to retard the formation of ice crystals in frozen dessert products is a result of this close fit with liquid water, but not with ice. Neighbouring oxygen atoms are 4.5 Å. Apart in ice at normal pressures. The anti-freeze properties of substances such as glycerol, $\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CH}_2\text{OH}$, and ethylene glycol, $(\text{CH}_2\text{OH})_2$, which have α -glycol oxygen atoms capable of similar orientation, may have a similar basis.

In solution, isolated glucose molecules will clearly have a strong stabilising effect on the clusters of water molecules around them and it is tempting to extend this concept

to the properties of polysaccharide-based systems. The discussions of gel structures in Chapter 3 should have left the reader in no doubt about the underlying strength of the polysaccharide–water interaction. For example, a good quality agar for microbiological use will give a firm gel at a polysaccharide concentration of 1.5% w/v. The average molecular weight of the monosaccharide units of agarose (the gel-forming and major component of agar, see Figure 3.9) is about 150. This implies that each monosaccharide unit is responsible for the immobilisation of at least 550 water molecules. The lifetime of an individual water cluster is far too brief to envisage the stability of the gel being the result of water clusters becoming ensnared in the polymer network. Capillary attraction is a far more plausible suggestion, with the gel having some of the characteristics of a wet sponge. As was suggested in Chapter 3, it will not be difficult to modify our perception of this model of gel structure to also account for the behaviour of gums, as well as the less soluble fibre polysaccharides.

It should come as little surprise that the interaction of water with non-polar molecules is a more aloof affair. It seems axiomatic that water and oil do not mix, but what is not so obvious is why this should be so. What is the advantage, in free energy terms, for two small oil droplets suspended in water to coalesce and become one larger droplet?

The answer lies in the collective strength of the hydrogen bonds of the water. If a single, small, non-polar molecule is suspended in water, it will have to occupy a cavity in a mass of associating water molecules. Numerous hydrogen bonds will need to be broken to form this cavity, requiring a large amount of free energy. The free energy of

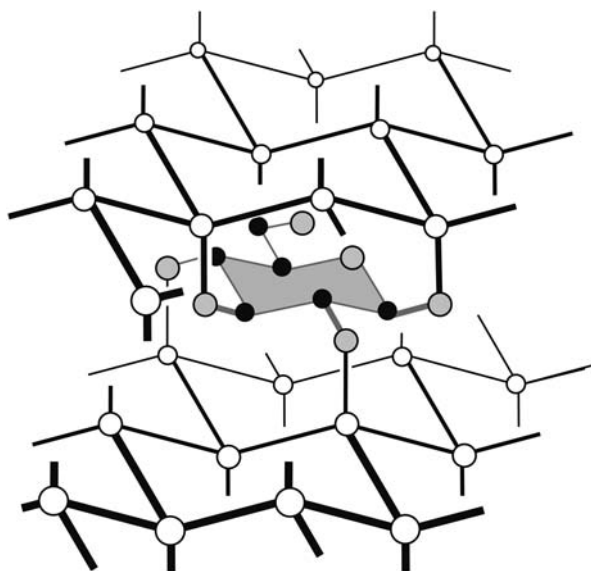


Figure 13.6 Insertion of a β -D-glucopyranose molecule into a water lattice. For clarity, only the oxygen and carbon atoms are shown. Open circles, water oxygen; grey circles, glucose oxygen; black circles, glucose carbon. Comparison with Figure 13.2 shows that at, at least in an ice-type lattice, the insertion of the glucose molecule has not disrupted the relationships of the water molecules in the layers above and below. The possibility of water molecules binding to the oxygen atom in the ring and the $-\text{CH}_2\text{OH}$ group of the glucose molecule remains unresolved.

the system is then partially reduced (by compensating changes in entropy) as the water molecules reorganise around the cavity to maximise the number of hydrogen bonds between them. There is evidence that this reorganisation can involve water structures based on strained five-membered rings, as suggested in Figure 13.7. At room temperature, the transfer of a small hydrocarbon molecule from an aqueous solution to a non-polar solvent is accompanied by a change in free energy, ΔG^0 , between -10 and -15 kJ mol^{-1} . The transfer of much larger molecules of typical food lipids can be expected to be driven by correspondingly larger and more negative changes in free energy.

When an isolated non-polar solute molecule is considered, it is clear that the larger the surface area of the solute molecule, the less soluble it will be in water; in other words, larger non-polar molecules tend to be more hydrophobic. However, when two non-polar molecules come to share the same cavity in the water, the total disruption of hydrogen bonding will be less than when they were apart and the free energy of the system will be lower. This principle readily extends to the coalescence of oil droplets suspended in water or floating on the surface.

