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Texture in food

Volume 1: Semi-solid foods

Edited by Brian M. McKenna

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Introduction

Texture is one of the most important attributes used by consumers to assess food quality. Texture influences the ‘mouthfeel’ of a product. Mouthfeel is the sensory experience derived from the sensations in the mouth and on the tongue after ingestion of a food. It is related to the density, viscosity, surface tension and other physical properties of the product. A change in the way in which a food moves or flows in the mouth and throat has a strong influence on perceptions of quality.

More and more foods are being developed that would be classed by the consumer as being semi-solid or semi-liquid. The definition of semi-solid foods, related to rate of deformation when stress is applied, will always be difficult. Solid and liquid foods can be seen as two extremes of a continuum. Indeed, it can sometimes be impossible to determine whether a material is behaving as a liquid or a solid. Many complex foodstuffs display viscous and elastic properties and have been categorised as ‘viscoelastic’. Others have been categorised according to their plastic properties. This volume discusses a range of these plastic and viscoelastic foods, from sauces and dressings to spreads and ice cream.

The first part of the book reviews key developments in our understanding of the structure of these kinds of food and its influence on texture. One of the principal types of semi-solid foods is emulsions. Food emulsions demonstrate a wide range of rheological characteristics. They include viscoelastic food products, such as salad dressings, and plastic foodstuffs such as butter and margarine. Chapter 1 reviews the latest research on emulsion rheology. This provides the foundation for improved control of the textural properties of these emulsion-based products which is also discussed later in the book.

The structure and texture of semi-solid foods are also strongly influenced by biopolymers such as proteins, polysaccharides and lipids which enhance viscosity and form networks. Understanding the phase behaviour of biopolymer mixtures makes it possible to design and control product microstructure more effectively.

Chapter 2 discusses phase transitions in biopolymers whilst Chapter 3 discusses phase separation. Chapter 4 focuses on starch as a biopolymer and its role as a texturogen in food systems such as sauces and purees. The combination of starches and other biopolymers such as celluloses, alginates and caseinates is increasingly important as fat mimics or substitutes in achieving the right texture in low fat foods. Chapter 5 discusses the use of biopolymer mixtures to give these products a specific flow behaviour and mouthfeel.

Understanding the structure and behaviour of emulsions or biopolymers depends on effective analytical techniques. Many of the advances in product development of semi-solid foods have been fuelled by improved methods of rheology measurement. Chapter 6 looks at general developments in rheology measurement whilst Chapter 7 concentrates on the important topic of in-line and on-line monitoring to ensure adequate process control and product quality.

Part II builds on the analysis of the structural characteristics of semi-solid foods in Part I. It considers key aspects of product development and enhancement, from processing to the use of ingredients. One of the most active areas of recent research has been in the influence of processing on the microstructure and rheology of semi-solid food systems. Building on the foundation laid by Chapters 1 and 2, Chapter 8 discusses how emulsion systems can be engineered to create appropriate functional and textural properties. It is complemented by Chapter 9 which reviews how different processing techniques can be used to create new protein gels which can improve the rheological properties of semi-solid foods such as spreads. The following group of chapters then looks at the use of ingredients to modify food structure and texture. Chapter 10 reviews the role of emulsifiers, whilst Chapters 11 and 12 consider the role and development of polysaccharides and other hydrocolloids to improve texture.

The final group of chapters demonstrates the results of the research in Parts I and II in controlling texture in particular products. There are chapters on yoghurt (Chapter 13), spreads (Chapter 14), ice cream (Chapter 15) and sauces and dressings (Chapter 16).

Part I

Food structure and texture

1

The rheology of emulsion-based food products

D. J. McClements, University of Massachusetts, USA

1.1 Introduction

Many familiar foods exist as emulsions at some stage during their production (Dickinson and Stainsby, 1982; Dickinson, 1992; Friberg and Larrson, 1997; McClements, 1999). These foods include natural products such as milk, and processed foods such as cream, butter, margarine, fruit beverages, soups, cake batters, mayonnaise, cream-liqueurs, sauces, desserts, salad cream, ice cream and coffee whitener (Swaisgood, 1996; Stauffer, 1999). The overall quality of a particular emulsion-based food product is determined by a combination of physicochemical and sensory characteristics, such as appearance, aroma, taste, shelf-life and texture. This chapter focuses primarily on the textural attributes of emulsion-based food products. Food emulsions exhibit a great diversity of rheological characteristics, ranging from low-viscosity Newtonian liquids (e.g. milk, fruit beverages), to viscoelastic materials (e.g. salad dressings, heavy cream) to plastic materials (e.g. butter, margarine). This diversity is the result of the different sorts of ingredients and processing conditions used to create each unique type of product. The creation of a food emulsion with specific quality attributes depends on the selection of the most appropriate raw materials (e.g. water, oil, emulsifiers, thickening agents, minerals, acids, bases, vitamins, flavors, colorants) and processing conditions (e.g. mixing, homogenization, pasteurization, sterilization) for that particular product. A better understanding of the fundamental principles of emulsion rheology would help to improve the economic production of high-quality products (Race, 1991; Barnes, 1994; Rao, 1995). This chapter aims to present the conceptual and theoretical framework required by food scientists to understand and control the rheological properties of emulsion-based food products.

1.2 General characteristics of food emulsions

1.2.1 Classifications and definitions

An emulsion consists of two immiscible liquids (oil and water), with one of the liquids dispersed as small spherical droplets in the other (Friberg and Larrson, 1997; McClements, 1999) (Fig. 1.1). In foods, the diameter of these droplets typically ranges between about 0.1 and 100 μm. A system that consists of oil droplets dispersed in an aqueous phase is called an oil-in-water (or O/W) emulsion, *e.g.* milk, cream, mayonnaise, soft drinks, soups and sauces. A system that consists of water droplets dispersed in an oil phase is called a water-in-oil (or W/O) emulsion, e.g. margarine, butter and some spreads. The material within the droplets is referred to as the dispersed, discontinuous or internal phase, whereas the material that makes up the surrounding liquid is called the continuous or external phase. It is also possible to create multiple emulsions, which can be either of the oil-in-water-in-oil type (O/W/O) or the water-in-oil-in-water type (W/O/W) (Dickinson and McClements, 1995; Garti and Benichou, 2001). The process of converting bulk oil and bulk water into an emulsion, or of reducing the size of the droplets in an existing emulsion, is known as homogenization (Walstra, 1993; Walstra and Smulders, 1998). In the food industry, homogenization is usually achieved by applying intense mechanical agitation to a liquid using a mechanical device known as a homogenizer, e.g. a high-speed blender, a high-pressure valve homogenizer, a colloid mill or an ultrasonic homogenizer (McClements, 1999).

Emulsions are thermodynamically unstable systems because the contact between oil and water molecules is unfavorable, and so they tend to break down with time (Dickinson, 1992; Friberg, 1997; Walstra, 1996a; McClements, 1999). The preparation of emulsions that are kinetically stable over a time period that is of practical use to the food industry (e.g. a few days, weeks, months or years) requires the incorporation of substances known as emulsifiers and/or thickening agents. An emulsifier is a surface-active substance that adsorbs to the surface of emulsion droplets to form a protective coating that prevents the droplets from aggregating with one another, e.g. certain proteins, polysaccharides, phospholipids, small molecule surfactants and solid particles (Stauffer, 1999). An emulsifier also reduces the interfacial tension and therefore facilitates the disruption of emulsion droplets during homogenization, which aids in the formation of emulsions containing smaller droplets (Walstra and Smulders, 1998). A thickening agent is a substance that either increases the viscosity of the continuous phase or forms a gel network within the continuous phase, thereby slowing down the movement of droplets due to gravity or Brownian motion, as well as providing the product with characteristics textural attributes (Imeson, 1997). Many types of polysaccharide and protein are suitable for use as thickening agents in food emulsions (Imeson, 1997).

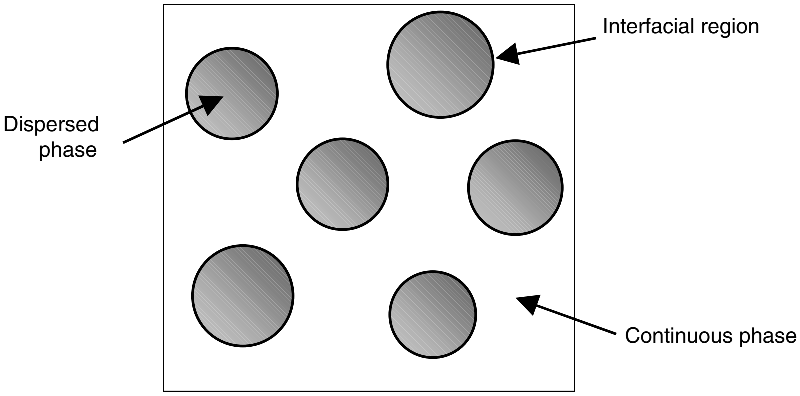

Fig. 1.1 Schematic representation of a polydisperse oil-in-water emulsion, consisting of oil droplets dispersed in an aqueous phase.

1.2.2 Instability mechanisms

A number of physicochemical mechanisms may be responsible for the breakdown of food emulsions (Dickinson, 1992; Walstra, 1996a; Friberg, 1997; McClements, 1999), the most important being gravitational separation, flocculation, coalescence, partial coalescence, Ostwald ripening and phase inversion (Fig. 1.2). Creaming is the process whereby droplets move upwards due to gravity because they have a lower density than the surrounding liquid. Sedimentation is the process whereby droplets move downwards due to gravity because they have a higher density than the surrounding liquid. Flocculation is the process whereby two or more droplets ‘stick’ together to form an aggregate in which the droplets retain their individual integrity. Coalescence is the process whereby two or more droplets merge to form a single larger droplet. Partial coalescence is the process whereby two or more partly crystalline droplets merge to form a single irregularly shaped aggregate due to the penetration of solid fat crystals from one droplet into a fluid region of another droplet. Ostwald ripening is the process whereby larger droplets grow at the expense of smaller droplets due to mass transport of dispersed phase material through the continuous phase. Phase inversion is the process whereby an oil-in-water emulsion changes to a water-in-oil emulsion, or vice versa. It should be noted that partial coalescence and phase inversion are integral parts of many food processing operations, such as the production of butter, margarine, ice cream and whipped cream (Dickinson and Stainsby, 1982; Berger, 1997; Buchheim and Dejmek, 1997; Walstra, 1996b). Generally, the term ‘emulsion stability’ refers to the ability of the emulsion to resist changes in its physicochemical properties over time. Nevertheless, it is always important to identify clearly the most important physical and/or chemical mechanisms responsible for the instability of a particular emulsion, since this will determine the most effective strategy to improve its stability.

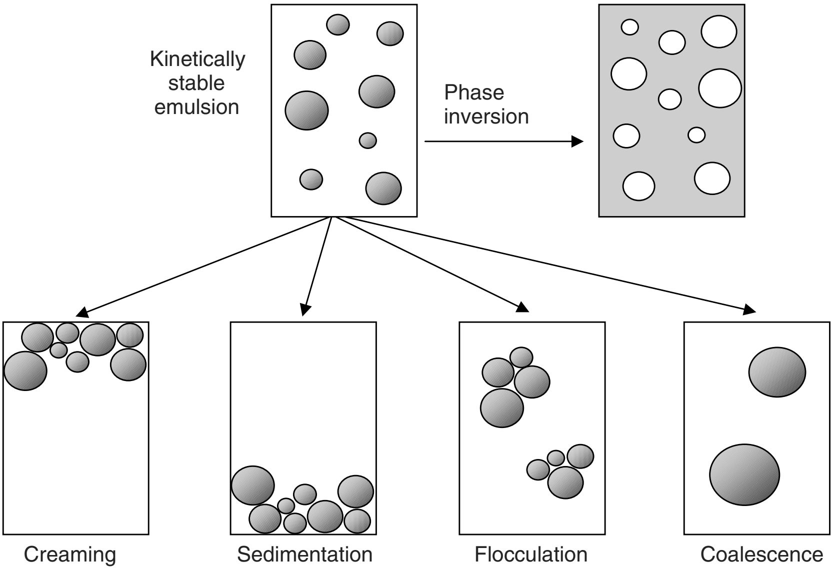

Fig. 1.2 The different physiochemical processes that can cause food emulsions to break down.

1.2.3 Colloidal properties

The rheological properties of food emulsions are strongly influenced by their colloidal nature, i.e. by the size, concentration, interactions and interfacial properties of the emulsion droplets (McClements, 1999). It is therefore useful to review briefly the colloidal characteristics of emulsions before discussing their rheological characteristics.

Droplet concentration

The concentration of droplets in an emulsion is usually characterized in terms of the dispersed phase volume fraction (ϕ) which is equal to the volume of emulsion droplets (V_D) divided by the total volume of emulsion (V_E): $\phi = V_D/V_E$. Practically, it is often more convenient to express the composition of an emulsion in terms of the dispersed phase mass fraction (ϕ_m), which is equal to the mass of emulsion droplets (m_D) divided by the total mass of emulsion (m_E): $\phi_m = m_D/m_E$. The relationship between ϕ_m and ϕ is given by the following equations:

$$\phi_m = \phi\left[\phi + (1-\phi)\frac{\rho_1}{\rho_2}\right]^{-1} \quad [1.1a]$$

$$\phi = \phi_m\left[\phi_m + (1-\phi_m)\frac{\rho_2}{\rho_1}\right]^{-1} \quad [1.1b]$$

where ρ_1 and ρ_2 are the densities of the continuous and dispersed phases, respectively. When the densities of the two phases are equal, the mass fraction is equivalent to the volume fraction. The droplet concentration may also be represented as either a dispersed phase volume percentage (= 100ϕ) or disperse phase mass percentage (= $100\phi_m$). It is particularly important to convert the droplet concentration to the appropriate units when comparing experimental work with theoretical predictions.

Droplet size

When all the droplets in an emulsion have the same size, the emulsion is referred to as 'monodisperse', and a single number, either the droplet radius or droplet diameter, can be used to characterize the droplet size (Hunter, 1986). In practice, all food emulsions contain a range of different droplet sizes, and are therefore referred to as being 'polydisperse' (Fig. 1.1). A polydisperse emulsion is characterized by its 'particle size distribution', which defines the concentration of droplets in different size classes (Hunter, 1986). The huge number of droplets in most food emulsions means that the particle size distribution can usually be considered to be continuous. Nevertheless, it is usually more convenient to present particle size distributions as histograms or tables by dividing the distribution into a number of size classes and recording the concentration of the droplets in each size class (McClements, 1999). When constructing or interpreting a particle size distribution it is important to pay attention to the manner in which the particle concentration and size are presented. The particle concentration is usually presented as either the volume percentage or number percentage of droplets within a particular size class, whereas the particle size is usually presented as either the mean droplet radius or the mean droplet diameter of the size class. Polydisperse emulsions may also be characterized as being 'monomodal', 'bimodal' or 'multimodal', depending on the number of peaks in the particle size distribution.

In some situations it is important to know the full particle size distribution of an emulsion, although in other situations it is only necessary to have an indication of the average droplet size and the width of the distribution. The particle size distribution of an emulsion can conveniently be described by the mean droplet diameter ($\bar{d}$) and the standard deviation of the droplet diameter (σ), e.g.:

$$\bar{d} = \sum n_i d_i / N \quad [1.2]$$

$$\sigma = \sqrt{\left[\sum n_{\mathrm{i}}(d_{\mathrm{i}} - \bar{d})^2\right]/N} \quad [1.3]$$

where n_{i} is the number of droplets with diameter d_{i}, and N is the total number of droplets. The above mean is often referred to as the mean 'length' diameter, d_{L}, because it represents the sum of the *length* of the droplets divided by the total number of droplets. It is also possible to express the mean droplet size in various other ways to reflect different aspects of an emulsion, e.g. average surface area, average volume, or ratio of total droplet surface area to total droplet volume (Hunter, 1986).

Droplet charge

The droplets in many food emulsions have an electrical charge because of adsorption of surface-active molecules that are ionized or ionizable, e.g. proteins, certain polysaccharides, ionic surfactants, phospholipids and some small ions (Dickinson and Stainsby, 1982; Bergenstahl and Claesson, 1997; Claesson *et al.,* 2001). The electrical charge on the droplets depends on the type and concentration of surface-active species present at the interface, as well as the pH and ionic composition of the aqueous phase. The charge on an emulsion droplet is important because it determines the nature of its interactions with other charged species (e.g. small ions, macromolecules or colloidal particles) or its behavior in the presence of an electrical field (e.g. electrophoresis). The droplets in many food emulsions are prevented from aggregating by using ionic emulsifiers that adsorb to their surface and prevent them from coming close together because of electrostatic repulsion (Friberg, 1997; McClements, 1999). The electrical charge on a droplet is characterized by its *surface electrical potential* (Ψ_0), *surface charge density* (σ) and *zeta potential* (ζ) (Hunter, 1986, 1989, 1993). The surface charge density is the amount of electrical charge per unit surface area, which depends on the net number of charged groups per surface-active molecule and the number of surface-active molecules per unit interfacial area. The surface electrical potential is the amount of energy required to increase the surface charge density from zero to σ, by bringing charges from an infinite distance to the surface through the surrounding medium. The surface electrical potential therefore depends on the ionic composition of the surrounding medium, usually decreasing as the ionic strength of the aqueous phase increases. The zeta potential (ζ) is the electrical potential at the 'shear plane', which is defined as the distance away from the droplet surface below which the counter-ions remain strongly attached to the droplet when it moves in an electrical field. In practice, the ζ-potential is often a better representation of the electrical characteristics of an emulsion droplet because it inherently accounts for the adsorption of any charged counter-ions. In addition, the ζ-potential is much easier to measure than the electrical potential or the surface charge density, and therefore droplet charges are usually characterized in terms of ζ (Hunter, 1993).

Interfacial properties

The droplet interface consists of a narrow region (usually a few nanometers thick) which surrounds each emulsion droplet, and contains a mixture of oil, water and emulsifier molecules (Hunter, 1986, 1989; Israelachvili, 1992). The interfacial region only makes up a significant fraction of the total volume of an emulsion when the droplet size is less than about 1 µm (McClements, 1999). Even so, it plays a major role in determining many of the most important bulk physicochemical and organoleptic properties of food emulsions. For this reason food scientists are particularly interested in elucidating the factors that determine the composition, structure, thickness, rheology and charge of the interfacial region (Dalgleish, 1996a,b; Dickinson, 1992, 2001; Dickinson and McClements, 1995; Patino and Nino, 1999; Wilde, 2000). The composition and structure of the interfacial region are determined by the type and concentration of surface-active species present, as well as by the events that occur both during and after emulsion formation, e.g. competitive adsorption. The thickness and rheology of the interfacial region influence the stability of emulsions to gravitational separation, coalescence and flocculation, and determine the rate at which molecules leave or enter the droplets (McClements, 1999; Dickinson, 2001).