The interaction of proteins with water has received a great deal of attention from biochemists over many years. However, this is mostly because of the importance of water to the structure and behaviour of proteins (*in vitro* as well as *in vivo*) rather than the influence of proteins on the behaviour of water. A protein molecule presents three kinds of surface group to its aqueous environment, depending on the nature of the amino acid side-chain: polar charged groups, such as those of glutamate ($-\text{CH}_2\text{CH}_2\text{COO}^-$) or lysine [$-(\text{CH}_2)_4\text{NH}_3^+$]; polar neutral groups, such as those of serine ($-\text{CH}_2\text{OH}$) or glutamic acid at low pH ($-\text{CH}_2\text{CH}_2\text{COOH}$); and non-polar groups, such as those of valine [$-\text{CH}(\text{CH}_3)_2$] or methionine ($-\text{CH}_2\text{CH}_2\text{SCH}_3$). For the reasons discussed here, the non-polar side-chains are usually buried in the interior of the protein and need not

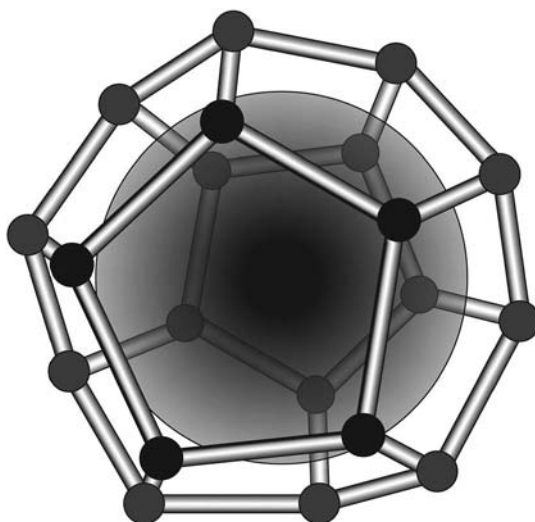


Figure 13.7 A possible arrangement of a single layer of water molecules around a hypothetical hydrophobic molecule, represented by the central grey sphere. As in Figures 13.2 and 13.5, only the oxygen molecules are shown here (as black circles).

concern us further. The pH value will obviously be a major influence on the properties of ionisable side-chains.

Studies using sophisticated physical methods have probed the water-binding properties of amino acid side-chains and have obtained results comparable with those for sugars. When ionised, the acidic side-chains, aspartate and glutamate, bind with about six molecules of water. On protonation, this figure decreases to two, a similar figure to that of the polar, but non-ionised, side-chains of asparagine, serine, *etc.* The positively charged side-chains of lysine and histidine each bind about four molecules of water. These numbers refer to the situation when the polypeptide chain is surrounded by an abundance of water. The high values for the charged side-chains probably result from the tendency of these to orientate multiple layers of polarised water molecules. The effect of pH on the water-binding capacity of amino acids is reflected in the behaviour of entire proteins, a phenomenon referred to in Chapter 5 in connection with the loss of water from fresh meat. A homogenised muscle preparation may double its water-binding capacity if the pH is lowered from 5.0 (approximately the mean isoelectric point of the muscle proteins) to 3.5.

As a general rule, only a small proportion of the polar groups of a native (*i.e.* not denatured) protein will be buried in the predominantly hydrophobic interior of the molecule and therefore unable to bind water. Thermal denaturation is assumed to lead to the unfolding of the polypeptide chain, which can cause some extra water-binding as the last few polar groups are exposed. The newly exposed hydrophobic regions of the polypeptide chain also show a limited water-binding capacity, partly due to the hydrophilic nature of the peptide bond regions of the backbone. Thus many proteins show a modest increase in water-binding on denaturation, from about 30 to 45% w/w. However, when denaturation leads to aggregation, as unfolded polypeptide chains become entangled around each other, there is sometimes a tendency for protein–water interactions to be usurped by protein–protein interactions, leading to an actual loss of water-binding capacity. The release of bound water when meat is roasted is a good example of this phenomenon.

The behaviour of protein solutions is markedly affected by the presence of low molecular weight ions, notably the anions and cations of inorganic salts. Up to a concentration of about 1 mol dm^{-3} , salts such as sodium or potassium chloride enhance the solubility of many proteins. Indeed, the old-fashioned division of proteins into albumins and globulins was based on the ability of albumins to dissolve in pure water, whereas globulins only dissolve in a dilute salt solution. The mechanism of this “salting-in” effect is straightforward. Charged groups on the surface of the protein attract and bind anions and cations more strongly than they do water. However, these ions will still bring with them ordered clusters of their own solvating water molecules, which will then function to maintain the protein molecule in solution.

The solubility-enhancing effect of increasing salt concentrations does not continue indefinitely. At concentrations higher than 1 or 2 mol dm^{-3} , many salts cause proteins to precipitate out of solution, a phenomenon known as “salting-out”. For biochemists, this is the basis of the time-honoured procedure for the fractionation of crude protein mixtures known as ammonium sulfate precipitation. The use of ammonium sulfate stems from its extreme solubility (1 L of water can dissolve 760 g of ammonium sulfate)

rather than from any special aspect of its interaction with proteins. The explanation is simple. As these salts reach high concentrations, the proportion of the total water that they bind starts to become significant. The ions of the salt compete with the protein for the water necessary for the protein to remain in solution and, as the salt concentration is increased, the different proteins precipitate out.

Salting-out only occurs at salt concentrations with little relevance to food systems, but salting-in is important in food processing. When pork is cured by the Wiltshire method (see Chapter 5), sodium and chloride ions are injected, as brine, into the muscle tissues. These bind to the muscle proteins, particularly the abundant actin and myosin of the myofibrils, and enhance their water-binding capacity. This gives the ham its attractive moistness, as well as incidentally increasing its weight. Many modern ham-type products, prepared for off-the-bone machine slicing for pre-packaged sale, are subjected to the additional process of “tumbling”, in which large pieces of ham are literally massaged for periods of up to half an hour in rotating vessels that closely resemble a concrete mixer. This has the effect of bringing dissolved myofibrillar proteins out of the confines of the cell structure so that they are able to give the moulded or pressed finished product a firmer texture based on the gels formed by these proteins.

In products such as luncheon meat and sausages, which, at least in the UK, include a major proportion of homogenised or macerated muscle tissue, the solubilisation of proteins by inorganic salts acquires special importance. Although water-binding is not unimportant in these products, the binding of fat is of great significance. The ability of the hydrophobic regions of proteins to bind fat molecules makes proteins excellent emulsifying agents. Getting myofibrillar proteins into solution and into action as emulsifiers is the task of added “emulsifying salts”. In addition to the ubiquitous sodium chloride, sodium and potassium phosphates and polyphosphates, citrates and tartrates also fulfil this role. The phosphates and polyphosphates were considered in greater detail in Chapter 11. We also find emulsifying salts in processed cheese, where they release casein molecules from their micelles to assist in binding the added fat.

13.3 Interactions of Water With Food Materials

When we consider the interaction between water and food materials as a whole, we must leave molecular niceties behind and look instead at the behaviour of water on a larger scale. Food scientists need to understand how water behaves because so many of the important properties of food are related to its presence, in particular the susceptibility of food to microbial growth. It is also clear, from data such as those in Table 13.1, that the water content of a foodstuff is not in itself the major influence on stability. What is relevant is the availability of the water to microorganisms, not its abundance. The concept of water activity is now universally adopted by food scientists to quantify the availability of water.

Water activity, a_w , is defined as follows:

$$a_w = p / p_0$$

where p is the partial pressure of water vapour enclosed above the sample (which can be solid or liquid) and p_0 is the partial pressure of water vapour above pure water at the same specified temperature. At equilibrium, which may take hours or even days to be achieved, there is a close relationship between the a_w of a food material and the equilibrium relative humidity (ERH) of the air enclosed above it:

$$a_w = \text{ERH} / 100$$

This relationship between ERH and a_w enables us to predict which foods will gain or lose water when exposed to air of a particular relative humidity.

Although the concept of water activity is sometimes regarded as lacking the full rigour of classical physical chemistry, it provides a good practical basis for describing the water relations of foodstuffs. Table 13.2 gives typical examples of water activity for a range of foods. An important feature of these data is the lack of correlation between water content and water activity, seen by comparison with Table 13.1.

The data in Table 13.3 illustrate the importance of water activity in controlling microbial growth. The halophilic (*i.e.* salt-loving) bacteria are those usually associated with marine environments, including the sea fish we eat. The xerophilic moulds and osmophilic yeasts are adapted to environments of low water activity. The cells of these specialised microorganisms contain high concentrations of particular solutes so that they do not lose water, by osmosis, to their surroundings. In bacteria, these protective solutes are usually the amino acids proline or glutamic acid and, in fungi, polyols, such as glycerol or ribitol. These fungi and yeasts can pose serious problems in the storage of some foods that are usually regarded as stable, notably dried fruit. Fortunately, they have not, so far, been associated with mycotoxin production, only the more obvious physical deterioration.

Table 13.2 Water activity of various foodstuffs.

Foodstuff	Typical water activity (a_w)
Fresh meat	0.98
Cheese	0.97
Preserves	0.88
Salami	0.83
Dried fruit	0.76
Honey	0.75
Dried pasta	0.50

Table 13.3 Minimum water activity for the growth of microorganisms.

Microorganism	Minimum water activity (a_w)
Normal bacteria	0.91
Normal yeasts	0.88
Normal moulds	0.80
Halophilic bacteria	0.75
Xerophilic moulds	0.65
Osmophilic yeasts	0.60

Microbial growth is not the only important phenomenon affected by water activity. The rates of both enzymic and non-enzymic reactions are also markedly affected. However, to gain a clearer understanding of the mechanisms of these influences, we need to examine the issue of water-binding.