Colloidal interactions

Colloidal interactions govern whether emulsion droplets aggregate or remain as separate entities, as well as determining the characteristics of any aggregates formed, e.g. their size, shape, porosity and deformability (Dickinson 1992, 2000; Dickinson and McClements, 1995; Lodge and Heyes, 1999). The rheological properties of many food emulsions depend on the extent of droplet aggregation and the characteristics of any aggregates formed (Dickinson, 1998; Manoj *et al.*, 1998b). The interactions between two emulsion droplets can be described in terms of an *interdroplet pair potential* (McClements, 1999). The interdroplet pair potential, $w(h)$, is the energy required to bring two emulsion droplets from an infinite distance apart to a surface-to-surface separation of h (Fig. 1.3). The overall interdroplet pair potential acting between two droplets is made up from contributions from many different types of interactions, including van der Waals, steric, electrostatic, depletion, hydrophobic and hydration interactions (McClements, 1999). These individual interactions vary in their sign (attractive or repulsive), magnitude (weak to strong) and range (short to long). Each of the individual interactions usually has a simple monotonic dependence on surface-to-surface separation, but the sum of the interactions can exhibit fairly complex behavior, exhibiting maximum or minimum values at certain separations (Fig. 1.3). Generally, droplets tend to aggregate when attractive interactions dominate, but remain as individual entities when repulsive interactions dominate (McClements, 1999).

1.3 Rheological properties of dilute emulsions

In this section, the relationship between the rheological properties of emulsions and their composition and microstructure is discussed, with special emphasis being given to the mathematical modeling of emulsion rheology. Initially, the

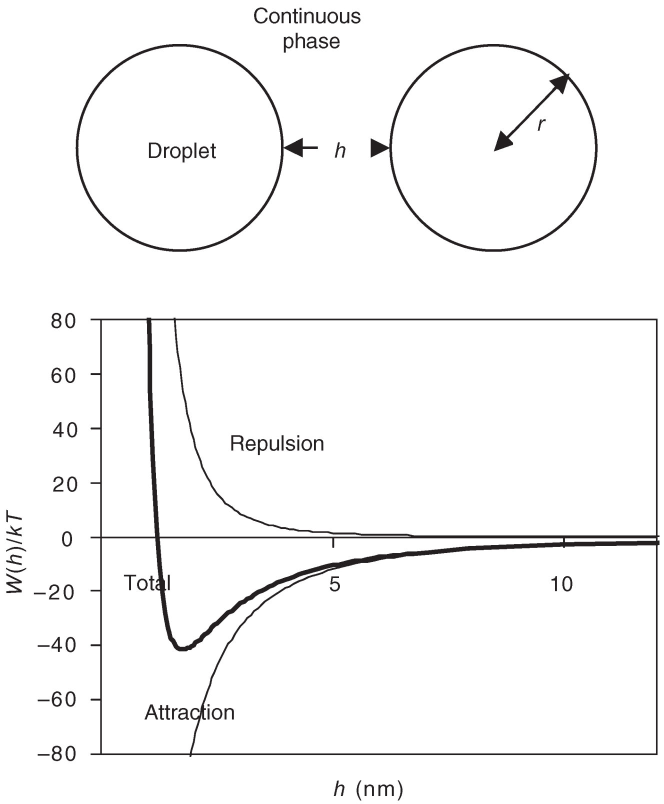

Fig. 1.3 The physiochemical properties of emulsions are strongly determined by the sign, range and magnitude of the colloidal interactions between the droplets. Droplet–droplet interactions can be characterized by an *interdroplet pair potential,* which describes the change in the interaction potential ($w(h)$) with droplet–droplet surface separation (h).

rheology of dilute suspensions of non-interacting rigid spheres is discussed, because the theory describing the properties of this type of system is well established (Hunter, 1986; Mewis and Macosko, 1994; Tadros, 1994; Hiemenz and Rajagopalan, 1997). Food emulsions may contain particles that are non-rigid, non-spherical, interacting and concentrated (Dickinson, 1992). For this reason, theories that have been developed to take each of these additional factors into account are discussed. A ‘dilute’ emulsion is one in which there are no appreciable colloidal or hydrodynamic interactions between the droplets, i.e. the droplets can be considered to behave independently of each other.

1.3.1 Rigid spherical particles

The simplest colloidal dispersion for which a mathematical relationship can be developed between the bulk rheology and the colloidal characteristics is one containing rigid spherical particles. Einstein derived an equation to relate the viscosity of a suspension of rigid spheres to the particle concentration (Larson, 1999):

$$\eta = \eta_0(1 + 2.5\phi) \tag{1.4}$$

where η_0 is the viscosity of the liquid surrounding the droplets and ϕ is the dispersed phase volume fraction. This equation assumes that the liquid is Newtonian, the particles are rigid and spherical, that there are no particle–particle interactions, that there is no slip at the particle–fluid interface and that Brownian motion effects are unimportant. The Einstein equation predicts that the viscosity of a dilute suspension of spherical particles increases linearly with particle volume fraction, and is independent of particle size and shear rate. The Einstein equation gives excellent agreement with experimental measurements for suspensions that conform to the above criteria, often up to particle concentrations of about 5% (Hunter, 1986; Hiemenz and Rajagopalan, 1997; Larson, 1999). It also predicts that the viscosity of dilute emulsions should be governed primarily by the viscosity of the continuous phase, rather than by the particle characteristics. For example, a colloidal dispersion containing 5% particles has a viscosity that is only about 12.5% larger than that of the continuous phase, whereas the viscosity of the continuous phase can be varied by orders of magnitude by adding small quantities of thickening agent.

For colloidal dispersions, it is convenient to define the *intrinsic viscosity* by the following expression (Hiemenz and Rajagopalan, 1997):

$$[\eta] = \lim_{\phi \to 0}(\eta - \eta_0)/\phi\eta_0 \tag{1.5}$$

The intrinsic viscosity is 2.5 for rigid spherical particles, but may be smaller for fluid particles and larger for non-spherical or aggregated particles (see below).

1.3.2 Fluid spherical particles

The droplets in food emulsions are usually fluid, rather than solid, and therefore it is necessary to consider how the rheology of the material within the droplets influences the overall rheology of an emulsion. When a droplet is in a flow field, a stress may be transmitted from the external fluid flowing past the droplet surface into the internal fluid contained within the droplets (Sherman, 1968; Dickinson and Stainsby, 1982; Larson, 1999). This shear stress causes the fluid within the droplets to circulate. Consequently, the velocity gradient at the surface of a liquid droplet is less than for a solid particle, which means that less energy is lost owing to friction, hence the viscosity of the suspension is less. The higher the viscosity of the fluid within a droplet, the more it acts like a rigid particle, and the higher is the emulsion's viscosity. The viscosity of a dilute emulsion containing spherical droplets is given by (Larson, 1999):

$$\eta = \eta_0 \left[1 + \left(\frac{\eta_0 + 2.5\eta_{\text{drop}}}{\eta_0 + \eta_{\text{drop}}} \right) \phi \right] \qquad [1.6]$$

where, η_{drop} is the viscosity of the liquid in the droplets. This equation assumes that the viscosities of both the continuous and dispersed phases are Newtonian. For droplets containing relatively high-viscosity liquids ($\eta_{\text{drop}}/\eta_0 >> 1$), the intrinsic viscosity tends to 2.5, and therefore Eqn [1.6] tends to the Einstein equation (Eqn [1.4]). For droplets containing relatively low-viscosity fluids ($\eta_{\text{drop}}/\eta_0 << 1$), such as air bubbles, the intrinsic viscosity tends to unity, and so the suspension viscosity is given by: $\eta = \eta_0(1 + \phi)$. One would expect the viscosity of emulsions to fall somewhere between these two extremes. In practice, the droplets in most food emulsions are coated by an interfacial membrane that retards the transmittance of tangential stresses from the continuous phase into the droplet, and therefore hinders the flow of fluid within the droplet (Pal *et al.,* 1992; Tadros, 1994). This may be due to the inherent viscoelasticity of the interfacial membrane (e.g. proteins or polysaccharides) (Dickinson, 2001) or due to the Gibbs–Marangoni effect (e.g. surfactants) (Walstra and Smulders, 1998). For this reason, most food emulsions contain droplets that act like rigid spheres, and so their viscosities at low concentrations can be described by the Einstein equation.

At sufficiently high shear stresses, the hydrodynamic forces can become so large that they overcome the interfacial forces holding the droplets together and cause the droplets to become deformed and eventually disrupted (Walstra and Smulders, 1998). Nevertheless, the shear stresses required to cause disruption of emulsion droplets are usually so high that the flow-profile may no longer be simple shear, and so it is not possible to make accurate viscosity measurements.

1.3.3 Non-spherical particles

Many of the particles in food emulsions may have non-spherical shapes, e.g. flocculated droplets, partially crystalline droplets, fat crystals, ice crystals or biopolymer molecules (Dickinson, 1992). Consequently, it is important to appreciate the effects of particle shape on the viscosity of food emulsions. The shape of many particles can be approximated as prolate spheroids (rod-like) or oblate spheroids (disk-like). A spheroid is characterized by its axis ratio $r_p = a/b$, where a is the major axis and b is the minor axis. For a sphere, $a = b$, for a prolate spheroid $a > b$, and for an oblate spheroid $a < b$. The flow profile of a fluid around a non-spherical particle causes a greater degree of energy dissipation than that around a spherical particle, which leads to an increase in viscosity (Hunter, 1986; Mewis and Macosko, 1994; Hiemenz and Rajagopalan, 1997). The magnitude of this effect depends on the rotation and orientation of the spherical particle. For example, the viscosity of a rod-like particle is much lower when it is aligned parallel to the fluid flow, rather than perpendicular, because the parallel orientation offers less resistance to flow.

The orientation of a spheroid particle in a flow field is governed by a balance between the hydrodynamic forces that act upon it and its rotational Brownian motion (Mewis and Macosko, 1994; Larson, 1999). The hydrodynamic forces favor the alignment of the particle along the direction of the flow field, because this reduces the energy dissipation. On the other hand, the alignment of the particles is opposed by their *rotational* Brownian motion, which favors the complete randomization of their orientations. The relative importance of the hydrodynamic and Brownian forces is expressed in terms of a dimensionless number, known as the *Peclet* number, *Pe*. For simple shear flow (Mewis and Macosko, 1994):

$$Pe = \frac{\dot{\gamma}}{D_R} \qquad [1.7]$$

where $\dot{\gamma}$ is the shear rate and D_R is the *rotational* Brownian diffusion coefficient, which depends on particle shape:

$$D_R = \frac{kT}{8\pi\eta r^3} \qquad \text{for } \textit{rigid spheres} \qquad [1.8a]$$

$$D_R = \frac{3kT}{32\pi\eta b^3} \qquad \text{for } \textit{circular disks} \qquad [1.8b]$$

$$D_R = \frac{3kT}{8\pi\eta r^3}(\ln 2r_p - 0.5) \qquad \text{for } \textit{long thin rods} \qquad [1.8c]$$

where r_p is a parameter that is related to the ratio of the polymer length to its thickness.

When the Peclet number is much less than unity ($Pe << 1$), the rotational Brownian motion dominates, and the particles tend to rotate freely in the liquid. This type of behavior is observed when the particles are small, the shear rate is low and/or the viscosity of the surrounding fluid is low. When the Peclet number is much greater than unity ($Pe >> 1$), the hydrodynamic forces dominate, and the particles become aligned with the flow field. This type of behavior is observed when the particles are large, the shear rate is high and/or the viscosity of the surrounding liquid is high.

The viscosity of a suspension of non-spherical particles therefore depends on the shear rate. At low shear rates (i.e. $Pe << 1$), the viscosity has a constant high value. As the shear rate is increased, the hydrodynamic forces become more important and so the particles become orientated with the flow field, which causes a reduction in the viscosity. At high shear rates (i.e. $Pe >> 1$), the hydrodynamic forces dominate and the particle remains aligned with the shear field and therefore the viscosity has a constant low value. Thus suspensions of non-spherical particles exhibit shear-thinning behavior. The shear rate at which the viscosity starts to decrease depends on the size and shape of the particles, as well as the viscosity of the surrounding liquid.

1.3.4 Flocculated particles

The droplets in many types of food emulsion are flocculated because the attractive forces between the droplets dominate the repulsive forces. A dilute suspension of flocculated particles has a higher viscosity than a dilute suspension containing the same concentration of isolated particles because the flocs entrap some of the continuous phase. Therefore the flocs have a higher *effective* volume fraction than the actual droplet volume fraction (Liu and Masliyah, 1996). To a first approximation, the rheology of dilute flocculated colloidal dispersions can be described by:

$$\eta = \eta_0(1 + 2.5\phi_{\text{floc}}) \qquad [1.9]$$

where ϕ_{floc} (= ϕ/ϕ_i) is the volume fraction of flocs present in the colloidal dispersion and ϕ_i is an internal packing parameter equal to the volume fraction of particles within the flocs (= $V_{\text{particle}}/V_{\text{floc}}$). The more open the packing of the particles within the flocs, the smaller the value of ϕ_i, and the larger the viscosity of the colloidal dispersion. Suspensions of flocculated particles tend to exhibit pronounced shear thinning behavior (Fig. 1.4). At low shear stresses, the hydrodynamic forces are not large enough to disrupt the bonds holding the particles together and so the flocs act like particles with a fixed size and shape, resulting in a constant viscosity. As the shear stress is increased, the hydrodynamic forces become large enough to cause flocs to become deformed and eventually disrupted. The deformation of the flocs results in them becoming elongated and aligned with the shear field, which results in a reduction in the viscosity. The disruption of the flocs decreases their effective volume fraction and therefore also contributes to a decrease in the suspension viscosity. The viscosity reaches a constant value at high shear stresses, either because all of the flocs are completely disrupted so that only individual particles remain or because the number of flocculated particles remains constant since the rate of floc formation is equal to that of floc disruption (Campanella *et al.*, 1995). The precise dependence of the apparent viscosity of a colloidal dispersion on shear stress depends on the strength of the attractive forces between the flocculated particles (Liu and Masliyah, 1996). Weakly flocculated particles will tend to become dissociated at lower shear stresses than strongly flocculated particles. Indeed, rheological measurements of the shear thinning behavior of colloidal dispersions can be used as a tool to provide information about the strength of colloidal interactions between particles (Sherman, 1970; Dickinson and Stainsby, 1982; Hunter, 1989).

For certain systems, it is possible to observe shear thickening behavior in colloidal dispersions due to particle flocculation induced by the shear field (de Vries, 1963; Larson, 1999). Some colloidal dispersions contain particles that are

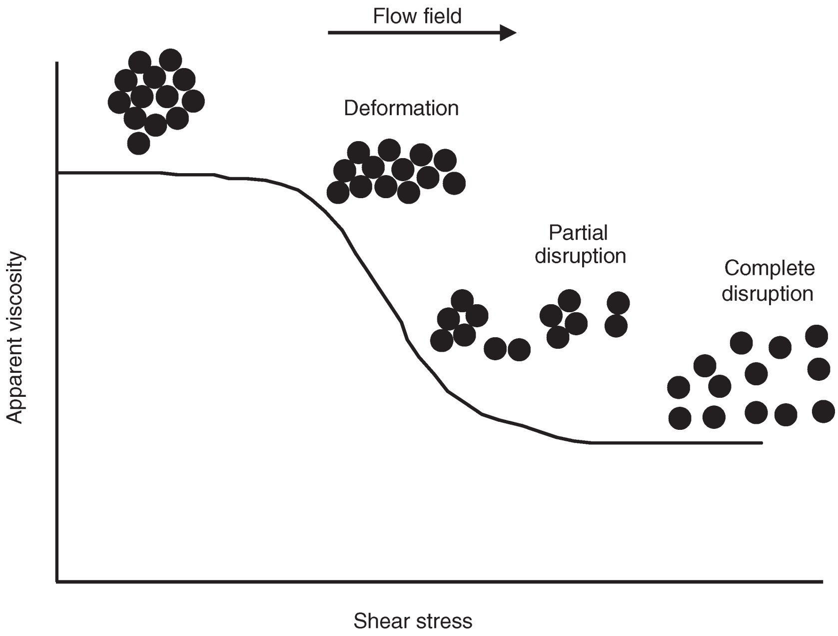

Fig. 1.4 An emulsion containing flocculated droplets exhibits shear thinning behavior because the flocs are deformed and disrupted in the shear field.

not flocculated under quiescent conditions because there is a sufficiently high-energy barrier to prevent the particles from falling into a primary minimum. However, when a shear stress is applied to the colloidal dispersion the frequency of collisions and the impact force between the particles increases, which can cause the particles to gain sufficient energy to ‘jump’ over the energy barrier and become flocculated, thereby leading to shear thickening.

1.4 Rheological properties of concentrated emulsions

A ‘concentrated’ emulsion is one in which colloidal or hydrodynamic interactions between droplets have an appreciable influence on the emulsion rheology (Hunter, 1986; Mewis and Macosko, 1994; Tadros, 1994; Larson, 1999). Concentrated emulsions can exhibit either fluid-like or solid-like behavior depending on the magnitude and nature of the interactions between the droplets. Fluid-like emulsions are usually characterized in terms of their apparent viscosity, whereas solid-like emulsions are characterized in terms of their dynamic shear modulus or their elastic modulus, yield stress and plastic viscosity.

1.4.1 Rigid spherical particles in the absence of long-range colloidal interactions

In this section, concentrated colloidal dispersions containing rigid spheres in the absence of long-range attractive or repulsive colloidal interactions are considered. In these systems, the rheology is influenced by hydrodynamic interactions associated with the relative motion of neighboring particles. Colloidal dispersions in which long-range colloidal interactions are also important are discussed in following sections.

At low particle concentrations, hydrodynamic interactions mainly occur between pairs of particles, but as the particle concentration increases three or more particles may be involved (Larson, 1999). As the particle concentration increases, the measured viscosity becomes larger than that predicted by the Einstein equation because these additional hydrodynamic interactions lead to a greater degree of energy dissipation. The Einstein equation can be extended to account for the effects of these interactions by including additional volume fraction terms (Pal *et al.,* 1992):

$$\eta = \eta_0(1 + a\phi + b\phi^2 + c\phi^3 + \ldots) \qquad [1.10]$$

The value of the constants, *a, b, c*, etc. can be determined either experimentally or theoretically (Larson, 1999). For a colloidal dispersion of rigid spherical particles the value of *a* is 2.5, so that Eqn 1.10 tends to the Einstein equation at low volume fractions. A rigorous theoretical treatment of the interactions between pairs of droplets has established that $b = 6.2$ for rigid spherical particles. Experiments have shown that Eqn 1.10 can be used up to particle concentrations of about 10% with $a = 2.5$ and $b = 6.2$ for colloidal dispersions in the absence of long-range colloidal interactions (Larson, 1999). It is difficult to theoretically determine the value of higher-order terms in Eqn 1.10 because of the mathematical complexities involved in describing interactions between three or more particles. In addition, each additional constant extends the applicability of the equation only to a slightly higher volume fraction. For this reason, it has proved to be more convenient to adopt a semi-empirical approach to the development of equations that describe the viscosity of concentrated colloidal dispersions. One of the most widely used equations was derived by Dougherty and Krieger and is applicable across the whole volume fraction range (Hunter, 1986; Mewis and Macosko, 1994):

$$\eta = \eta_0\left(1 - \frac{\phi}{\phi_c}\right)^{-[\eta]\phi_c} \qquad [1.11]$$

where $[\eta]$ is the intrinsic viscosity (Eqn 1.5) and ϕ_c is the *maximum packing* volume fraction, which is usually taken to be an adjustable parameter that is determined experimentally. Physically, ϕ_c is related to the particle volume fraction at which the spheres become close packed. The intrinsic viscosity is 2.5 for spherical particles, but may be appreciably larger for non-spherical or aggregated particles (Hiemenz and Rajagopalan, 1997). Typically, the value of ϕ_c is between about 0.6 and 0.7 for spheres which do not interact via long-range colloidal interactions (Hunter, 1986), but it may be considerably lower for suspensions in which there are strong long-range attractive or repulsive interactions between the droplets (see following sections). This is because the effective volume fraction of the particles in the colloidal dispersion is greater than the actual volume fraction of the particles, so that the maximum packing volume fraction is reached at lower particle concentrations.