13.4 Water-binding

The term “bound water” is often used in discussions of the properties of food materials, but it rarely means exactly the same thing to two different scientists. Linked to this term is another, “water-holding capacity”, which refers with similar vagueness to the maximum quantity of bound water a material is able to contain. Fennema has described a sequence of four categories for the water present in a solid food material to cover the range of possible definitions that might be applied to bound water. The percentage figures given for each category indicate its contribution to the total water in a typical high-moisture food, *i.e.* one with a water content of about 90% w/w.

- Vicinal water ($0.5 \pm 0.4\%$). This is water held at specific hydrophilic sites on molecules in the material, either by hydrogen bonding or by the attraction between charged groups and the water dipole. Vicinal water may give an uninterrupted monolayer surrounding a hydrophilic molecule. There are suggestions that it is organised into a lattice structure similar to those described earlier in this chapter. Vicinal water is regarded as non-freezable down to at least $-40\text{ }^{\circ}\text{C}$ and is unable to act as a solvent.
- Multilayer water ($3 \pm 2\%$). This is the water forming additional layers around the hydrophilic groups of the material. Water-solute hydrogen bonds can occur, but water-water bonds predominate. Most multilayer water does not freeze at $-40\text{ }^{\circ}\text{C}$ and the remainder still has a very low freezing point. Multilayer water does have limited solvent capacity.
- Entrapped water (up to about 96%). This water has properties similar to that in dilute salt solutions, with only a slight reduction in its freezing point and an almost normal solvent capability. However, it is prevented from flowing freely by the matrix of gel structures or in tissues where it is held by capillary attraction.
- Free water (up to about 96%). This has properties essentially similar to those of pure water. Although it will remain held within the structure of a food material as a consequence of capillary action, or enclosed in layers of fatty material, it does not require much pressure to squeeze it out.

Starting with a completely dry food material, one can visualise the gradual addition of water filling each of these categories in turn. The layer of vicinal water is considered to be complete at a_w values of about 0.25. The boundary between multilayer water and either entrapped or free water lies at an a_w value of about 0.8. This sequence of events is usually expressed in the form of a sorption isotherm, a plot of water content against water activity. Examples of these plots are shown in Figure 13.8.

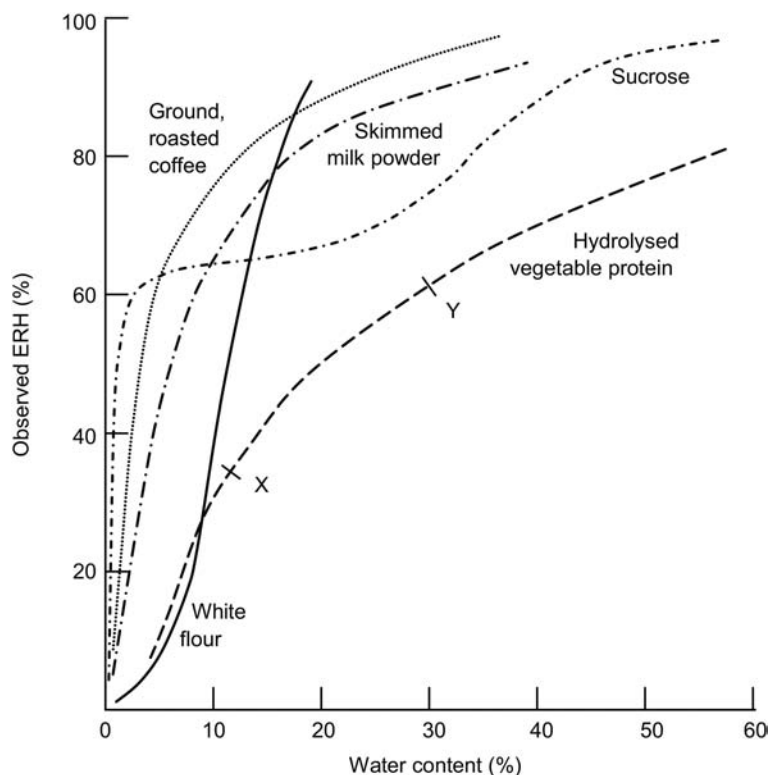


Figure 13.8 Sorption isotherms. These are representative curves for the food materials shown, at or near room temperature. The points marked X and Y on the hydrolysed vegetable protein isotherm mark the transition, first from a free-flowing powder to an increasingly sticky mass (at or near X) and then to a viscous solution (at or near Y).

It is tempting to offer speculative explanations for the differences between the shapes of these curves in terms of the molecular properties of the different materials present. However, the reader's guesses are unlikely to be any less reasonable than the author's, so none will be offered. The value of plots like these lies more in their practical application rather than in their theoretical interpretation. Whenever two or more ingredients that start with different a_w values are confined together in an airtight space, water will move between them, from the higher a_w ingredient to the lower until the a_w values are equal. Their biological, chemical and physical properties are thus liable to change in ways that may not have been expected unless their respective sorption isotherms had been checked beforehand. An obvious problem area is dehydrated instant meals, which may have pieces of meat, vegetables, *etc.*, all together inside the same compartment. To make matters even more complicated, the sorption isotherm is just that, an isotherm, by definition recorded at one particular temperature. Low-temperature storage of a product could well change the relationships that had previously been established at the temperature of the production line.