Concentrated colloidal dispersions exhibit appreciable shear-thinning behavior above a certain particle concentration (~45%) due to Brownian motion effects (Pal *et al.,* 1992; Mewis and Macosko, 1994; Liu and Masliyah, 1996). At low shear stresses, the particles have a three-dimensional isotropic and random distribution because of their Brownian motion (Hunter, 1993). As the shear stress increases, the particles become more ordered along the flow lines to form 'strings' or 'layers' of particles that offer less resistance to the fluid flow and therefore cause a decrease in the suspension viscosity. The resulting decrease in viscosity with increasing shear stress can be described by the following equation (Larson, 1999):

$$\eta = \eta_{\tau\to\infty} + \frac{\eta_{\tau\to 0} - \eta_{\tau\to\infty}}{1 + (\tau / \tau_i)} \qquad [1.12]$$

where τ_i is a critical shear stress that is related to the size of the droplets: $\tau_i = kT/\beta r^3$, k is Boltzmann's constant, T is the absolute temperature and β is a dimensionless constant with a value of about 0.431 (Hunter, 1989). The value of τ_i is a characteristic of a particular system which describes the relative importance of the *translational* Brownian motion and hydrodynamic shear forces. When $\tau << \tau_i$, Brownian motion dominates and the particles have a random distribution, but when $\tau >> \tau_i$, the shear forces dominate and the particles become organized into strings or layers along the lines of the shear field, which causes less energy dissipation. Equation 1.12 indicates that the viscosity decreases from a constant value at low shear stresses ($\eta_{\tau\to 0}$) to another constant value at high shear stresses ($\eta_{\tau\to\infty}$). The apparent viscosity can decrease by over an order of magnitude with shear stress for highly concentrated oil-in-water emulsions due to Brownian motion effects (Dickinson, 1998). The shear rate at which the viscosity starts to decrease from its η_0-value is dependent on the particle size (Larson, 1999). For large particles (r > 1 μm), τ_i may be so low that shear thinning behavior is not observed over the shear stresses normally used in shear rheometers (0.001–10 Pa). On the other hand, for small particles (r < 0.04 μm), τ_i may be so high that it is also not observed. Many food emulsions contain intermediate-sized droplets, and so pronounced shear thinning behavior is observed in concentrated emulsions (Dickinson, 1998; Chanamai and McClements, 2000a).

The viscosity of concentrated colloidal dispersions can still be described by the Dougherty–Krieger equation when shear stresses are applied, but the value of ϕ_c used in the equation depends on the shear stress. This is because droplets can pack more efficiently at higher shear stresses and therefore the maximum packing volume fraction (ϕ_c) increases with shear stress (Hunter, 1989). In the low shear stress limit, the maximum packing volume fraction (ϕ_{cL}) has been found to be about

0.57 for rigid spherical particles, whereas in the high shear stress limit the value (ϕ_{cH}) has been found to be around 0.68 (Larson, 1999).

A variety of other semi-empirical equations have been developed to describe the rheological characteristics of concentrated emulsions in addition to the Dougherty–Krieger equation. The usefulness of these various equations has been reviewed by Pal (2000a,b, 2001).

1.4.2 Rigid spherical particles in the presence of repulsive colloidal interactions

In this section, the influence of long-range repulsive interactions on the rheology of colloidal dispersions is considered. The major types of relatively long-range repulsion interactions that operate in food emulsions are electrostatic and steric interactions (Friberg, 1997; Bergenstahl and Claesson, 1997; McClements, 1999; Claesson *et al.*, 2001). These repulsive interactions prevent the droplets from coming into close contact when they collide with each other, and therefore increase the effective volume fraction of the droplets (Tadros, 1994; Mewis and Macosko, 1994; Larson, 1999):

$$\phi_{\text{eff}} = \phi\left(1 + \frac{\delta}{r}\right)^3 \qquad [1.13]$$

where δ is equal to half the distance of closest separation between the two droplets.

For sterically stabilized particles δ is approximately equal to the thickness of the adsorbed layer (Tadros, 1994). For electrostatically stabilized particles δ is related to the Debye screening length κ^{-1} (Larson, 1999). At low shear stresses, the value of δ is given approximately by the following equation (Mewis and Macosko, 1994):

$$\delta = \kappa^{-1} \ln\left\{\frac{\alpha}{\ln[\alpha / (\ln\alpha)]}\right\} \qquad [1.14]$$

where $\alpha = 4\pi\,\varepsilon_0\varepsilon_R\Psi_0 r^2\kappa \exp(2r\kappa)/kT$, ε_0 is the dielectric permitivity of a vacuum, ε_R is the relative dielectric permitivity of the continuous phase, Ψ_0 is the electrical potential at the droplet surface, r is the radius, κ^{-1} is the Debye length, k is Boltzmann's constant and T is the absolute temperature. For electrically charged oil droplets, the distance of closest contact therefore decreases as the surface charge decreases or as the ionic strength of the aqueous phase increases.

It is convenient to categorize droplets with repulsive interactions as being either 'hard' particles or 'soft' particles (Liu and Masliyah, 1996). A hard particle is incompressible and so its effective size is independent of shear rate or droplet concentration. On the other hand, a soft particle is compressible and so its effective size may be reduced at high shear rates or high droplet concentrations. Sterically stabilized droplets with dense interfacial layers are usually considered to act like hard particles because the layer is relatively incompressible, whereas electrostatically stabilized droplets or sterically stabilized droplets with open interfacial layers are usually considered to act like soft particles because the layer is compressible.

Two approaches can be taken to describe mathematically the viscosity of concentrated colloidal dispersions containing particles with repulsive interactions using the Dougherty–Krieger equation (Eqn 1.11). In the first approach, the actual volume fraction of the particles (ϕ) normally used in the Dougherty–Krieger equation is replaced by the effective volume fraction of the particles (ϕ_{eff}). For hard particles the value of ϕ_c is taken to be the same as for non-interacting particles, but for soft particles the value of ϕ_c is increased because the particles may be compressed at higher volume fractions and therefore pack more efficiently. As a consequence the viscosity of an emulsion containing soft particles is lower than one containing hard particles at the same *effective* volume fraction. In the second approach, the value of ϕ_c normally used in the Dougherty–Krieger equation is treated as an adjustable parameter that is determined experimentally. In this case, ϕ_c decreases with an increase in particle–particle repulsion because the particles become close packed at lower actual particle volume fractions.

The influence of repulsive interactions on the rheology of emulsions depends on the magnitude of δ relative to the size of the particles. For relatively large particles (i.e. $\delta << r$), this effect is negligible, but for small droplets or droplets with thick layers around them (i.e. $\delta \approx r$), this effect can significantly increase the viscosity of a suspension (Tadros, 1994; Weiss and McClements, 2000).

Above the maximum packing volume fraction, the colloidal dispersions behave as viscoelastic or plastic materials, which can be characterized in terms of their dynamic shear modulus or their elastic modulus, yield stress and plastic viscosity. The modulus of these particle gels normally increases with decreasing droplet size, increasing droplet concentration, and increasing strength and range of the repulsive interactions. A variety of theories have been developed to relate the rheological characteristics of this type of particle gel to their colloidal characteristics (Larson, 1999).

1.4.3 Rigid spherical particles in the presence of attractive colloidal interactions

The particles in many types of colloidal dispersion aggregate into flocs because of the existence of relatively strong attractive forces between the particles (McClements, 1999; Claesson *et al.*, 2001). In concentrated colloidal dispersions, the flocs are close enough together to interact with each other, through hydrodynamic interactions, colloidal interactions and/or entanglement. To a first approximation, the viscosity of a colloidal dispersion containing strongly flocculated particles can be described by the Dougherty–Krieger equation by replacing the particle volume fraction (ϕ) with the floc volume fraction (ϕ_{floc}) (Tuinier and de Kruif, 1999). As mentioned earlier, the volume fraction of flocs in a colloidal dispersion depends on the structural organization of the particles within the flocs: $\phi_{floc} = \phi/\phi_i$, where ϕ_i is the internal packing parameter:

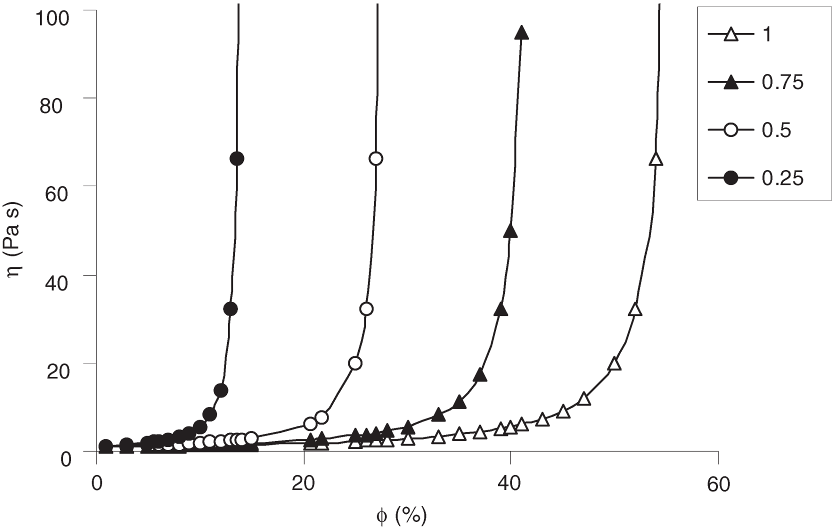

Fig. 1.5 Influence of droplet flocculation on the apparent viscosity of emulsions containing flocs with different internal packing parameters.

$$\frac{\eta}{\eta_0} = \left(1 - \frac{\phi_{\text{floc}}}{\phi_c}\right)^{-[\eta]\phi_c} = \left(1 - \frac{\phi}{\phi_c\phi_i}\right)^{-[\eta]\phi_c} \qquad [1.15]$$

Predictions made using this equation are shown in Fig. 1.5 for emulsions with different internal packing parameters. As the flocs become more open in structure (smaller ϕ_i), the viscosity at low particle concentrations increases and the actual droplet volume fraction where the viscosity increases dramatically due to close packing of the droplets decreases. This latter effect occurs because a lower concentration of particles is needed to form a network of connecting particles that can transmit stresses in a flocculated colloidal dispersion. These predictions have been qualitatively confirmed by experimental measurements on monodisperse oil-in-water emulsions containing flocculated droplets (Chanamai and McClements, 2000b).

When the floc volume fraction exceeds the maximum packing volume fraction, the system forms a particle network consisting of a three-dimensional network of aggregated particles (Sherman, 1970; Goodwin and Ottewill, 1991; Pal, 1996; Larson, 1999). The more open the structure of the particles within the flocs, the lower the value of the actual droplet volume fraction where the network is formed. Network formation causes a suspension of particles to exhibit plastic and/or viscoelastic characteristics (Pal, 1996; Weiss and McClements, 2000). The network of aggregated droplets acts like a solid at low shear stresses because the applied forces are not sufficient to overcome the forces holding the droplets together. Once a critical shear stress is exceeded, the bonds between the droplets are disrupted and so the droplets can flow past one another. If some of the bonds are capable of reforming during the shearing process then the emulsion will exhibit viscoelastic behavior (Sherman, 1968). At higher shear stresses, the rate of bond disruption greatly exceeds that of bond formation and the emulsion acts like a liquid. Consequently, a suspension containing a three-dimensional network of aggregated particles often has a yield stress, below which it acts like an elastic solid and above which it acts like a liquid. Above the yield stress, the suspension often exhibits strong shear thinning behavior as more and more flocs are deformed and disrupted. The magnitude of the yield stress depends on the strength of the attractive forces holding the particles together: the greater the attractive forces, the greater the yield stress (Pal, 1996). The rheology of the system is also sensitive to the structural organization of the particles, e.g. whether they are loosely or densely packed, and the number of bonds per particle (Bremer *et al.*, 1993; Pal, 1996; Narine and Marangoni, 1999; Marangoni and Narine, 1999; Larson, 1999; Marangoni, 2000).

A variety of theoretical and computational models have been developed to describe the rheological characteristics of particle networks (Papenhuijzen, 1972; Shi *et al.*, 1990; Bremer *et al.*, 1993; van Vliet, 1995; Lodge and Heyes, 1999; Larson, 1999; Dickinson, 2000). These models attempt to relate the bulk rheological properties of particle networks (elastic modulus, shear modulus, yield stress, viscosity) to the structural organization of the particles within the network and the strength of the attractive forces between the particles. The main differences between the models are the assumptions made about the structural organization of the particles and the nature of the colloidal interactions acting between the particles.

1.4.4 Emulsions with semi-solid continuous phases

A number of food emulsions consist of droplets dispersed in a continuous phase that is either partly crystalline or gelled (Sherman, 1970; Dickinson and Stainsby, 1982; Dickinson, 1992; Moran, 1994). For example, butter and margarine consist of water droplets suspended in a liquid oil phase that contains a three-dimensional network of aggregated fat crystals. Many meat products, desserts, dressings, dips and sauces consist of oil droplets suspended in an aqueous phase of aggregated biopolymer molecules (e.g. proteins or polysaccharides). The rheology of these systems depends on the complex interplay between the rheology of the continuous phase and the characteristics of the suspended particles (Larson, 1999). In systems where the particle concentration is relatively low ($\phi < 0.05$), the overall rheology is usually dominated by the rheology of the continuous phase. In systems where the particle concentration is high enough to form a particle gel, the overall rheology may be dominated by the properties of the particle network (Dickinson, 1998; Larson, 1999). In many systems, the overall rheology depends on both the rheology of the continuous phase and the particle network (Dickinson, 1998).

1.5 Measuring the rheological properties of emulsions

1.5.1 Instrumentation for measurement of emulsion rheology

Food emulsions exhibit a broad range of different rheological characteristics, including, liquid, solid, plastic and viscoelastic behavior (Dickinson and Stainsby, 1982; Dickinson, 1992). A variety of instrumental methods have therefore been developed to characterize their rheological properties (Bourne, 1982; Whorlow, 1992; Macosko, 1994; Rao, 1995; Rao *et al.*, 1995). These instruments vary according to the type of deformation they apply to the sample (shear, compression, elongation or some combination), the property they measure (viscosity, yield stress, modulus), their cost, their sophistication and their ease of operation (Whorlow, 1992). For industrial applications, it is usually important to have instruments that make rapid, low-cost, simple and reproducible measurements, rather than giving fundamental data (Sherman, 1970; Rao, 1995; Rao *et al.*, 1995). On the other hand, in research and development applications it is often important to use instruments that provide fundamental information about the rheological constants of the material being tested, so data can be compared with previous measurements or with theoretical predictions (Daubert and Foegeding, 1998). Many of the instruments described in other chapters can be used for characterizing the rheological properties of food emulsions. Shear viscometers and dynamic shear rheometers are commonly used for testing fluid and viscoelastic emulsions, whereas unaxial compression devices are commonly used for testing viscoelastic, plastic or solid-like emulsions. The rheology of fluid emulsions is usually characterized in terms of the dependence of their apparent viscosity on shear stress. The rheology of fluid, viscoelastic and solid emulsions can be characterized in terms of their complex dynamic shear modulus (G^* and δ or G' and G''). The rheology of solid emulsions can also be characterized in terms of their elastic modulus, breaking stress and breaking strain during uniaxial compression. The rheology of plastic emulsions is often characterized in terms of their modulus, yield stress and apparent plastic viscosity, using either shear or compression tests. These rheological characteristics of emulsions are often measured as a function of time or temperature.

1.5.2 Possible sources of experimental error

It should be noted that the rheological characterization of emulsion-based products using shear viscometers and rheometers does present a number of specific challenges (Sherman, 1970; Hunter, 1989; Pal *et al.,* 1992; Larson, 1999). This section highlights a number of possible sources of experimental error that should be avoided or taken into account when carrying out rheology measurements on food emulsions. Mewis and Macosko (1994) discuss other sources of error that are common to all types of rheology measurement.

Rheometer gap effects

The gap between the cylinders or plates should be at least 20 times greater than the diameter of the droplets, so that the emulsion appears as a homogeneous material within the device (Pal *et al.,* 1992). On the other hand, the gap must be narrow enough to ensure a fairly uniform shear stress across the whole of the sample.

Wall slip effects

A phenomenon known as *wall slip* may occur within a viscometer or rheometer, which can cause serious errors in rheological measurements if not properly taken into account (Sherman, 1970; Franco *et al.*, 1998; Sanchez *et al.,* 2001). It is normally assumed that the liquid in direct contact with the surfaces of the measurement cell moves with them at the same velocity (Hunter, 1986). This assumption is usually valid for simple liquids because the small molecules are caught within the surface irregularities on the walls and are therefore dragged along with them. For an emulsion, this assumption may not hold because the droplets or flocs are greater in size than the surface irregularities. Under these circumstances, phase separation occurs at the cylinder surface and a thin layer of continuous phase acts as a lubricant so that *slip* occurs. Wall slip effects can be taken into account by roughening the surfaces of measurement cells, by using a range of different gap widths or by using rotating vane rheometers (Barnes and Nguyen, 2001; Hunter, 1986; Pal *et al.,* 1992; Franco *et al.*, 1998; Sanchez *et al.,* 2001).

Sample history

The rheological properties of many food emulsions depend strongly on their thermal and shear history, and so this must be carefully controlled in order to obtain reproducible measurements (Franco *et al.*, 1998). For example, the viscosity of many flocculated food emulsions decreases substantially upon shearing due to disruption of particle flocs, and the recovery of the initial viscosity takes a certain length of time to achieve after the shear stress is removed. For these systems, it is extremely important to establish a consistent thermal and shear sample history prior to starting any rheological measurements. For example, it may be necessary to place an emulsion in a thermostated measurement cell, apply a fixed shear stress for a constant time, allow it to sit for a fixed time, and then begin the rheological measurements. The objectives of this process are to break down and reform the structure of the emulsion in a reproducible and consistent manner.

Gravitational separation

Many emulsions are susceptible to creaming or sedimentation during the course of an experiment, which causes the vertical distribution of droplets in the emulsion to become inhomogeneous (Mewis and Macosko, 1994). For example, in emulsions where the droplet density is less than the density of the surrounding liquid, creaming leads to the formation of a droplet-rich layer at the top of the emulsion and a droplet-depleted layer at the bottom (McClements, 1999). The separation of an emulsion into a creamed and a serum layer should be avoided because the rheological characteristics of a separated emulsion may be appreciably different from those of a homogeneous emulsion. The importance of this effect depends on the geometry of the measurement cell. In a concentric cylinder measurement cell, the formation of a viscoelastic or plastic creamed layer may dominate the rheology of the whole emulsion because the shear stress is applied to the sides of the emulsion. On the other hand, in a cone-and-plate or parallel plate rheometer, the formation of a viscoelastic or plastic creamed layer may have a different effect because the shear stress is applied to the top and bottom of the emulsions. An approximate criterion which has been proposed to ensure that gravitational separation effects do not greatly affect measurements, is that the droplets should move less than 10% of the emulsion height in the rheometer during the course of a measurement (Mewis and Macosko, 1994):

$$t_{10\%h} = \frac{0.45h\eta_0}{gr^2\left|\left(\rho_{\text{drop}} - \rho_0\right)\right|} \tag{1.16}$$

where h is the height of the emulsion in the rheometer, ρ_{drop} is the density of the droplets, ρ_0 is the density of the continuous phase, g is the acceleration due to gravity and r is the droplet radius.