Another complication is that, with many substances, the isotherm obtained as a material is dried (the desorption isotherm) differs from the isotherm obtained as the

material regains water (the resorption isotherm). As a general rule, the a_w at a given water content will be higher when a material gains water than when it loses water. There are numerous explanations for this, including the time dependency of the equilibration processes, capillary collapse and swelling phenomena. It seems probable that all will be found to be involved, to some degree or other.

The growth of microorganisms is by no means the only process affected by the water activity of a foodstuff. A number of important enzyme-catalysed reactions and chemical reactions are also affected. For example, the rate of enzymic hydrolysis of lipids in foods such as meat becomes negligible at a_w values <0.4 . Chemical reactions can show other effects. The rate of the Maillard reaction (see Chapter 2) increases sharply as the water activity decreases to about 0.8 and only decreases again below about 0.3. The usual explanation for this is that enhancement of the reaction rate initially occurs when the removal of water effectively concentrates the reactants. The subsequent fall is the result of the loss of reactant mobility in the residual multilayer water. This loss of reactant mobility appears to be the dominant influence on reaction rates at low water activities. An interesting exception to this general trend is the autoxidation of unsaturated lipids in food materials (see Chapter 4). Although this process shows a steady decrease in rate down to an a_w of about 0.4, it then increases again to about 10% of its maximum rate at a_w values approaching zero. It is assumed that the removal of vicinal water from hydrophilic sites in the material actually exposes vulnerable lipid molecules to oxygen.

Knowledge of the sorption isotherm of a material thus enables food processors to identify the water content at which the effect of degradative reactions is minimised, usually the point at which multilayer water has been removed, but the monolayer of vicinal water is still intact.

13.5 Water Determination

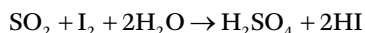
It will now be evident that there are two distinct measurements of the water in food materials. The first is to determine the amount of water the material actually contains. The primary motive of this is often to establish what proportion of the food material is available for more valuable nutrients. The second is to determine the activity of the water in the material to obtain useful information about other properties and the behaviour of the material. In many cases, the water content is sufficient to predict much about its behaviour. For example, we knew how far the water content of cereal grains should be reduced to prevent mould growth or sprouting long before the concept of water activity was introduced.

There are three basic methods for the determination of total water content of a food material: gravimetric, volumetric and chemical. Gravimetric methods simply depend on the difference in weight of a sample before and after drying. The actual drying method must be chosen with care. High temperatures will obviously hasten water loss, but at the risk of causing the decomposition of some food components, such as sugars; other volatiles such as short-chain fatty acids may also be driven off. Before drying, it is often necessary to reduce the particle size of the material. Cereal grains need to be milled and wet materials, such as jam, must be dispersed onto a particulate matrix

(e.g. sand). Without these steps, the drying period can be inordinately long. Whichever drying method is adopted, it is essential that it is carried out to constant weight, *i.e.* until the sample shows no further weight loss when drying is continued. For many purposes, especially where accounting for the final 1% of the moisture content is not critical, an ordinary laboratory fan oven is sufficient. For precise work, a vacuum oven is preferable. Using temperatures around 100 °C and pressures <50 mm Hg, the drying time for cereals can be brought to within 16 h. It must never be forgotten that, having been dried at elevated temperatures, the sample must be cooled in a dry environment such as a desiccator before being weighed.

Volumetric methods depend on distilling off and condensing any water present when the sample is subjected to reflux distillation with a water-immiscible solvent such as toluene.

The only chemical method for water determination in regular use is the Karl Fischer titration. This can be applied to most foodstuffs, apart from those with heterogeneous structures (such as fruit, vegetables and meat), which cannot be dispersed in the solvent. It is especially effective with heat-sensitive materials, those rich in volatiles and sugary materials in which the water is particularly tightly bound. The method depends on the reaction between sulfur dioxide and iodine in the presence of water, pyridine and methanol:



Although not shown in this equation, basic pyridine appears to form addition compounds with the acidic products, pulling the equilibrium to the right. All the reagents must be specially prepared to be totally free from water. The titration apparatus is designed to exclude atmospheric water vapour. The food sample is dispersed or dissolved in a reagent consisting of sulfur dioxide dissolved in a mixture of dry methanol and pyridine and then titrated with standardised iodine in methanol.

Although the range of materials to which they can be applied is limited, there are two additional instrumental methods of water determination that deserve a mention. One of these is near-infrared spectrophotometry, mentioned in Chapter 5 in connection with protein determination. Water has a number of absorbance bands in the near-infrared, of which the band at 1.93 µm is most often used. Comparison of the absorbance at this wavelength (or at 1.94 µm where interference is minimal) with that at 2.08 µm (outside the major absorbance bands) provides a sensitive and very rapid estimate of the water content of cereal grains, flour and similar materials. Low-resolution nuclear magnetic resonance spectrometry can also be used for the determination of water. Although the instrumentation is expensive and the theory of the method intimidating, this method is proving increasingly popular, especially in the cereal industry, because it is both rapid (one minute may suffice for an entire determination) and non-destructive. Even whole grains can be examined.

The determination of the water activity of food materials is less easy. All the methods available depend on the relationship between water activity and ERH and measure the tendency of the test material to gain or lose water when exposed to air at a specific temperature and fixed relative humidity. The usual technique is to place the sample in a small

Table 13.4 Water activity of saturated salt solutions at 25 °C.

Salt	a_w	Salt	a_w
Potassium dichromate	0.980	Potassium chloride	0.843
Potassium nitrate	0.925	Ammonium sulfate	0.790
Barium chloride	0.902	Sodium chloride	0.753
Sodium benzoate	0.880	Ammonium nitrate	0.618

open vessel alongside another containing vessel a solution of known a_w . Both vessels are placed together in a sealed container and kept at constant temperature for a few days. At intervals, the container is opened and the weights of the vessels compared to determine whether the sample is gaining or losing moisture. If a range of different standard solutions is available, then it is possible to arrive at the a_w value of the sample by interpolation. Table 13.4 shows the a_w values of several useful salt solutions. Other values may be obtained using standard solutions of concentrated sulfuric acid or glycerol.

The instruments marketed for the determination of a_w use a variation of this approach. The sample is allowed to equilibrate with a small known volume of air, the relative humidity of which can be monitored by a special sensor built into the sample container. This is often fitted with a small fan to speed up the measurements. Several different types of sensors are used. Most common is the dewpoint hygrometer. The temperature at which dew forms on a clean surface is directly related to the relative humidity of the air. The hygrometer has a mirror that is cooled until dew condenses, as detected by an optical sensor. Other instruments measure the relative humidity in the test chamber electrically, measuring the conductivity or capacitance of a hygroscopic polymer, such as polyamide.

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Appendix I

Nutritional Requirements and Dietary Sources

This book is not a textbook of nutrition. Extensive consideration of the amounts of different food components required as nutrients in a healthy diet is beyond its scope. Nevertheless, it is obviously valuable to be able to place the quantity, stability and availability of the nutrients discussed in this book within the context of what nutritionists would regard as desirable. Such data were at one time presented as Recommended Daily Amounts (RDAs). Unfortunately, RDA values were frequently misused, being interpreted as absolute values to be applied without qualification in decisions on the adequacy of individual diets. In 1991, the UK Government Committee on Medical Aspects of Food (COMA) introduced the concept of Dietary Reference Values (DRVs). For any given nutrient (*e.g.* a particular vitamin), and within a defined section of the population (*e.g.* boys aged 11–14 years), there are normally three DRVs:

- (i) **Estimated Average Requirement (EAR).** Statistically, approximately half the population will require less than the EAR daily and the other half more.ⁱ
- (ii) **Reference Nutrient Intake (RNI).** This is the daily nutrient intake that is enough, or more than enough, for almost all the population. This is not dissimilar from the old RDA and is set at two standard deviations above the EAR. This statistical terminology assumes that the spread of actual requirements among individual members of the population follows, as it usually does, regular statistical rules, *i.e.* it has a normal distribution, and with the implication that “almost all” actually means 97.5%.
- (iii) **Lower Reference Nutrient Intake (LRNI).** This is the daily nutrient intake that is enough, or more than enough, for only a “small proportion” of the population. This is arbitrarily set at two standard deviations below the EAR, so that “small proportion” actually means 2.5%.

The RNI and LRNI values for vitamins and minerals that are currently (*i.e.* since 1991) agreed by the Department of Health in the UK are shown in Table A1, together with the corresponding figures for vitamin and mineral consumption for the same age group in

ⁱReaders familiar with statistics will appreciate that the average (or mean) is not the same as the median, which would precisely divide the population into two numerically equal halves.

Table A1 Dietary Reference Values (DRVs) and daily consumption data for the intake of minerals and vitamins from food sources. The consumption data were obtained from a sample of 1126 adult men aged 19–64 years. The data for women in the same age group are similar. These data were derived from the UK National Diet and Nutrition Survey Rolling Programme (2008/2009–2011/12) published online in 2014 by Public Health England and the Food Standards Agency. No data for vitamin D is included in this table because the formation of this vitamin in the skin makes a significant, but variable, contribution to the levels of the vitamin in the tissues.