1.6 Factors controlling emulsion rheology

In this section, the major factors influencing the rheological properties of food emulsions are briefly discussed.

1.6.1 Disperse phase volume fraction

The viscosity of a non-flocculated emulsion increases with disperse phase volume fraction (Fig. 1.6) because the presence of the droplets increases the energy dissipation associated with fluid flow (Mewis and Macosko, 1994; Larson, 1999). At low droplet concentrations the viscosity increases linearly with ϕ (Eqn 1.4), but at higher droplet concentrations the viscosity increase is steeper (Eqns 1.10 and 1.11). Above a critical disperse phase volume fraction, ϕ_c, the droplets are packed so closely together that they cannot easily flow past each other. Above this droplet concentration the emulsion viscosity increases steeply and the emulsion gains gel-like properties, e.g. elasticity, viscoelasticity, plasticity. The precise nature of the dependence of the viscosity on volume fraction is mainly determined by the nature of the colloidal interactions between the droplets. For example, when there is a relatively strong repulsion or attraction between the droplets, their *effective* volume fraction may be much greater than their *actual* volume fraction, which leads to a large increase in emulsion viscosity (Section 1.3). For products whose rheological characteristics are not dominated by the effects of strong colloidal interactions, real food products can be used to illustrate the influence of droplet volume fraction on emulsion viscosity. Full-fat milk ($\phi \sim 0.04$) is a fairly low-viscosity fluid, heavy cream ($\phi \sim 0.36$) is a fairly high-viscosity fluid and mayonnaise ($\phi \sim 0.80$) is a viscoelastic gel. In principle, it is therefore possible for food manufacturers to control the rheology of food emulsions by varying their

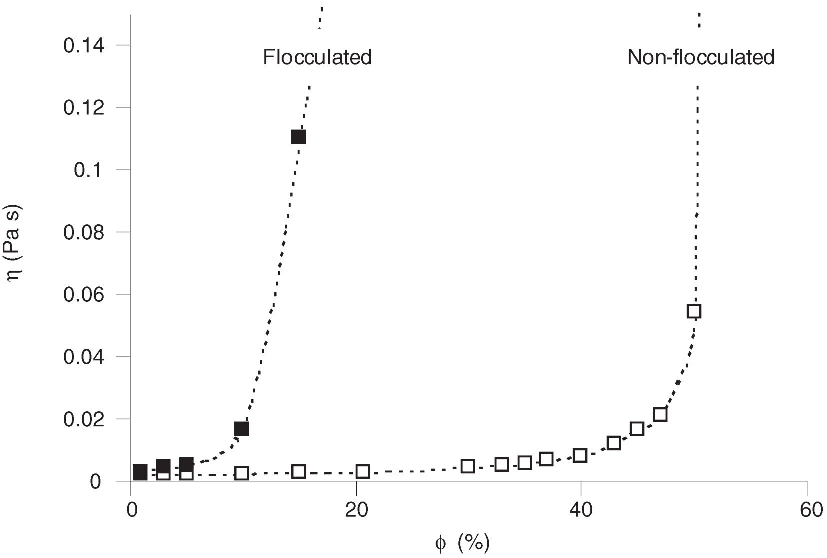

Fig. 1.6 Dependence of the apparent viscosity on droplet volume fraction for flocculated (SDS = 80 mM) and non-flocculated (SDS = 7 mM) monodisperse *n*-hexadecane oil-in-water emulsions (shear stress = 0.1 Pa). The emulsions contained 0.86 μm diameter droplets. Droplet flocculation was induced by adding excess SDS micelles into the continuous phase.

disperse phase volume fraction. In practice, this is rarely feasible because of constraints associated with ingredient cost, nutritional attributes, flavor or shelf-life. Consequently, it is more common for food manufacturers to modify emulsion rheology using other methods, e.g. by adding thickening or gelling agents (see below).

1.6.2 Rheology of component phases

Theoretically, the viscosity of a fluid emulsion is directly proportional to the viscosity of the continuous phase (Eqns 1.4, 1.10 and 1.11). Consequently, any alteration in the rheology of the continuous phase will have a corresponding influence on the rheology of the whole emulsion. For food emulsions with droplet concentrations below the maximum packing volume fraction (ϕ_c), the most effective means of modifying their rheology is to add thickening or gelling agents. It is for this reason that polysaccharides and proteins are often incorporated into the aqueous phase of food emulsions to modify their texture or improve their creaming stability, e.g. beverages, salad dressings, sauces, dips and desserts (Pettit *et al.*, 1995; Pal, 1996; Ford *et al.*, 1997). Similarly, the rheology of water-in-oil emulsions, such as margarine or butter, is largely determined by the rheological characteristics of the fat crystal network present in the oil phase (Moran, 1994;

Buchheim and Dejmek, 1997). Hence, the overall rheology of these products is usually manipulated by altering the structure, concentration or interactions of the fat crystals, e.g. by altering the temperature, history or composition of the fat phase.

It should be noted that in emulsions where the overall rheology is dominated by the rheology of the continuous phase, the presence of emulsion droplets can still have a significant impact. For example, the rheology of many salad dressing products is largely determined by the presence of biopolymers in the aqueous phase (Dickinson, 1992, 1998). These biopolymers act as thickening agents that give the salad dressings high viscosities, shear-thinning behavior and stability against droplet creaming. Nevertheless, many biopolymers promote the formation of a three-dimensional network of aggregated droplets due to their ability to induce depletion flocculation (Dickinson, 1998; McClements, 1999). This droplet network has been shown to modify significantly the overall rheological characteristics of model salad dressing products (Manoj *et al.*, 1998a,b; Dickinson, 1998). For example, the formation of a network of aggregated droplets tends to increase the viscosity and the extent of shear-thinning in oil-in-water emulsions containing non-adsorbed biopolymers in the aqueous phase (Dickinson, 1998). Biopolymers that promote droplet aggregation through a bridging mechanism may also have a pronounced influence on emulsion rheology (Dickinson, 1998).

Another example of the influence of droplets on the rheology of emulsions whose properties are dominated by the rheology of the continuous phase is provided by filled whey protein gels (McClements *et al.,* 1993; Dickinson and Hong, 1995; Dickinson and Yamamoto, 1996). These filled gels are created by heating oil-in-water emulsions that contain a significant amount of whey protein in the continuous phase above a temperature where the proteins unfold and form a three-dimensional network of aggregated molecules. The oil droplets can either act as structure *promoters* or structure *breakers* depending on the nature of their interaction with the gel network. When the droplets are stabilized by whey protein, the attractive interactions between the adsorbed proteins and those in the network reinforce the network and increase the gel strength. Conversely, when the droplets are stabilized by small molecule surfactants (which do not interact strongly with the protein network) the presence of the droplets tends to weaken the network and decrease the gel strength. Quite complex rheological behavior can be observed in emulsions containing mixtures of proteins and small molecule surfactants (Dickinson and Hong, 1995; Dickinson and Yamomoto, 1996). The shear modulus of filled-gels containing protein-stabilized oil droplets increases dramatically when a small amount of surfactant is added to the system, but decreases at higher surfactant values. This is probably because surfactants can interact directly with the proteins, thereby modifying their gelation characteristics, and because surfactants can displace proteins from the droplet surface, thereby altering the interactions of the droplets with the gel network. The influence of the emulsion droplets on the overall rheology also depends on the size of the droplets relative to the size of the pores in the gel network (McClements *et al.,* 1993; Yost and Kinsella, 1993). If the droplets are larger than the pore size, they tend to disrupt

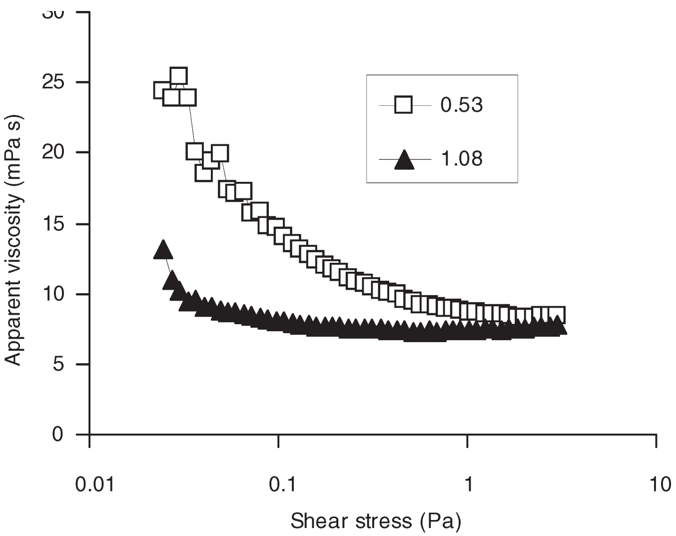

Fig. 1.7 Shear thinning behavior of concentrated non-flocculated monodisperse *n*-hexadecane oil-in-water emulsions with different droplet diameters.

the network and decrease the gel strength, but if they are smaller than the pore size they are easily accommodated into the network without disrupting it.

The rheology of the dispersed phase has a minor influence on the rheology of most fluid food emulsions because the droplets are covered by a fairly viscoelastic membrane, which means they behave similarly to rigid spheres (Tadros, 1994; Walstra, 1996a,b). However, in concentrated oil-in-water emulsions, such as mayonnaise, the rheology of the droplets can play an important role. In these systems, the droplets may deform on the application of a stress and therefore an emulsion containing fluid droplets would be expected to have a lower resistance to deformation than a suspension containing rigid particles.

1.6.3 Droplet size

The influence of the droplet size and the droplet size distribution on the rheology of an emulsion depends on the disperse phase volume fraction and the nature of the colloidal interactions. In the absence of appreciable colloidal interactions, droplet size alters emulsion rheology because of its influence on the relative importance of Brownian motion and shear stress effects (Mewis and Macosko, 1994). This effect is appreciable only in emulsions with relatively high droplet concentrations, i.e. $\phi > 0.45$ (Pal *et al.*, 1992; Pal, 1996; Dickinson, 1998). Nevertheless, if there are appreciable long-range repulsive interactions between droplets, this type of shear-thinning effect can occur at lower droplet concentrations (Chanamai and McClements, 2000a). For example, Fig. 1.7 shows that there is strong shear thinning behavior in monodisperse oil-in-water emulsions containing 40 vol%

electrically charged droplets, and that the apparent viscosity decreases appreciably when the droplet diameter increases from 0.53 to 1.08 μm. The influence of droplet size on emulsion viscosity becomes less important at higher shear stresses because shear forces then dominate Brownian motion effects (Fig. 1.7).

The mean droplet size and degree of polydispersity has a particularly significant influence on the rheology of highly concentrated emulsions (Liu and Masliyah, 1996; Pal, 1996). In emulsions containing non-flocculated droplets the maximum packing factor (ϕ_c) depends on the polydispersity. Droplets are able to pack more efficiently when they are polydisperse and therefore the viscosity of a concentrated polydisperse emulsion is less than that of a monodisperse emulsion with the same droplet volume fraction. It is partly because of this fact that it is possible to make mayonnaise products that have droplet concentrations above the theoretical maximum packing volume fraction of monodisperse droplets.

1.6.4 Colloidal interactions

The nature of the colloidal interactions between the droplets in an emulsion is one of the most important factors determining its rheological behavior. When the interactions are long range and repulsive, the effective volume fraction of the dispersed phase may be significantly greater than its actual volume fraction ($\phi_{eff} = \phi(1+\delta/r)^3$), and so the emulsion viscosity increases (Section 1.4.2). When the interactions between the droplets are sufficiently attractive, the effective volume fraction of the dispersed phase is increased due to droplet flocculation, which also results in an increase in emulsion viscosity (Section 1.4.3). The rheological properties of an emulsion therefore depend on the relative magnitude and range of the attractive (mainly van der Waals, hydrophobic and depletion) and repulsive (mainly electrostatic, steric and thermal fluctuation) interactions between the droplets (McClements, 1999). Manipulation of the colloidal interactions between droplets can therefore be used to control the rheological properties of food emulsions effectively. The viscosity of oil-in-water emulsions can be increased appreciably by promoting droplet flocculation (Dickinson, 1998). Droplet flocculation can be achieved in a variety of different ways depending on the nature of the system involved. For example, droplet flocculation can be induced by adding biopolymers to increase the depletion attraction (Dickinson and Golding, 1997; Manoj *et al.*, 1998a,b; Tuinier and de Kruif, 1999), by adding biopolymers to cause bridging flocculation (Dickinson, 1998), by altering the pH or ionic strength to reduce electrostatic repulsion (Hunt and Dalgleish, 1994; Demetriades *et al.,* 1997), and by heating protein-stabilized emulsions to increase hydrophobic attraction (Demetriades and McClements, 1998). The influence of droplet flocculation on the viscosity of monodisperse oil-in-water emulsions is clearly shown in Fig. 1.6. The droplets in the flocculated emulsion were made to flocculate by adding surfactant micelles (sodium docecyl sulphate; SDS) to the continuous phase to promote depletion flocculation. The emulsion containing flocculated droplets had a higher viscosity than the emulsion containing non-flocculated droplets at low droplet concentrations, and exhibited stronger shear thinning behavior. In addition, the droplet volume fraction at which the emulsion viscosity increased sharply was considerably reduced when the emulsion droplets were flocculated. The reasons for the change in emulsion rheology caused by droplet flocculation are discussed in Section 1.4.3.

1.6.5 Droplet charge

Many food emulsions contain droplets that have an electrical charge due to the adsorption of ionizable surface-active components, such as ionic surfactants, proteins or polysaccharides (McClements, 1999). The charge on a droplet can influence the rheological properties of an emulsion in a number of ways. First, droplet charge influences emulsion rheology due to the *primary electroviscous effect* (Pal, 1996; Larson, 1999). As a charged droplet moves through a fluid, the cloud of counter-ions surrounding it becomes distorted. This causes an attraction between the charge on the droplet and that associated with the cloud of counter-ions that lags slightly behind it. This attraction opposes the movement of the droplets and therefore increases the emulsion viscosity because more energy is needed to cause the droplets to move at the same rate as uncharged droplets. Mathematical analyses of this phenomenon have shown that the primary electroviscous effect is fairly small in most colloidal dispersions (Hiemenz and Rajagopalan, 1997), particularly at the relatively high salt levels present in most food emulsions.

Secondly, the droplet charge influences the emulsion rheology through the *secondary electroviscous effect,* which accounts for the fact that electrically charged emulsion droplets cannot approach as closely together as can uncharged droplets because of electrostatic repulsion (Hiemenz and Rajagopalan, 1997). Consequently, the effective diameter of the droplets is increased, which leads to an increase in emulsion viscosity and a decrease in the droplet volume fraction where the viscosity increases steeply (Eqns 1.13 and 1.15). Secondary electroviscous effects can have a pronounced impact on the rheology of emulsions when the Debye screening length is of the same order of magnitude as the droplet radius, i.e. $\kappa^{-1} \sim r$. Dickinson (1998) has shown that the viscosity of oil-in-water emulsions stabilized by an anionic surfactant (SDS) are considerably larger than those stabilized by a non-ionic surfactant (Tween 20) at high droplet volume fractions because of this effect. The secondary electroviscous effect has a dramatic effect on the rheological properties of emulsions near the maximum packing volume fraction. Figure 1.8 shows the droplet size dependence of the dynamic shear rheology (G^* and δ) of 25 wt% octadecane oil-in-water emulsions containing negatively charged droplets (Weiss and McClements, 2000). When the droplet radius was below about 80 nm the emulsions had solid-like characteristics ($\delta \sim 0^{\circ}$) and a yield stress, but when the radius was above about 90 nm the emulsions had fluid-like characteristics ($\delta \sim 90^{\circ}$). This dramatic change in emulsion rheology occurred because the effective volume fraction of the droplets increased as the droplet radius decreased (Eqn 1.13), and so the maximum packing volume fraction was exceeded below a particular droplet radius.

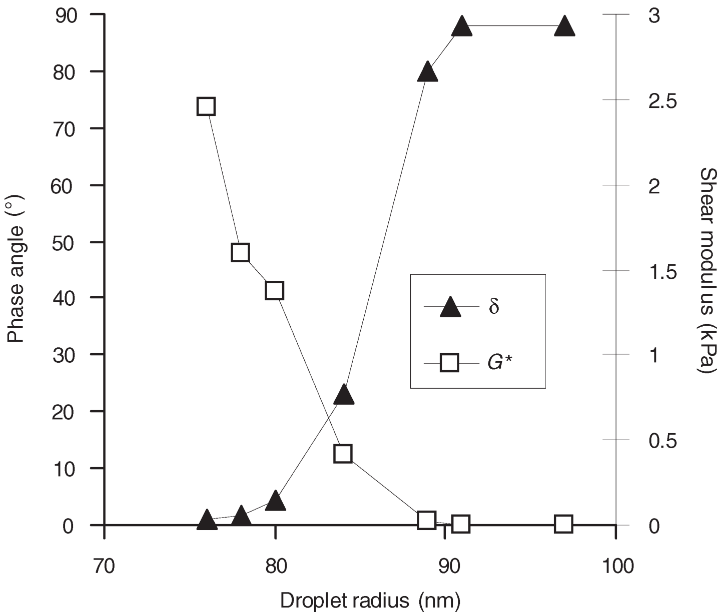

Fig. 1.8 Dependence of complex shear modulus and phase angle of 25 wt% *n*-octadecane oil-in-water emulsions on mean droplet radius.

Thirdly, the droplet charge may influence emulsion rheology through the *tertiary electroviscous effect* (Hiemenz and Rajagopalan, 1997), which accounts for the fact that the thickness of adsorbed layers may change with the ionic environment (pH, ionic strength). This effect will have the biggest impact on the rheology of emulsions stabilized by relatively thick layers of charged biopolymers, e.g. some proteins and polysaccharides. As the pH or ionic strength of the aqueous phase is altered, the electrostatic interactions between the biopolymer chains adsorbed to the interface change, which may lead to either an increase or decrease in the thickness of the adsorbed layer.

Finally, it should be noted that one of the most dramatic influences of droplet charge on emulsion rheology occurs in electrostatically stabilized systems. If the pH or the ionic strength of the aqueous phase is altered so that the electrostatic repulsion between the droplets is no longer sufficient to overcome the attractive interactions, the droplets will flocculate. Droplet flocculation causes an appreciable increase in emulsion viscosity and causes the emulsion to become strongly shear thinning (see Section 1.6.4).

1.7 Future trends

There has being a growing emphasis on understanding the colloidal basis of the rheology of food emulsions, rather than just treating them as a 'black box' whose properties could be characterized in terms of certain rheological parameters. Researchers are attempting to relate quantitatively the rheological properties of food emulsions to the characteristics, interactions and spatial distribution of the droplets they contain. A wide variety of analytical, mathematical and computational techniques are being developed and utilized to achieve this objective. Powerful commercial instruments are widely available to quantify the colloidal characteristics of both dilute and concentrated emulsions, e.g. droplet size, concentration and electrical charge (Hunter, 1998; Meyers, 2000; Hills *et al.,* 2001). Theoretical, computational and experimental work is providing a much better understanding of the various types of colloidal interactions that operate between emulsion droplets (Bergenstahl and Claesson, 1997). The cost, sensitivity and range of commercial rheometers are continually improving (Mewis and Macosko, 1994). New rheometers are being developed that will enable researchers to measure changes in the structure of emulsions as they are being sheared or compressed, e.g. rheo-optics (Mewis *et al.*, 1998), rheo-acoustics (Chanamai *et al.*, 2000) and rheo-NMR (nuclear magnetic resonance) techniques (Sinton *et al.,* 1994; McCarthy and Kerr, 1998). Traditional microscopic techniques are being refined so that they can be used to characterize the microstructure of delicate materials, such as emulsions (Dickinson, 1995). In addition, new microscopic technologies are being developed to characterize the organization of molecules at an interface (Patino and Nino, 1999; Pluchnett *et al.*, 2001; Morris *et al.*, 2001).

Advances in our understanding of the relationship between emulsion rheology and colloidal characteristics are also being made through development of more comprehensive physical theories (Bremer *et al.*, 1993; Larson, 1999; Marangoni, 2000) and the utilization of powerful computational techniques (Lodge and Heyes, 1999; Dickinson, 2000; Whittle and Dickinson, 2001). The application of these new concepts and tools will eventually lead to a much better understanding of the colloidal basis of emulsion rheology. This knowledge will enable food manufacturers to design foods in a more rational fashion, which should eventually lead to improvements in product quality and reductions in manufacturing costs.

1.8 Sources of further information and advice

There is a vast literature on the rheological properties of colloidal dispersions which can be used by food scientists to understand the rheological properties of food emulsions. Much of this work has been summarized in review articles (Tadros, 1994), book chapters (Hunter, 1986; Mewis and Macosko, 1994; Dickinson, 1998; Hiemenz and Rajagopalan, 1997) and monographs (Sherman, 1968; Larson, 1999). In addition, many commercial manufacturers of analytical instruments provide extensive literature about the principles of rheology in the form of application notes and manuals.

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2

Phase transitions, food texture and structure

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2.1 Introduction

Foods are composed of bioploymers and aqueous solutions of water containing dissolved sugars and ions. The former are large molecules, often called macromolecules, such as proteins, polysaccharides, and lipids from a wide range of plant and animal sources. Foods may be viewed as edible structures that are created as a result of the responses of proteins, polysaccharides, and lipids in aqueous media to different processing conditions. Most, if not all, of the responses are physical in nature. Water is an important component in foods and plays a major role in the creation of edible structures and their storage stability.

2.1.1 Transitions in foods

The three basic physical states in food are: solid, liquid, and gas (air, carbon dioxide). While one can envision liquids and gases unambiguously, among solid foods there is a range of hardness, from soft, sometimes called semi-solid or gel, to very hard that must still be amenable to comminution in the mouth for consumption. The expression 'transition' refers to a change in physical state and in foods the transition of concern is often either from liquid to solid or solid to liquid.

The transitions are caused primarily by a change in temperature (heating and/or cooling) or pressure (Roos, 1995). However, auxiliary conditions, such as pH and presence of divalent ions, as well as enzymatic action, aid liquid to solid transitions. For example, gels can be created from casein either by enzymatic action followed by precipitation with Ca^{2+} or by acid coagulation. The thermodynamic definition of a phase transitions is based on changes occurring in Gibbs free energy, G, and chemical potential, μ, at the transition temperature (Sperling, 1986, 2001; Roos, 1998). A first-order transition is defined as one in which the first derivatives of G and μ with respect to temperature exhibit discontinuities at the transition temperature. Concomitantly, a step change occurs in enthalpy, entropy, and volume at the transition temperature.

Important first-order transitions in foods include crystallization, melting, protein denaturation, and starch gelatinization. In pure materials, first-order transitions occur at well-defined and material-specific temperatures. However, invariably in a food, many compounds are present, so that a transition may occur over a range of temperatures instead of a fixed temperature. A second-order transition is defined as one in which the second derivatives of G and μ with respect to temperature exhibit discontinuities at the transition temperature. Although glass-transition of amorphous foods has the properties of a second-order transition, there are no well-defined second-order transitions in foods (Roos, 1998).

2.1.2 Phase transition temperatures

In order to study and understand a phase transition, it is important to know the magnitudes of the temperatures over which the transition takes place. The differential scanning calorimeter (DSC) is used extensively to determine first-order and glass transition temperatures, more so than other techniques. A DSC measures the rate of heat flow into or out of a cell containing a sample in comparison to a reference cell in which no thermal events occur. It maintains a programmed sample cell temperature by adjusting heat flow rates. It should be noted that data obtained with a DSC depend to some extent on the heating/cooling rate that should be specified when discussing the data; common heating rates are 5 °C/min and 10 °C/min. Heat flow versus temperature diagrams are known as thermograms.

The DSC output for gelatinization of starch in a Russet Burbank potato sample under excess water conditions, based on data reported by Bu-Contreras (2001), and an idealized output curve of starch with intermediate water content are shown in Fig. 2.1. For the excess moisture curve, drawing a tangent at the start and end points of transition, one can determine well-defined gelatinization initiation, T_o, peak, T_p, and end, T_e, temperatures, respectively. Further, the area under the curve is a measure of the energy required for the transition, ΔH. For gelatinization under intermediate water conditions two endothermic peaks are observed. Often, the temperature at the end of gelatinization is called the starch melting temperature. Because foods are complex mixtures of several compounds, their freezing and melting occur over a range of temperatures. We note here that a thermogram of melting is similar in shape to that of gelatinization under excess water conditions and the melting temperature, T_m, corresponds to T_p.

At the glass transition temperature, T_g, the amorphous portions of a polymer soften and flow. Owing to coordinated molecular motion, in the glass transition

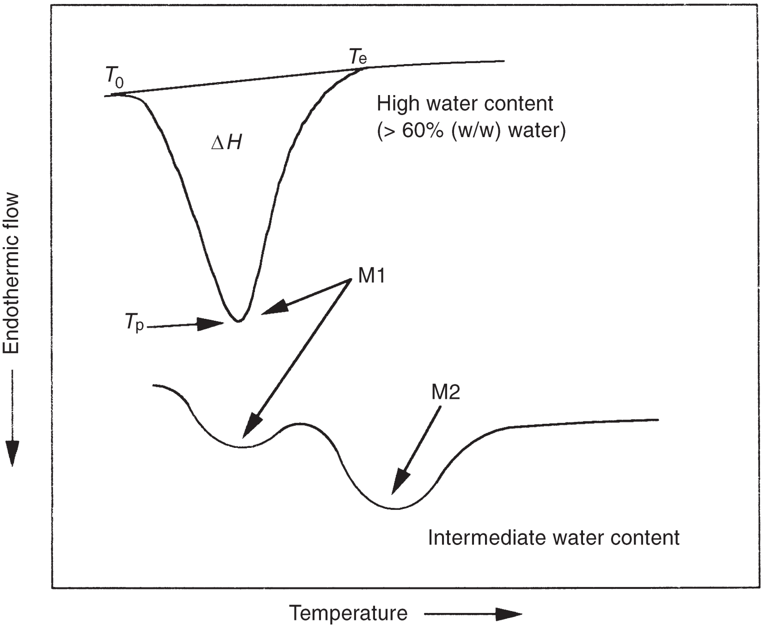

Fig. 2.1 DSC output for gelatinization of starch under excess water conditions (Bu-Contreras, 2001), and an idealized output curve of starch with intermediate water content. Gelatinization temperatures are: initiation, T_0, peak, T_p, and end, T_e. At low water content the endotherm shows two peaks (Hoseney, 1998), M1 and M2, respectively.

region, the polymer softens and the modulus decreases by about three orders of magnitude. Figure 2.2 illustrates a DSC curve for an idealized glass transition in which T_g can be taken as the temperature at which half the change in heat capacity, ΔC_p, has occurred (Sperling, 1986). Besides DSC, other experimental techniques that have been used to determine T_g include dilatometry, static and dynamic mechanical measurements, as well as dielectric and magnetic measurements (Sperling, 1986, 2001). Acceptance of the important role of glassy and rubbery states, and glass transition to understand better processing, storage, and stability of foods was largely due to the efforts of Levine and Slade; much useful information can be found in their reviews (e.g. Levine and Slade, 1992; Slade and Levine, 1998).

2.1.3 Length scale of food molecules and foods

The structure of a food is the result of specific and non-specific interactions at levels ranging from the molecular (–0.1 to 100 nm) to the supramolecular (2×10^3 to 10^7 nm) (Clark and Ross-Murphy, 1987; Aguilera and Stanley, 1999). Specific interactions at the molecular level are between distinct atoms that result in covalent bonds, hydrogen bonding, enzyme–substrate coupling, as well as hydrophobic

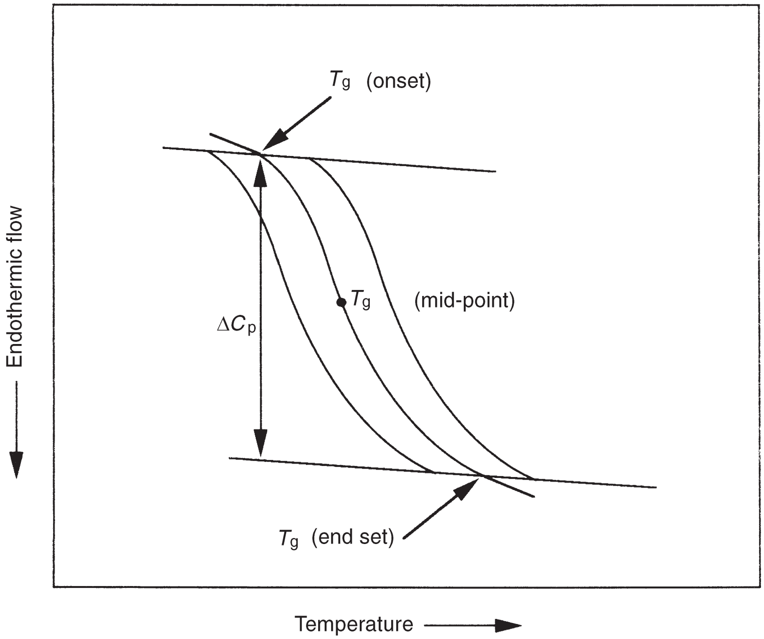

Fig. 2.2 A DSC curve for an idealized glass transition in which T_g can be taken as the temperature at which one-half of the change in heat capacity, ΔC_p, has occurred (Sperling, 1986).

interactions. Much of the work in understanding foods, especially biopolymer gels, has been based on studies at the molecular level from which the structural details at the supramolecular level have been inferred.

While different spectroscopic methods are suitable for studies at the molecular length scales, for supramolecular structures, that have length scales >2000 nm, available techniques include electron and light microscopy, and rheology. For various microscopy techniques one may consult the text by Aguilera and Stanley (1999) or other sources.

2.2 Rheological techniques for studying phase transitions

In this chapter, rheological techniques applicable to studies on phase transitions will be discussed in brief and additional discussion on rheology can be found in Chapter 6. Among the rheological techniques, small amplitude oscillatory tests, popularly known as dynamic rheological tests, have found extensive use in studies on phase transitions. They have been used to determine gel times, gel and melting temperatures, structure development during gelation, and structure loss during melting, and to characterize starch gelatinization and glass transition.

In a dynamic rheological experiment, a sinusoidal oscillating stress or strain with a frequency ω is applied to the material, and the phase difference between the oscillating stress and strain as well as the amplitude ratio are measured. The information obtained should be equivalent to data from a transient experiment at time $t = \omega^{-1}$. In dynamic tests, a food sample is subjected to a small sinusoidally oscillating strain or deformation $\gamma(t)$ at time t. The applied strain generates two stress components in the viscoelastic material: an elastic component in line with the strain and a 90° out-of-phase viscous component. For deformation within the linear viscoelastic range, the generated stress can be expressed in terms of an elastic or storage modulus G' and a viscous or loss modulus G''. The storage modulus G' expresses the magnitude of the energy that is stored in the material or recoverable per cycle of deformation. G'' is a measure of the energy which is lost as viscous dissipation per cycle of deformation.

For a viscoelastic material the resultant stress is also sinusoidal but shows a phase lag of δ radians when compared with the strain. The phase angle δ covers the range of 0 to $\pi/2$ as the viscous component increases. It should be noted that if G' is much greater than G'', the material will behave more like a solid, i.e. the deformations will be essentially elastic or recoverable. However, if G'' is much greater than G' the energy used to deform the material is dissipated viscously and the material's behavior is liquid-like. The viscoelastic moduli determined over a range of frequencies will indicate transition zones corresponding to relaxation processes dependent on the material's structure (Ferry, 1980).

For a specific food, magnitudes of G' and G'' are influenced by frequency, temperature, and strain. For strain values within the linear range of deformation, G' and G'' are independent of strain. The loss tangent is the ratio of the energy dissipated to that stored per cycle of deformation. One can also employ notation using complex variables and define a complex modulus $G^*(\omega)$:

$$|G^*| = \sqrt{(G')^2 + (G'')^2} \qquad [2.1]$$

We note that the dynamic viscosity and the dynamic rigidity are components of the complex dynamic viscosity, η^*:

$$\eta^* = (G^*/\omega) = \eta' - i(G'/\omega) \qquad [2.2]$$

where ω is the frequency of oscillation; i is $\sqrt{-1}$; the real part of the complex viscosity η' is equal to (G''/ω) and the imaginary part η'' is equal to (G'/ω). One can also determine the loss modulus G'', from oscillatory shear data using the expression:

$$G'' = \omega\eta' \qquad [2.3]$$

2.2.1 Dynamic rheological tests for phase transitions

Dynamic rheological tests are non-destructive and do not interfere with either gel/structure formation or softening of a structure, the time involved in the measurements is short relative to the characteristic times of the gelation and softening processes, and the results are expressible in fundamental terms so that they can be related to the structure of the network (Ross-Murphy, 1984).

An experiment in which the strain is varied over a range of values is essential to determine the linear viscoelastic range. The limit of linearity can be detected when dynamic rheological properties (e.g. G' and G'') change rapidly from their almost constant values. There are two tests that can be used to examine phase transitions in foods (Rao, 1999):

1. **Temperature sweep studies** in which G' and G'' are determined as a function of temperature at fixed frequency (ω). This test is well suited for studying gel formation during cooling of a heated food polymer (e.g. pectin, gelatin) dispersion, and gel formation by a protein dispersion and gelatinization of a starch dispersion during heating.
2. **Time sweep studies** in which G' and G'' are determined as a function of time at fixed ω and temperature. This type of test, often called a gel cure experiment, is well suited for studying structure development in physical gels.

Additionally, frequency sweep studies in which G' and G'' are determined as a function of frequency (ω) at a fixed temperature are useful for probing the structure of viscoelastic foods.

Once a food gel has been created, a frequency sweep, conducted over a wide range of oscillatory frequencies, can be used to examine its properties. The behavior of the moduli G' and G'' over a wide range of frequencies provides insights into the nature of the gel, such as whether it is a ‘weak’ or a ‘strong’ gel.

Specific important first-order transitions in food will be described next, followed by glass transition phenomena. Among first-order transitions, starch gelatinization will be covered separately because rheological behavior of starch dispersions before and after gelatinization is, to a large extent, governed by the state of starch granules.

2.3 Starch gelatinization

Starch is used widely as a thickening/gelling agent in foods. Although found in many plants, starches from the cereal grains corn (maize) and wheat are used extensively in the food industry (Hoseney, 1998). However, starches from other sources, such as potato and sago palm, are also being used. In addition, modified starches, such as those cross-linked chemically, are used for special applications.

Starch is a homopolymer made up of the monomer D-glucose, which, strictly speaking, should be called α-D-glucopyranose. Two types of polymer are distinguishable: amylose, a linear polymer, and amylopectin, which is highly branched. Amylose, the linear starch polymer, typically has molecular weights ranging from 10^5 to 10^6 and 500 to 5000 glucose residues per molecule. It has been determined that some α-(1→6)-branching exists in the amylose molecule on the order of 2–8 branch points per molecule, with side chains ranging in length from 4 to >100 glucose units (Galliard and Bowler, 1987). Native starches contain approximately 25% amylose, yet there are some mutant varieties of corn that contain 85% amylose (high-amylose corn) or no amylose (waxy maize) (Whistler and Daniel, 1985). Amylopectin has a molecular weight greater than 10^8 with 5% of its structure containing α-(1→6)-branch points (Galliard and Bowler, 1987). It is generally agreed that the amylopectin chains are assembled in a cluster structure. Starch also contains other constituents that affect properties of starch. For phase transitions and for texture, it is important to note that native starches are semi-crystalline materials, with crystallinity between 30 and 40% (Roos, 1998), and are found in the form of granules.

2.3.1 Gelatinization temperature and extent of gelatinization

Temperature versus enthalpy profiles similar to those shown in Fig. 2.1 are obtained when starches are heated either in excess water, i.e. when water : starch ratios are equal to 2 or greater, or under intermediate water content. The start of the endothermic peak at T_o corresponds to loss of birefringence, in the form of the typical 'Maltese cross,' when the starch granule is viewed under polarized light. A single endotherm, such as that obtained under excess water conditions, is referred to as the M1 endotherm. Based on data obtained with DSC (Roos, 1995), it can be said that many starches heated in excess water conditions exhibit gelatinization temperature ranges of about 7–10 °C and the gelatinization initiation temperatures (T_o) range from about 50 to 68 °C.

As the amount of water is reduced, the DSC peak for starch gelatinization widens. For example, for wheat starch, the range of temperatures for gelatinization is about 7 °C in excess water, and at low water content (water:starch, 0.35:1) it is greater than 30 °C and the endotherm shows two peaks (Hoseney, 1998), M1 and M2, respectively. In addition, the starch does not gelatinize completely even at 100 °C. Therefore, when foods containing starch, such as rice and spaghetti, are boiled, the starch granules at or near the surface gelatinize easily, but those in the interior of the food may not gelatinize. The extent of gelatinization of starch inside a food will depend on the temperature and water content which in turn depend on diffusion of water. For wheat starch and water, based on enthalpies of gelatinization, Fukuoka *et al.* (2002) proposed an empirical equation for extent of gelatinization (EG) as a function of moisture content (m, g water/g starch) and temperature (T, °C):

$$\mathrm{EG}\,(m, T) = \frac{3.15m/(1 + m) - 0946}{1 + \exp[-0.1792(T - 69.1)]} \qquad [2.4]$$

It should be noted that Eqn 2.4 is valid for $0.54 \leq m \leq 1.5$.