	LRNI for men aged 19–64 years	RNI for men aged 19–64 years	Average daily consumption by men aged 19–64 years
Thiamin	0.23 mg ^a	0.8 mg	1.59 mg
Niacin	4.4 mg ^a	13 mg	4.23 mg
Pyridoxine	11 mg ^b	1.2 mg	2.5 mg
Riboflavin	0.8 mg	1.1 mg	1.73 mg
Folic acid	100 µg	200 µg	287 µg
Cobalamin	1.0 µg	1.5 µg	5.7 µg
Ascorbic acid	10 mg	40 mg	84.3 mg
Vitamin A ^c	250 µg	600 µg	948 µg
Calcium	400 mg	700 mg	888 mg
Magnesium	190 mg	300 mg	283 mg
Potassium	2.0 g	3.5 g	3.04 g
Zinc	5.5 mg	9.5 mg	9.7 mg
Copper ^d		1.2 µg	1.4 mg
Iron	4.7 mg	8.7 mg	11.7 mg
Iodine	70 µg	140 µg	180 µg
Selenium	40 µg	75 µg	53 µg

^aPer 1000 kcal of energy from food.

^bPer 1 g of protein consumed.

^cAs retinol equivalents.

^dNo LRNI value has been set for copper.

the time period 2008–2012. At the time of writing (2020–2022), no significant change had been made to these values.ⁱⁱ

An important feature of this table is that the DRVs for three of the vitamins are also quoted in terms of the intake of other nutrients. This is a reflection of the close involvement of these vitamins in particular aspects of metabolism – thiamin and niacin with energy metabolism, and pyridoxine with protein metabolism. All three DRVs have not been established for some vitamins and minerals because the rarity of deficiency symptoms in the UK (and elsewhere) leads to the conclusion that even poor diets are able to supply adequate quantities. Another problem is that for some nutrients (particularly iron), the statistical distribution of requirements is not “normal” in the statistical sense, but skewed, which makes the over-rigorous application of these simple statistical concepts invalid.

In the case of pantothenic acid, the normal intake rate of 3–7 mg per day is reasonably assumed to be both safe and adequate. A similar conclusion is drawn for biotin intakes of 10–200 µg per day. Most people synthesise their own vitamin D, as discussed in Chapter 7, but for people confined indoors the RNI is set at 10 µg per day. The special needs of infants for vitamin D are reflected in RNI values of 8.5 µg per day for the first six months and 7 µg per day for the following 18 months. The requirement for vitamin E is highly dependent on the polyunsaturated fatty acid (PUFA) content of the diet, but the indications are that at a typical PUFA intake 3–4 mg per day is adequate. New born

ⁱⁱhttps://www.nutrition.org.uk/attachments/article/261/Nutrition%20Requirements_Revised%20August%202019.pdf.

infants are particularly prone to vitamin K deficiency and for them an RNI of 10 µg per day is suggested. For adults, a figure related to body weight (1 µg per day per kg) is recommended as adequate and safe.

Although the chapters on vitamins and minerals give information on the amounts of these nutrients in particular foods, to appreciate such data fully one needs to understand the contribution of different foods to the average diet. Figure A1 summarises data from the survey on the contribution of various classes of foodstuffs to the supply of vitamins and minerals in the UK diet.

The previous survey conducted in the UK, in 2000–2001, included items of information that have not been updated since, but do demonstrate the limitations of applying this type of data to individual people. Examples reported in 2000–2001 included the observations that:

cooked carrots supplied 30% of total carotene, but adult men obtained 26% of their vitamin A from liver. Beer and lager made a significant contribution to the B vitamin intake of adult men (e.g. riboflavin 7%, niacin 9%, pyridoxine 13% and folic acid 11%), but the corresponding figures for women are all ≤3%.

Further Reading

McCance and Widdowson's *The Composition of Foods, Seventh Summary Edition*, Royal Society of Chemistry, Cambridge, 2014.

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<https://www.efsa.europa.eu/en/topics/topic/dietary-reference-values> (accessed 2022).

National Academy of Sciences, *Dietary Reference Intakes for Thiamine, Riboflavin, Niacin, Vitamin B₆, Folate, Vitamin B₁₂, Pantothenic Acid, Biotin and Choline*, Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Washington DC, 1998.

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Food Standards Agency, *National Diet and Nutrition Survey*, HMSO, 2002–2003, vol. 1–3.