It has been known for some time that addition of sucrose and other sugars increases the starch gelatinization temperature. In addition, starch granule swelling is impeded so that at very high sugar concentration the granules hardly swell. For wheat starch, as the water activity of a sugar solution decreased, gelatinization temperature increased. Further, at equal water activities different sugars increased the gelatinization temperature to different extents. Thus, one can control the gelatinization temperature of a starch by adding a different amount and/or type of sugar (Hoseney, 1998).

Salts also affect gelatinization temperature, but as they also affect the taste of a product they are not useful agents for controlling gelatinization temperature. The effect of a salt on gelatinization temperature depends on whether it is chaotropic or non-chaotropic. It is well known that when calcium chloride, a chaotropic salt, is added in proper amount, starch gelatinizes at room temperature. Fats and emulsifiers have a limited effect on gelatinization temperature, but they affect granule swelling after gelatinization.

2.3.2 Flory–Huggins relationship

The Flory–Huggins relationship (Eqn 2.5) relates the melting point of a polymer after the addition of a diluent, T_m, and that in the pure state, T_m^0 (Sperling, 1986; author notes that the same discussion on the Flory–Huggins relationship can be found in the third edition of Sperling's book, except that there is a typographical error in the equation):

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \frac{R}{\Delta H_m} \frac{V_u}{V_1}(v_1 - \chi_2 v_1^2) \qquad [2.5]$$

where R is the gas constant, ΔH_m is the heat of melting per mole of crystalline repeating unit, v_1 is the volume fraction of diluent, χ_1 is the Flory solvent interaction parameter, and V_u and V_1 are molar volumes of the polymer repeat unit and the solvent, respectively. The quantity χ_1 is a function of the energy of mixing per unit volume and has been interpreted in several ways (Sperling, 1986).

Based on Eqn 2.5, a plot of $1/T_m$ against v_1, the volume fraction of diluent, results in a straight line. The Flory–Huggins equation has been used to relate starch gelatinization temperatures as a function of water content (Donovan, 1979; Biliaderis *et al.*, 1986) and sugar content (Lelievre, 1976). One practical use of Flory–Huggins plots is that starch gelatinization temperatures can be estimated at different moisture and sugar concentrations within the range of the experimental variables. However, caution should be exercised in using the Flory–Huggins equation because starch is not a pure compound, and gelatinization is a non-equilibrium and non-isothermal phenomenon.

2.3.3 Rheological and structural changes during starch gelatinization

When a starch dispersion (STD) is heated continuously in excess water, a series of events takes place, including: loss of birefringence and swelling of starch granules, additional swelling of the granules and partial solubilization of starch, and rupture of the granules. Swelling of the starch granules results in an increase in their volume fraction, while rupture results in a decrease. The average initial and

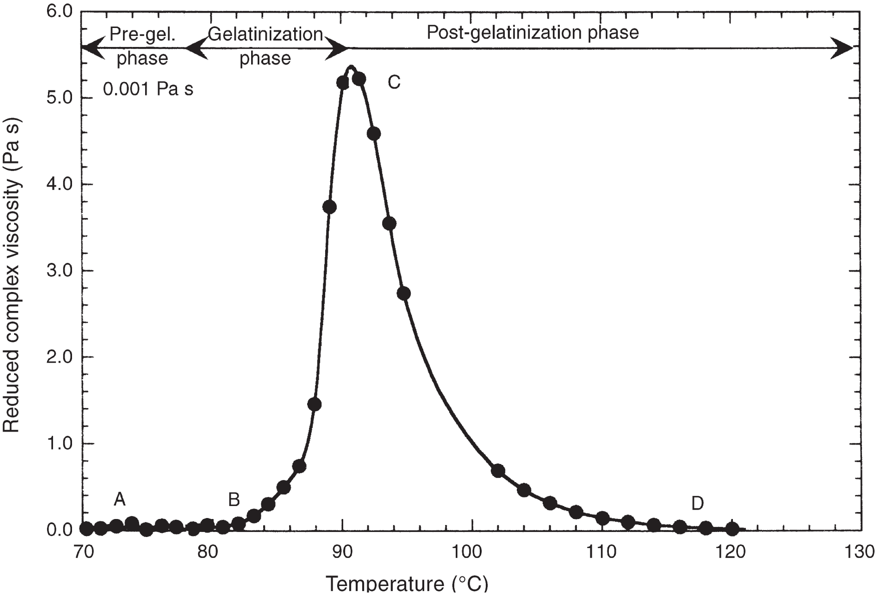

Fig. 2.3 Shape of viscosity versus temperature curve for a 5% corn starch dispersion; other native starches also exhibit such curves. Reduced complex viscosity is obtained by dividing the complex viscosity at a given oscillatory frequency by that at a reference frequency.

maximum diameters before rupture vary with the botanical source of starch. For example, the average diameters of hydrated raw granules of corn, cowpea and cross-linked waxy maize starches were reported to be 13.5, 18.7, and 16.3 μm, while those heated at 80 °C for 30 minutes were: 32.8, 67.0, and 39.8 μm, respectively (Rao *et al.*, 1997).

Based on studies by Yang and Rao (1998) and Liao *et al.* (1999), the general shape of viscosity versus temperature for a corn starch dispersion is shown in Fig. 2.3. In these studies, dynamic rheological data were obtained during temperature sweeps at several oscillatory frequencies using a parallel plate (gap 500 μm) geometry of a controlled-stress rheometer. Because the profiles of η^* versus temperature at a specific frequency were similar in shape, by choosing an arbitrary reference frequency (ω_r), all the η^*-temperature curves at the different frequencies were reduced to a single curve. Figure 2.3 is the master curve of reduced complex viscosity η_R^* data obtained on a 3.5% corn STD at different heating rates and frequencies.

The shape of the curve in Fig. 2.3 reflects the aforementioned changes in the volume fraction of starch granules, which in turn are due to changes in the size of the granules as a starch dispersion is heated. Initially at low temperatures, the granules are in the raw state and the volume fraction is low. As the granules swell, owing to water absorption as they are heated (segment A–B–C), the volume fraction increases and reaches a maximum (C). With further heating, the granules rupture and disintegrate, resulting in a gradual decrease in the volume fraction of the granules in the dispersion (segment C–D). The rupture of granules also results in the release of amylose that contributes to the viscosity of the continuous phase of the starch dispersion. Thus, in Fig. 2.3, the segment C–D is not a mirror image of the segment ABC. The leached amylose and the granule remnants contribute to a viscosity at point D that is higher than that at point A. Similar viscosity versus temperature profiles will be exhibited by other native STDs, while cross-linked STDs, owing to limited granule rupture, do not exhibit a sharp decrease in viscosity similar to segment CD.

During the initial stages of gelatinization of a native starch, granule swelling is accompanied by an increase in the granule size distribution in the STD, which is reflected as an increase in the standard deviation of the distribution (Okechukwu and Rao, 1995). This change in the polydispersity of the starch granules introduces important consequences in the rheological behavior of a suspension. For instance, dispersions of either raw or minimally heated starch granules exhibit shear-thickening rheological behavior, while those heated at relatively high temperatures and for long times exhibit shear-thinning behavior. A gelatinized starch dispersion may be regarded as a composite material consisting of swollen granules and granular fragments dispersed in a continuous biopolymer matrix (Evans and Haisman, 1979; Eliasson, 1986). Therefore, properties of the dispersed phase, the continuous phase, and interactions between them are three important considerations for understanding the rheology of gelatinized starch suspensions.

As mentioned earlier, the volume fraction of granules plays an important role in the magnitudes of rheological properties of starch dispersions (Evans and Haisman, 1979; Bagley and Christianson, 1982). However, because of their deformable nature, it is difficult to determine volume fraction of starch granules accurately and it is preferable to work with starch granule mass (SGM) fraction (cQ), where c is the dry starch concentration, w/w, and Q is the mass of hydrated starch granules per unit weight of dry starch. The SGM fraction takes into consideration both solids concentration and volume of starch granules after water absorption, and plays an important role in the viscosity (consistency) and yield stress (Bagley and Christianson, 1982; Okechukwu and Rao, 1995) as well as the viscoelastic properties (G', G'') of starch dispersions (Evans and Lips, 1992).

2.4 Food polymer gels

Flory (1953) defined a gel to be made up of cross-linked polymeric molecules linked to form a tangled interconnected network immersed in a liquid medium. At the molecular level, gelation is the formation of a continuous network of polymer molecules, in which the stress-resisting bulk properties (solid character) are imparted by a framework of polymer chains that extends throughout the gel phase. Most food polymers form physical gels, structured by the weak interactions: hydrogen, electrostatic, and hydrophobic, and are often thermoreversible and almost invariably occur in the presence of excess water or aqueous electrolyte.

Table 2.1 Structural characteristics of important gelling food polymers

Polymer	Structural characteristics
Carrageenans	Alternating β-1,3- and α-1,4-linked galactose residues; presence of 3,6-anhydride residues; presence of sulfated residues
Agarose	Alternating 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose
Alginates	Alternating blocks of β-1,4-linked D-mannuronic acid and α-1,4-linked L-guluronic acid residues
Pectins	α(1,4)-D-Galacturonic acid residues, partially methyl esterified; presence of 1,2-linked L-rhamnosyl residues
High-methoxyl	Degree of methylation higher than 50%
Low-methoxyl	Degree of methylation lower than 50%
Gellan gum	Glucose, glucuronic acid and rhamnose residues (2:1:1); presence of *O*-acetyl and *O*-L-glyceryl substituents
Gelatin	High content of pyrolidine residues (pro + hypro); presence of glycine as every third residue; isoelectric point affected by acid or alkali treatment
Bovine serum albumin	Globular protein (MW ~66 500 Da); isoelectric point ~ 5.1; characteristic secondary structure, with a specific content of α-helix, β-sheet and disordered peptide chain conformations; presence of disulfide bonds and one free sulfydryl group.
β-lactoglobulin	Globular protein (MW ~18 500 Da); characteristic secondary structure, with a specific content of α-helix, β-sheet and disordered peptide chain conformations; isoelectric point ~ 5.4–5.5; presence of two disulfide bridges and a free thiol group.

Source: Lopes da Silva *et al*. (1998).

Several food gel systems can be found (Table 2.1), whose gelling mechanisms range from systems formed purely by topological entanglements to the complex networks formed by ordered fibrous assemblies (Lopes da Silva *et al.*, 1998).

2.4.1 Classification of food gels

Gelling biopolymers may be divided in to 'cold-setting' or 'heat-setting', based on the two main gelation mechanisms. In the former, gelation is induced by cooling, and includes those biopolymer gels that occur in nature and provide structures in biological systems. In the latter, gelation occurs by heating, and includes those systems where gelation involves extensive denaturation of the biopolymer, e.g. thermally unfolded globular proteins. Clark and Ross-Murphy (1987) divided gels based on the level of order of the macromolecule both before and during the network formation: (1) those formed from disordered biopolymers, such as carrageenans, pectins, starch, gelatin, and (2) those networks that involve specific interactions between denser and less flexible particles, such as thermally denatured globular proteins and aggregated proteins from enzymatic or chemical action.

Depending on the macroscopic behavior of these systems, a useful distinction can be made between those gels that are free-standing as a consequence of the development of the three-dimensional network, called 'true gels', and those characterized by a tenuous gel-like network which is easily broken when submitted to a high enough stress, called 'weak gels' (Doublier *et al.*, 1992). Clark and Ross-Murphy (1987) indicated that 'strong gels' are those networks that have 'finite energy', and 'weak gels' are those systems that are transient in time. It is easy to distinguish between the entanglement network, the 'weak gel', and the 'strong gel' by using either a frequency sweep test or by examining strain dependence of the storage and loss moduli (Clark and Ross-Murphy, 1987). In the former, over the time scales studied, because the molecular rearrangements within the network are reduced, G' is higher than G'' throughout the frequency range, and is almost independent of frequency (ω). In a 'weak gel' there is a higher dependence on frequency for the dynamic modulus, suggesting the existence of relaxation processes occurring even at short time scales, and lower difference between values of G' and G'', indicating that a lower percentage of the stored energy is recovered. A well-known example of weak gel behavior is the viscoelastic behavior of xanthan gum dispersions.

2.4.2 Percolation theory

Useful theoretical treatments of food gels may be divided into: (1) those based on the pioneering theoretical work of Flory and Stockmayer, and subsequent modification incorporating the cascade approach of network formation, and (2) the percolation approach that describes the geometrical transition between disconnected and connected phases as the concentration of bonds in a lattice increases (de Gennes, 1979; Stauffer *et al.*, 1982). These theories, especially the cascade approach to food gels have been well reviewed by Clark and Ross-Murphy (1987), Clark (1992), and Lopes da Silva *et al.* (1998). Here, the percolation approach will be covered because it predicts critical exponents, including viscoelastic behavior at sol–gel transition.

Percolation describes the geometrical transition between disconnected and connected phases as the concentration of bonds in a lattice increases. It is the foundation for the physical properties of many disordered systems and has been applied to gelation phenomena (de Gennes, 1979; Stauffer *et al.*, 1982). At just above the gelation threshold, denoting the fraction of reacted bonds as p and $p = p_c + \Delta p$, p_c being the critical concentration (infinite cluster), the scaling laws (critical exponents) for gel fraction (S_∞) and modulus (E) are:

$$S_\infty \cong (p - p_c)^\beta;\ E \cong (p - p_c)^t \qquad [2.6]$$

Theoretical values for the exponents from the percolation theory are: $\beta = 0.39$, $t \sim 1.7–1.9$. The magnitude of the exponent t is greater than that of β because of dangling chains.

According to the percolation model, the chains in a swollen gel need not be

Gaussian, a requirement in the classical theory. For a true gel, where $\sigma >> E$, the stress (σ) versus strain (λ) relationship from the percolation model is:

$$\sigma = E\lambda^{5/2} \tag{2.7}$$

The viscoelastic properties of the system that characterize the sol–gel transition are also important features of the percolation model:

$$\eta_o \sim \varepsilon^{-s} \tag{2.8}$$

$$G_o \sim \varepsilon^{t} \tag{2.9}$$

$$G'(\omega) \sim G''(\omega) \sim \omega^{\Delta} \tag{2.10}$$

$$\eta^*(\omega) \sim \omega^{\Delta-1} \tag{2.11}$$

where ε is the relative distance to the gel point, $\varepsilon = (|p - p_c|/p_c)$ and $\Delta = t/(s + t)$. From Eqn 9, it follows that tan δ is independent of frequency at the gel point:

$$\tan\delta = G''/G' = \text{constant} = \tan(\Delta\pi/2) \tag{2.12}$$

The predictions of the scalar percolation theory have been experimentally confirmed for chemical gelation (Durand *et al.*, 1989; Adam, 1991) and physical gelation (Tokita *et al.*, 1984; Axelos and Kolb, 1990; Wang *et al.*, 1991).

2.4.3 Gel point and sol–gel transition

During gel formation, a polymer undergoes a phase transition from a liquid to a gel. The sol–gel transition is a critical point where one characteristic length scale (viz. the size of the largest molecule) diverges. It is a transition in connectivity between a sol where the ‘monomers’ are not connected, to a gel where they are connected. Therefore, irrespective of the system studied or the mechanism involved, gelation is a critical phenomenon where the transition variable will be the connectivity of the physical or chemical bonds linking the basic structural units of the material (Lopes da Silva *et al.*, 1998).

Gelation requires a critical minimum concentration of polymer molecules, C_0, to form a gel matrix. When a gelling polymer concentration is above C_0, one may think about gel point either as an instant in time, t_c, or as a specific temperature, T_{gel}. Before the gel point the connectivity is small and the material typically relaxes rapidly. Near the gel point the relaxation time rises sharply and at the gel point it diverges to infinity (or at least to very long times for a finite sample); in addition, the relaxation spectrum no longer contains a characteristic time. After the gel point, if the network has reached a high degree of development, the maximum relaxation time of the final network is also very short (Lopes da Silva *et al.*, 1998). By means of dynamic rheological tests, the continuous evolution of the viscoelastic properties throughout the gelation process can be followed. Because the magnitude of the strain is kept small, modification of gel structure caused by shear is minimized. The criteria that have been used to determine gel points based on dynamic rheological tests are discussed next.

G′ *and* G″ *crossover*

Based on studies on thermosetting resins the gel point may occur at the time at which G' and G'' cross each other at a given frequency. This criterion was applied to the gelation of several biopolymers, such as gelatin (Djabourov *et al.*, 1988), β-lactoglobulin (Stading and Hermansson, 1990), maltodextrins (Kasapis *et al.*, 1993) and κ-carrageenan gels (Stading and Hermansson, 1993). However, the time of G'–G'' crossover was found to be dependent on the oscillatory frequency in the case of both synthetic polymers (Winter *et al.*, 1988; Muller *et al.*, 1991) and biopolymers (Djabourov *et al.*, 1988). Because gel time is an intrinsic property of a material, it should not be dependent on the frequency of the dynamic rheological experiment. Another concern is that in low-concentration gelling systems, the viscoelastic moduli may be too low to give a measurable signal by a conventional rheometer. Nevertheless, when applicable data can be obtained, it is likely that the G'–G'' crossover time might be close to the sol–gel transition time.

Relaxation modulus

Winter and co-workers (Winter and Chambon 1986; Chambon and Winter, 1987; Winter *et al.*, 1988) found experimental evidence for power law behavior of the relaxation modulus at the gel point. In addition, they showed that scaling of the dynamic moduli and dynamic viscosity with oscillatory frequency is according to power laws. Power law relaxation at the gel point appears to be a general property of both chemical gelation and physical gelation involving either synthetic polymers or biopolymers, respectively. However, because different values of the relaxation exponent $\Delta = 0.13$ to 0.84 were found for different gelling polymers, it is not a universal parameter for gelation. Even for the same polymeric material, relatively wide ranges of values have been obtained for this critical exponent and for some physical gels it was found to be dependent on polymer concentration and thermal history (Michon *et al.*, 1993; Lopes da Silva and Gonçalves, 1994), as well as chain stiffness (Matsumoto *et al.*, 1992). As the exponent Δ seems to depend on the molecular structure of the gelling polymer, it may be useful to distinguish between different gel structures (Lopes da Silva *et al.*, 1998). Nevertheless, Eqn 2.12 is the most generally valid gel point criterion and it has been successfully applied for detection of the gel point of a wide variety of polymers (te Nijenhuis and Winter, 1989; Lin *et al.*, 1991; Muller *et al.*, 1991; Scanlan and Winter, 1991; Michon *et al.*, 1993).

Threshold G′ *value*

For many biopolymer systems gelation begins from a sol state characterized by a very low viscosity that is often below the instrument resolution in small strain oscillatory instruments. Many authors have assessed the gel point as being when G' has increased to a value greater than the experimental noise level, such as 2 or 3 Pa.

Although this approach is not very rigorous, this criterion was used for amylose (Doublier and Choplin, 1989), β-lactoglobulin (Stading and Hermansson, 1990), gelatin (Ross-Murphy, 1991), and bis(trimethylsilyl)-acetamide (Richardson and Ross-Murphy, 1981; Tobitani and Ross-Murphy, 1997). In the latter study, it was shown that this criterion systematically indicated a gel time less than that measured on the basis of viscosity changes.