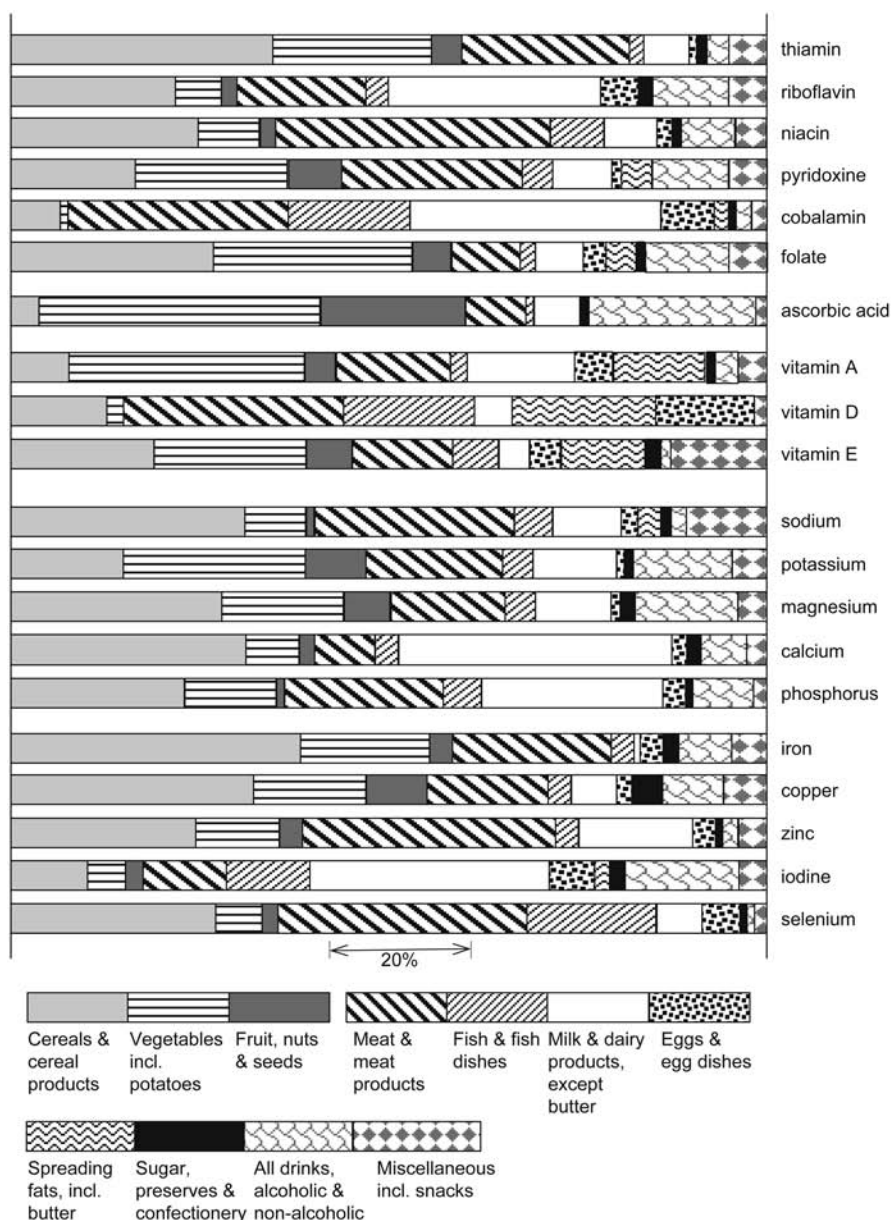


Figure A1 Percentage contribution of different foods to the vitamin and mineral content of the UK diet surveyed during the time period 2008–2012. The survey covered males and females in age groups from 4 to 65+ years, but the data shown here are restricted to the same group of 1126 adult men aged 19–64 years in Table A1. Full details of the survey, including data, extensive details of the foods that make up the broad classes in this figure, and commentary, were accessed in 2020 at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/594361/NDNS_Y1_to_4_UK_report_full_text_revised_February_2017.pdf.

Appendix II

Food Additive Regulations

The online sources of the regulations applying to the use of food additives referred to in Chapters 1, 5, 6, 8 and 10 are listed here. These sites were all last accessed in 2022.

European Union and UK

<https://www.food.gov.uk/business-guidance/approved-additives-and-e-numbers>

USA

<https://www.fda.gov/food/food-additives-petitions/food-additive-status-list>

Canada

<https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/lists-permitted.html>

Australia and New Zealand

<https://www.foodstandards.gov.au/consumer/additives/additiveoverview/Documents/Food%20additives%20-%20alphabetical%20May%202019.pdf>

Japan

https://www.jetro.go.jp/ext_images/en/reports/regulations/pdf/foodext2010e.pdf

South America, The Mercosur grouping of Argentina, Brazil, Paraguay and Uruguay

<http://www.assal.gov.ar/documentacion/aditivos-permitidos-mercosur.pdf>

Subject Index

Numerical prefixes in chemical names have been omitted and others (*e.g.* N-, β) are included but ignored in ordering the entries. Salts are indexed under the names of either their cation, or metal component (*e.g.* “sodium”) or anion (*e.g.* “nitrate”) whichever is more significant in the context of food chemistry.

The well-known observation that Britain and the USA “are two nations separated by a common language” is especially true in matters of food. To the best of the author’s limited abilities the American names of foods have been included in this index with the appropriate “translations”.

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