Extrapolation of G′ *value*

Extrapolation of the rapidly rising values of G', during gelation, to the time axis was also suggested as an accurate estimate of the gel point, t_c (Clark, 1991). Hsieh *et al.* (1993) determined T_{gel} of whey and egg proteins by extrapolation of the rapidly rising values of G' to the temperature axis. In addition, the gel time was calculated for the gelation of bovine serum albumin by monitoring the increasing viscosity as a function of time (Richardson and Ross-Murphy, 1981) and taking the time corresponding to the asymptotic value of viscosity. In this method, although small shear rates were used, the high strains necessarily imposed probably affected the gel critical point.

2.4.4 Effect of concentration and temperature on gel time

It is of practical interest to understand how concentration of a gelling polymer and temperature affect gel times of that polymer. The effect of concentration on gel time of gelatin gels was described (te Nijenhuis, 1981) by the equation:

$$\ln(t_c) = a_1 + a_2 c \qquad [2.13]$$

Oakenfull (1984), based on gelation of high-methoxyl pectins, suggested the equation:

$$t_c = \frac{1}{kc^n} \qquad [2.14]$$

where k is a reaction rate constant and n is the order of reaction. It should be understood that reaction orders higher than four are not encountered and a minimum (critical) concentration is necessary for gelation. Also of relevance is the work of Clark (1994) and Clark *et al.* (1994) which is based on kinetics and the cascade theory mentioned earlier.

Tobitani and Ross-Murphy (1997) used kinetic and scaling concepts to arrive at the concentration dependence of gel time as:

$$t_c \approx \frac{nN_{b0}}{kN_{a0}^n} \qquad [2.15]$$

$$t_c \sim \frac{1}{N_{a0}^n} \sim \frac{1}{c^n} \qquad [2.16]$$

where N_{a0} is the initial number of active sites of the polymer per unit volume, N_{b0} is the initial number of junctions composed of n reacted sites, and the symbol ~ is

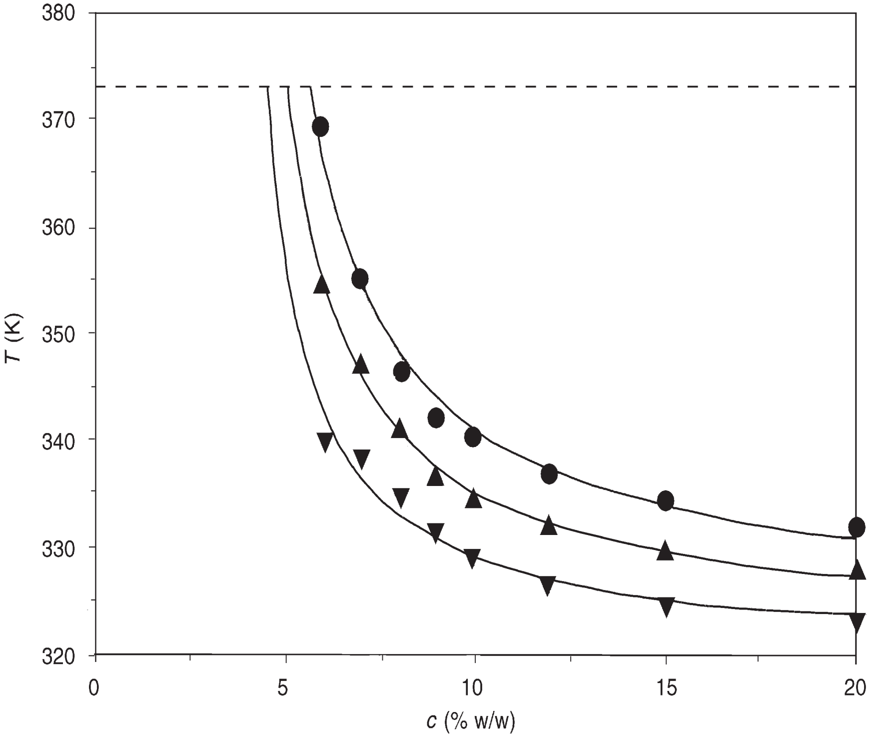

Fig. 2.4 Experimental gel threshold times: ● 100 s, ▲ 1000 s and ▼ 10 000 s for bovine serum albumin as a function of temperature and concentration. Lines are values calculated using a model (Reprinted with permission from: Tobitani and Ross-Murphy, 1997; copyright American Chemical Society).

used to indicate that the two terms on either side can be equated by scaling, but not by absolute values. The influence of temperature, T (K), was taken into account in the reaction rate constant, k:

$$k = A \exp\left(\frac{-E_a}{RT}\right) \qquad [2.17]$$

where A is the pre-exponential factor, E_a is the apparent activation energy, and R is the gas constant. Lopes da Silva *et al.* (1995) also found that the effect of temperature on gelation rates followed the Arrhenius equation. Tobitani and Ross-Murphy (1997) combined the above equations and then modified the resulting expression to take into account the need for the cross-term of (ln c) and temperature:

$$\ln(t_c) = a_1 + a_2 \ln(c) + a_3T + a_4T \ln(c) \qquad [2.18]$$

where a_1, a_2, a_3 and a_4 are coefficients to be determined from experimental data.

The experimental gel threshold times for bovine serum albumin determined by Tobitani and Ross-Murphy (1997) are shown as a function of temperature and bovine serum albumin concentration in Fig. 2.4. They were determined as the time at which G' of a sample reached a threshold value of between 2 and 3 Pa. Further, values calculated using Eqn 2.18 are shown as lines in the figure; in addition, the upper region represents the gel state and the lower region the sol state of a sample. For a given set of values of concentration and temperature, the threshold time for gel formation can be determined from the figure. In addition, the diagram can be used to predict the possibility of gel formation for any given concentration and temperature.

2.4.5 Structure development during biopolymer gelation

Aqueous food polymer gels exhibit a general characteristic behavior when the structure development is evaluated by dynamic rheological measurements (Clark and Ross-Murphy, 1987). The beginning of the gelation process is dominated by the viscous behavior of the system ($G''>G'$) and the final stages by the elastic behavior ($G'>>G''$). Both moduli increase as a result of increasing density of junction zones, but G' rises more sharply until it intersects and then exceeds G''. After an initial rapid increase, G' increases continuously as a result of the slower formation and rearrangement of junction zones, resulting in a net decrease in the length of elastically active chains and eventually reaches a pseudo plateau region. In many biopolymer gelation processes, e.g. gelatin (te Nijenhuis, 1981) and HM pectin/fructose and sucrose systems (Rao and Cooley, 1993; da Silva and Gonçalves, 1994), because of the non-equilibrium behavior, even after aging for long periods, G' continues to increase steadily as a result of a continuous reorganization of the network. Therefore, the kinetics applicable at longer times are quite different from those observed close to the gel point.

Structure development rate (SDR), defined as dG'/dt, can be measured under isothermal conditions, at different aging temperatures (cure curves), and also during the gelation process promoted by decreasing the temperature of the biopolymer dispersion (non-isothermal conditions) (Rao and Cooley, 1993). The analysis of structure development rates under isothermal conditions, as opposed to non-isothermal conditions, is useful because it avoids non-equilibrium effects and the effect of thermal history of a gelling system (Lopes da Silva *et al.*, 1998).

2.4.6 Effect of temperature on gel modulus

Many food polymer gels, including alginates, k-carrageenan, agarose/κ-carrageenan mixed gels, globular proteins, and agarose/κ-carrageenan mixed gels (Lopes da Silva *et al.*, 1998), exhibit a decrease in viscoelastic parameters with increasing temperature, which is the general behavior of viscoelastic amorphous polymers (Ferry, 1980). This behavior is typical of polysaccharide gels where hydrogen bonding or electrostatic interactions are the only significant interactions that stabilize the polymer network. For most biopolymer gels the rupture of junction zones stabilized by thermolabile interactions may explain the decrease in modulus with increasing temperature, in addition to the increase in flexibility of the polymer chains. Activation energies associated with the breakage of junction zones in biopolymer gels have been estimated by fitting the Arrhenius relationship to the temperature-dependent data on different rheological parameters; for protein and polysaccharide gels activation energies ranging from 10 to 275 kJ mol^{-1} have been reported (Lopes da Silva *et al.*, 1998).

Eldridge–Ferry plot

Eldridge and Ferry (1954) studied the relationship between the melting point (T_m), and concentration (c) and molecular weight (M) of gelatin gels. They found linear relationships between ln(c) and $1/T_m$, and between ln(M) and T_m. Assuming equilibrium binary association of polymer chains and using the van't Hoff law, they suggested the following relationship, from which it is possible to calculate the enthalpy (ΔH_m) of the cross-linking process and to get some information about the stability of the junction zones into the gel:

$$-\left[\frac{\mathrm{d}\ln c}{\mathrm{d}T_m}\right]_{MW} = \frac{\Delta H_m}{RT^2} \qquad [2.19]$$

Equation 2.19 was found to be applicable to other biopolymer gel systems, including agarose (Watase and Nishinari, 1987a), high-methoxyl (HM) pectin/dimethylsulfoxide (DMSO) gels (Watase and Nishinari, 1993), κ-carrageenan (Watase and Nishinari, 1987b), and low-methoxyl (LM) pectin/Ca^{2+} systems containing sucrose and sorbitol (Fu and Rao, 1999). The enthalpy change obtained in this way characterizes the enthalpy associated with the strongest network junctions averaged over the concentration range, i.e. the junctions zones that remain stable until the melting temperature is reached (Braudo *et al.*, 1984).

The temperature dependence of the modulus of a gel well before melting indicates the breakdown enthalpy of network junctions (ΔH_{b_T}) that may be considered to be a measure of the variation in the cross-link strength (i.e. in the number of cross-links) within the gel (Braudo *et al.*, 1984). Both ΔH_m and ΔH_{b_T} can serve as measures of stability of a gel's junction zones, but each of them reflects different breakdown processes and their magnitudes are not necessarily equal for all gels. For example, values of ΔH_m for LM pectin/calcium gels were close to those for κ-carrageenan and κ-furcellaran gels, but much lower than those of either gelatin or agarose gels. The κ-carrageenan and κ-furcellaran gels are formed by helical structures aggregated by potassium ions (Morris *et al.*, 1980), a mechanism that energetically is similar to the gelation of LM pectin via cross-linking by calcium ions. In the case of gelatin and agarose, gelation is associated with helix formation which results in a large entropy decrease that must be compensated by a large enthalpy decrease. The difference between agarose and κ-carrageenan gels may be due to the different sulfate content (Watase and Nishinari, 1987a). The smaller values of ΔH_m obtained for the LM pectin gels suggest that the junction zones in LM pectin/calcium are less heat-resistant. In contrast, the enthalpy ΔH_{b_T} obtained for LM pectin/calcium gels was higher than that obtained for HM pectin gels, which suggests a cooperative process in the formation or disruption of effective junction zones in the pectin/calcium system (Garnier, 1992).

2.5 Phase transitions in fats

Lipids are a group of molecules that are poorly soluble in water and soluble in organic solvents. The term ‘fats’ refers to a subset of lipids. Most fats in foods are present as saturated, monounsaturated, or polyunsaturated species of fatty acids esterified into triglycerides. Reversible crystallization and the development of plastic fats contribute to the desirable functionality of fats in foods, such as: shortening of doughs, melting of chocolate, and texture of cakes and confections. Further, crystallization takes place in two discrete steps: nucleation and crystal growth, and it affects the consistency and mouthfeel of a high-fat food. During crystallization the non-equilibrium condition induced by process variables leads to crystal formation.

For consumers, textural attributes of fats that arise from their molecular states are of primary importance. Fats affect texture by forming structures of crystalline networks and by disruption of structure by interfering with non-fat networks. Fats undergo solid–liquid and liquid–solid transitions that are reversible melting and crystallization, respectively. However, a natural fat is never completely solid, but is a mixture of a liquid phase trapped in a solid phase made up of microscopic triglyceride crystals. Fats appear to be solids and have specific textural properties called plastic properties.

The ability of a fat to exist in different crystal forms is called polymorphism. There are three main polymorphic forms: α, β, and β', which represent different packing arrangements and increase in both molecular ordering, packing tightness, crystal size and morphology, melting point, and thermodynamic stability. Butter, processed from milk fat, provides desirable texture, mouthfeel, and flavor to many processed foods. To simulate butter’s functional characteristics, margarines, shortenings, and other functional fats that are used extensively in the food industry have been developed. Stick margarines are formulated such that spreadability and melting in the mouth are similar to those of butter, and soft margarines are formulated to be softer and more spreadable than stick margarines. Low-fat spreads show poor meltability in the mouth and slow flavor-release characteristics (Borwankar *et al.*, 1992).

Techniques for studying fat crystallization include: dilatometry, wide-line nuclear magnetic resonance (NMR), magnetic resonance imaging (MRI), and DSC, and for studying fat crystal structures various spectroscopic and microscopic techniques can be used (German and Simoneau, 1998). To a large extent, textural properties of fats are determined using empirical and imitative tests: cone and cylinder penetrometry, texture profile analysis, and extrusion force measurement (German and Simoneau, 1998). However, the temperature-sweep dynamic rheological technique has been used to study meltability of butter and maragarines (Borwankar *et al.*, 1992). Table 2.2 contains values of the temperature at which 90% melting was achieved in a DSC and when the complex modulus, G^*, reached 100 Pa during temperature sweeps for butter in a parallel-plate rheometer, and high-fat and diet margarines. In the DSC experiments, the samples were first cooled from 4.4 °C to 0 °C at –40 °C/min and then they were heated at 10 °C/min.

Table 2.2 Temperatures (°C) for G^* to reach 100 Pa and 90% melting with a DSC during heating of butter and margarines

Sample	Temperature at G^*=100 Pa	Temperature at 90% melting
High-fat products		
Stick butter	32.5	31.7
Stick margarine	33.0	33.5
Soft margarine	31.6	32.9
Soft diet margarines		
Sample with 80% fat	30.9	33.4
Sample with 75% fat	30.3	32.2
Sample with 50% fat	35.4	34.2
Sample with 40% fat	~45	33.0
Sample with 28% fat	~45	37.3
Sample with 20% fat	~60	33.0

Source: Borwankar *et al.* (1992).

In these rheological experiments, the oscillatory frequency was 0.1 Hz, the samples were heated from 5 °C to 60 °C in steps of either 5 °C or 10 °C, and G^* was measured at each step after the temperature reached a steady value (Borwankar *et al.*, 1992). In Table 2.2, it can be seen that values of temperature using DSC and those obtained from rheological data were about the same for butter and high-fat margarines, but different for soft and diet margarines.

In chocolate processing the three main polymorphic forms α, β, and β′ also play important roles. Desired textural properties are obtained by providing processing conditions that generate the correct number, size, shape, and polymorph of lipid crystals prior to enrobing or molding. For example, in the tempering process, the chocolate mass is first cooled rapidly from 50 °C to 26 °C and later heated to about 32 °C to create the more stable β polymorph (Hartel, 1998).

2.6 Crystallization kinetics

As stated earlier, crystallization takes place in two discrete steps: nucleation and crystal growth. It may occur above the glass transition temperature, but below the melting temperature. Crystallization of amorphous compunds affects food quality and contributes to deteriorative changes. This is because at temperatures below the glass transition temperature, the viscosity is very high and diffusion of a solute is very low, and at the melting temperature nucleation and cystal growth cease because the thermodynamic equilibrium shifts to the liquid state (Roos, 1995).

The kinetics of crystallization are often described by the Avrami equation (Sperling, 1986; Roos, 1995):

$$X_t = 1 - \exp(-kt^n) \qquad [2.20]$$

where X_t is the volume fraction of crystalline materials, known widely as the degree of crystallinity, t is time, and k and n are constants. The shape of increase in crystallinity with time is a sigmoid. Eqn 2.20 can be written in logarithmic form:

$$\ln[-\ln(1 - X_t)] = \ln k + n \ln t \tag{2.21}$$

thus a plot of $\ln[-\ln(1 - X_t)]$ against $\ln t$ results in a straight line with slope n and intercept $\ln k$.

2.7 Structural and textural changes due to glass transition

Glass transition affects mechanical properties of amorphous food materials that in turn alter textural characteristics, such as stickiness, caking, collapse, and crispness (Roos, 1998). Rates of changes in mechanical properties can be analyzed in terms of relaxation times. Relaxation refers to the time required to respond to a change in temperature or pressure; it also implies some measure of the molecular motion, especially near a transition condition (Sperling, 1986). The empirical Williams–Landel–Ferry (WLF) equation can be used to describe the temperature dependence of the mechanical properties above T_g. It is based on the assumption that the relaxation times of mechanical changes are altered similarly in relation to a reference temperature, T_s. However, use of T_g as the reference temperature is not recommended because of the difficulty in measuring relaxation times at and around T_g. The ratio of the relaxation time, τ, to that at a reference temperature, τ_s, denoted as a_T, follows the WLF equation. In addition, the relaxation times can be related to the corresponding viscosities, η, and η_s.

$$\ln a_T = \ln \frac{\tau}{\tau_s} = \ln \frac{\eta}{\eta_s} \tag{2.22}$$

$$\ln \frac{\eta}{\eta_s} = \frac{-C_1(T - T_s)}{C_2 + (T - T_s)} \tag{2.23}$$

Much later, the WLF equation was derived from the free volume theory that predicts the value of free volume at T_g of any polymer at 2.5%. In addition, other useful information on glass transition was obtained from applying kinetic and thermodynamic theories (Sperling, 1986). For several inorganic and organic glasses, the decrease in viscosity above T_g could be described by the WLF equation (Eqn 2.23) (Ferry, 1980). In addition, in the WLF equation, values $C_1 = -17.44$ and $C_2 = 51.6$ were satisfactory for describing changes in viscosities of the studied materials. However, for a specific food, it is recommended that values of C_1 and C_2 that best fit a given set of data be used.

2.7.1 Glass transition temperature of mixtures

Because most foods are made up of several components, it is useful to know the effect of composition on T_g. An empirical equation for calculating T_g for a binary

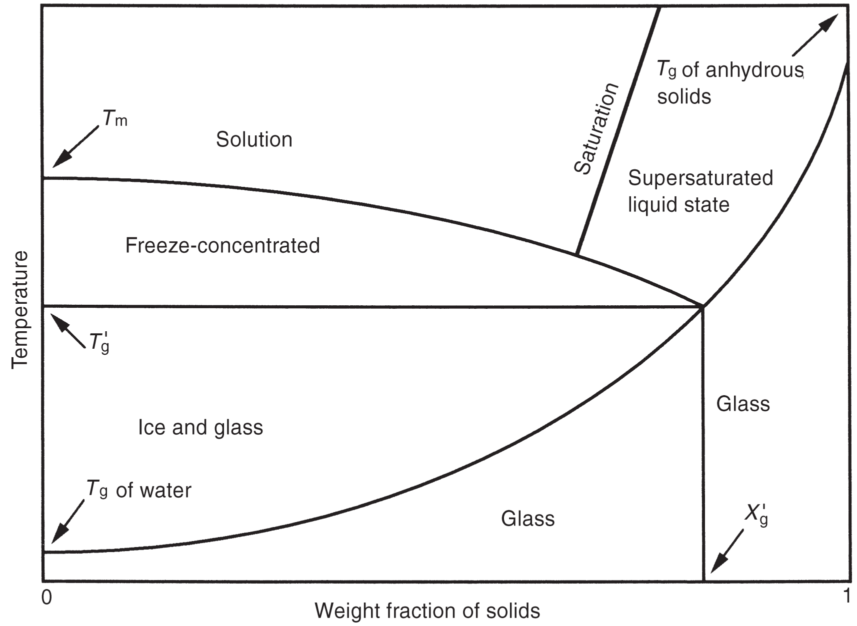

Fig. 2.5 A general state diagram that illustrates glass transition (T_g) and melting (T_m) temperatures as well as the different phases. T'_g is the glass transition temperature of the unfrozen solute phase and X'_g is the corresponding solids weight fraction.

mixture of components 1 and 2, proposed by Gordon and Taylor in 1952, was shown to have thermodynamic basis by Couchman and Karasz in 1978 (see Roos, 1995):

$$T_{gm} = \frac{x_1 T_{g1} + (\Delta C_{p1}/\Delta C_{p2})\, x_2 T_{g2}}{x_1 + (\Delta C_{p1}/\Delta C_{p2})\, x_2} \qquad [2.24]$$

where x_1, T_{g1} and ΔC_{p1}, and x_2, T_{g2} and ΔC_{02} are the mole fraction, glass transition temperature, and change in heat capacity at the glass transition temperature of components 1 and 2, respectively.

Because foods are complex mixtures of several compounds, owing to variation in the composition of samples, it is often difficult to determine their glass transition temperatures accurately. At the least several determinations should be made on a given food and it would be desirable to report an average value of T_g and the associated standard deviation. The T_g of water is about –135 °C, so that for many high-moisture foods their T_g will be well below the temperatures of practical interest.

Glass transition, freezing, and melting temperatures of a food may be plotted on a temperature versus moisture content diagram, called a state diagram, in which different phases may be illustrated. A general state diagram is shown in Fig. 2.5, which illustrates glass transition, freezing, and melting temperatures as well as the different phases. In addition, especially for aqueous solutions, solubility and water vaporization curves can be included in a state diagram.

2.8 Future trends

A number of new food processing, especially non-thermal, techniques are being investigated, such as high-pressure and high-shear processing, ohmic heating, and pulsed electric preservation. Phase transitions in foods using these processing/ preservation techniques need to be understood for production of high-quality foods. Theoretical and computer simulation studies on structure formation of foods based on polymers are being conducted, and these will be refined and improved in the future. Experimental techniques, such as the development of atomic force microscopy and magnetic resonance imaging, will improve our knowledge of food polymers and reactions at the molecular level. Together with improvements in instrumentation for studies at the macromolecular and supramolecular level, our understanding of phase transitions in foods will continue to improve.

2.9 Sources of further information and advice

A number of references that were cited in this chapter contain much useful information. Aguilera and Stanley (1999) covered techniques for examining food microstructure, structure formation, and role of food structure on properties. Clark and Ross-Murphy (1987) provide a comprehensive review of the chemical structure of many food polymers, gelation mechanisms, and dynamic rheological techniques. Classical texts of Flory (1953), de Gennes (1979), and Ferry (1980) contain a wealth of information on polymers and their gels. Both the first (1986) and third (2001) editions of Sperling's book contain much information on polymer physics, including their phase transitions. The papers presented on various aspects of phase transitions in several foods at a symposium are in Rao and Hartel (1998). For the latest developments on phase transitions in foods, consult scientific journals, such as *Carbohydrate Polymers*, *Food Hydrocolloids*, *Journal of Agricultural and Food Chemistry*, *Journal of Food Science*, *Journal of Texture Studies*, and *Macromolecules.*

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3

Phase separation in foods

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3.1 Introduction

It has become increasingly critical for the food industry to have sufficient scientific understanding of the phase behaviour of biopolymer mixtures in order to design and control product microstructure. A solid physical and microstructural understanding of single and mixed biopolymers will enable a more efficient use of existing food ingredients and novel materials in microstructural engineering of high-quality food. Biopolymers, such as proteins and polysaccharides, are used to impart texture, consistency and stability because of their ability to enhance viscosity and form networks. Network formation and the phase properties of mixed biopolymer systems influence the rheology of the system and, hence, considerable research effort has been directed towards understanding mechanisms for network formation and, for mixed systems, the incompatibility of biopolymers (Harding *et al.*, 1995). Consumers' concern about food additives also leads to demands for increased understanding of the functionality and phase behaviour of natural additives and new materials, which will allow controlled usage to impart desired textural attributes. Lately, the importance of the manufacturing process technology (e.g. shear forces and cooling) on the resulting microstructure and rheology of mixed systems has been recognised (Brown *et al.*, 1995; Foster *et al.*, 1996a,b), showing that that process significantly influences the properties of the food material. Further understanding of the influences of alternative processes on food material and the resulting effect on functionality of the ingredients will continue to be beneficial for the consumer, manufacturer and the environment, in cost and energy-effective processes used for required product quality. The importance of phase separation is reflected in the large number of publications over the last decade (see, e.g. Morris, 1990; Brown *et al.*, 1995; Foster *et al.*, 1996a,b; Tolstoguzov, 1983, 1996, 1997, 1998; Grinberg and Tolstoguzov, 1997; Zasypkin *et al.*, 1997; Semenova and Savilova, 1998; Lundin *et al.*, 2000; Norton and Frith, 2001).

In this chapter we will try to summarise the basic rules of how to take account of phase separation when formulating and developing a food product. We discuss phase separation terminology, rheology of two-phase systems, factors influencing the phase behaviour of mixtures and phase separation in food products.

3.2 Properties of mixed biopolymer systems

The absence of phase separation in aqueous biopolymer mixtures is relatively unusual, particularly in systems in which the components undergo ordering and gelling transitions. However, it has been observed, in mixtures where the drive for phase separation is low and the gelation rapid, that mixed gels are formed that are indeed interpenetrating at the molecular level. Such interpenetrating networks (IPNs) have been the subject of investigation (Clark *et al.*, 1999; Amici *et al.*, 2000) but have not, as yet found widespread application in designed textural applications. More commonly, upon mixing biopolymer solutions, some kind of demixing does occur. In these cases it is convenient to distinguish between associative and segregative phase separation.

3.2.1 Associative phase separation

Associative phase separation has classically been thought of as a result of complex coacervation of two oppositely charged biopolymers (Bungenberg de Jong, 1949a,b). Complexes formed in this fashion are enriched in one phase, which is in equilibrium with a biopolymer-depleted phase. The formation of these complexes is governed by the properties of the individual polyelectrolytes (e.g. conformation, charge density, position of the ion site) and physicochemical environment (e.g. solvent, salt concentration, pH and temperature) (Tsuchida and Abe, 1982). In most situations the driving forces for the formation of polyelectrolyte complexes are an attractive Coulombic force between the polymers and an overall entropic effect involving the counterions, i.e. the counter-ions will gain entropy as the polymers are associated. Associative phase separation can also occur (in rare cases) without an effective attraction between the different polymers if the polymer solvent conditions are poor compared with the polymer–polymer interaction (Piculell *et al.*, 1995).

The full structural, textural and functional potential of mixed biopolymer complexes in food materials are not fully appreciated and elucidated. To date, associative interactions between proteins and polysaccharides have been cleverly used for protein fractionation and as flocculents for the precipitation of protein suspensions (Albertsson, 1995). Furthermore, the surface-active properties of polyelectrolyte complexes are utilised to encapsulate oil as well as to stabilise foams (Tolstoguzov, 1997). Several different biopolymer systems which form polyelectrolyte complexes have been utilised to encapsulate solid and liquid phases (Finch 1993), e.g. gelatin:gum arabic (Burgess and Carless, 1984, 1985), gelatin:carboxymethylcellulose (Sungur *et al.*, 1992; Lii *et al.*, 2002), gelatin:pectin(McMullan *et al.*, 1982, 1984; Joseph and Venkataram, 1995), gelatin:carrageenan (Michon *et al.* 1995, 2000, 2002; Antonova and Goncalves, 1999) chitosan:xanthan (Sakiyama *et al.*, 1999; Shu *et al.*, 2001), chitosan: carrageenan (Hugerth *et al.*, 1997; Shumilina and Shchipunov, 2002). In the presence of solid or liquid particles, polyelectrolyte complexes are known to deposit on and coalesce around these core particles and encapsulate the material (Tolstoguzov, 1997, 1998). It is generally believed that the increased hydrophobicity of the polyelectrolyte complexes as they are formed drives the encapsulation process.

3.2.2 Segregative phase separation

In terms of the exploitation of phase separation phenomena in order to generate desired product texture attributes, it is fair to say that segregative phase separation has been the primary tool of choice. In most cases the phase behaviour of mixed biopolymers has been established at high temperatures (see, e.g. Foster *et al.*, 1996a; Kasapis *et al.*, 1993a,b; Grinberg and Tolstoguzov, 1997). However, for many mixed biopolymer systems a reduction in temperature will influence the phase separation. In many cases, cooling causes one or more of the biopolymers in the mixture to order, aggregate and form a network. The onset of ordering initiates phase separation as the ordering and aggregation process changes the thermodynamic properties of the constituents and thus drives the demixing process (Clark, 1995). For mixed biopolymer systems other properties such as salt content, charge and molecular weight also influence both the phase behaviour and the gelation process of the polymers (Picullel and Lindman, 1992; Albertsson, 1995; Piculell *et al.*, 1995; Grinberg and Tolstoguzov, 1997; Semenova and Savilova, 1998). Not only can the initiation of a biopolymer structuring process initiate phase separation, it also invariably slows down the phase separation process by structurally trapping the system via gelation. In order to be able to control the microstructural and rheological properties of such mixed systems it is important to understand the interplay between phase separation and network formation, i.e. to understand how temperature, conformational ordering and gelation affect the trapping of these microstructures at various stages of separation or ripening (Lundin *et al.*, 2000; Lorén *et al.*, 2001b).

3.3 Mechanisms of phase separation

For all practical purposes food systems incorporate mixtures of at least two biopolymers and a solvent. Formally, mixtures of two biopolymers and a solvent should be considered as ternary systems. The solvent, which normally is water,

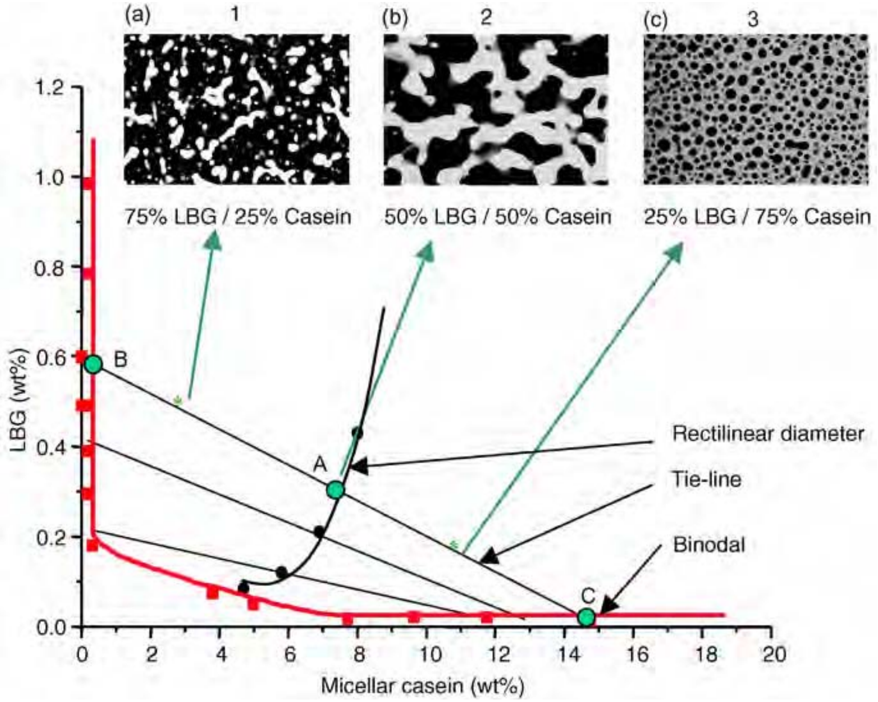

Fig. 3.1 Phase diagram for micellar casein and locust bean gum at 5 °C and pH ~6.8, 0.08 ionic strength, image width 500 µm (Schorsch *et al.*, 1999b).

often constitutes >90% of the samples. Hence, it is most common to present phase diagrams by plotting the biopolymer concentrations as *x–y* plots rather than triangular diagrams. An example of a phase diagram for micellar casein and locust bean gum (LBG) is presented in Fig. 3.1.

The phase diagram consists of a binodal (or cloud point) curve that divides the miscible region at low polymer concentrations from the phase separated, immiscible region at higher polymer concentrations. Samples that have a composition falling inside the miscible region will not phase separate. For those compositions, the biopolymers will be miscible on a molecular level. For mixtures whose compositions fall inside the immiscible region, the biopolymers will be segregated into two phases. A sample made with the composition A will segregated into two phases B and C. The compositions of these phases are determined by the slope of the tie-line on which the composition sits, and can be estimated at the cross-over between the tie-line and the binodal; see B and C in Fig. 3.1. The relative position of A on the line B–C determines the phase volumes of the two phases. The line B–A corresponds to the phase volume of micellar casein-rich phase and the line A–C to the phase volume of the locust bean gum-rich phase. The microstructure of three phase separated mixtures with nominal compositions chosen from the same tie-line is also depicted in Fig. 3.1. Since the compositions are on the same tie-line, all three samples will have the same compositions in the two enriched phases, i.e. their effective concentrations will be the same. However, the phase volumes of the phases will vary. The phase volumes for the three samples, 1, 2 and 3, are 75% LBG:25% micellar casein, 50% LBG:50% micellar casein and 25% LBG:75% micellar casein, respectively. In the confocal images the protein-enriched phase is white and the polysaccharide phase is black. It can be observed that in sample 1 the locust bean gum phase constitutes 75% of the total phase volume and hence is the continuous phase with inclusions of the lower phase volume micellar casein phase. In contrast, sample 3 has a micellar casein phase with a phase volume of 75% that is the continuous phase and an included locust bean gum phase of 25% phase volume. The general rule is that the phase continuity is governed by the biopolymer with highest phase volume (Foster *et al.*, 1996a). The cross-over points between the rectilinear diameter and the tie-lines show the compositions where the phase separated systems will have equal phase volumes, i.e. 50:50 as in sample 2. Such mixtures often show a bi-continuous microstructure where both phases are striving to be continuous.

As indicated above, the concentrations of the biopolymers in the phases in which they are enriched are effectively increased from their nominal (starting) values. For our example: sample A; the nominal concentrations were 7% micellar casein and 0.3% locust bean gum; after phase separation, the concentrations of the milk protein-enriched phase are 14.8% micellar casein:<0.025% locust bean gum and for the locust bean gum-enriched phase <0.25% micellar casein:0.6% LBG. In this example the binodal is close to the axes. Therefore, each phase contains little of the second biopolymer. However, this is not always true, which adds further complexity to the situation, particularly if the concentration of the second compatible biopolymer is above its critical concentration for gelation. This process of gelation, as a function of, for example, ordering and/or aggregation, as a result of changing the experimental environment (e.g. temperature changes), can induce secondary phase separation, discussed in further detail below.

Under evolving conditions, it should be remembered that both the phase diagrams and associated microstructure images, shown in Fig. 3.1, are snap-shots relevant to a particular time. From a food texture point of view the structural rearrangements during processing and over time during storage are of great importance. Table 3.1 gives the structural and physical events associated with phase separation. We suggest that is convenient to distinguish between initial phase separation, ripening and bulk phase separation.

Two mechanisms are used to describe the phase separation process, namely nucleation and growth, and spinodal decomposition. The conditions the system is subjected to determine by which mechanism the mixture phase separates. The nucleation and growth mechanism is operative near to the binodal, whereas deeper quenches into the incompatible region tend to yield spinodal phase separation. The important points that distinguish the two mechanisms are, for nucleation and growth, that the phase volumes of the two phases continuously change, while the equilibrium (effective) concentration of the phases are established immediately. However, in spinodal decomposition the phase volume is established at the early

Table 3.1 Structural and physical events associated with phase separation

	Time scale		
	0–10 s	seconds	hours days
	Phase separation	Ripening*	Bulk phase separation
Nucleation and growth			
Phase volume	//	–	–
Concentration of phases	–	–	–
Structural evolution	/	/	–
Spinodal decomposition			
Phase volume	–	–	–
Concentration of phases	//	–	–
Structural evolution	/	/	–

* Coalesence, Oswald ripening and sedimentation.
– = change at slow rate, / = change at moderate rate, // = change at high rate.

stages and the biopolymer phase concentrations evolve with time. The initial rate of volume change for nucleation and growth and concentration change for spinodal decomposition are, in most cases, very high. For liquid, non-gelling systems, the equilibrium conditions are, in general, established within tens of seconds (Tromp *et al.*, 1995; Lorén *et al.,* 2001a,b; Williams *et al.,* 2001). During this initial stage of phase separation, the structure is, therefore, continuously evolving. This stage is followed by a ripening stage. During ripening, processes such as droplet coalescence, Oswald ripening, creaming and sedimentation occur. Structural rearrangements can occur over the time scales of seconds to hours, days, weeks or even years (Foster *et al.*, 1996a). If these processes are not slowed down or arrested the mixture will ultimately separate into two bulk phases. In Fig. 3.2 these processes are schematically depicted for a phase separated system where the included phase sediments under non-shear conditions. Droplet coalescence and the rate of sedimentation or creaming are dependent on the relative rheology of the different phases, the interfacial tension and the density difference between the two phases, and, if applicable, the amount of shear the system is subjected to (Grace, 1982; Foster *et al.*, 1996a,b; Tanaka, 1997; Cao *et al.,* 2001; Butler, 2002a).

For further reading about the mechanism of phase separation, the reader is referred to Harding *et al.* (1995).

For all practical purposes, it is the product developer's task to try to slow down or even stop structural rearrangements when the desired microstructural and textural properties are obtained. The structural ripening effects described above will invariably be slowed by gelation of one or both of the biopolymers. If gelation of the polymer in the continuous phase in a system such as that depicted in Fig. 3.1c is induced at an early stage in the ripening process, the resulting microstructure will consist of a gel filled with spherical inclusions, which may or may not be in a gelled state (depending on the biopolymer in question). For such a system the rate of

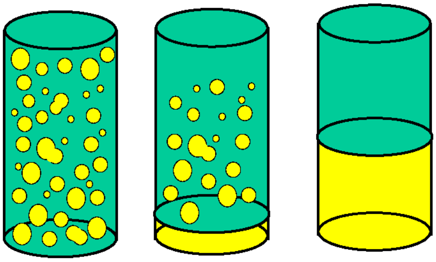

Fig. 3.2 Schematic showing the structural ripening process (coalescence and sedimentation) in a phase separated system.

structural changes is drastically slowed down. However, if the mixture is subjected to shear during the gelation and the continuous phase is the first to gel, it will be broken up in the shear field and become the included phase (Foster *et al.*, 1996b).

3.3.1 Rheological implications of phase separation

For phase separated systems the mechanical properties are dependent on the following structural and physical parameters:

- Phase continuity – based on the phase diagram, it is possible to determine which of the biopolymers forms the continuous phase. In general, the structuring of the composite is governed by the continuous phase (Clark *et al.*, 1983; Brown *et al.*, 1995; Foster *et al.,* 1996a,b; Zasypkin *et al.*, 1997).
- Effective concentration of the biopolymers in the phases – the phase diagram also gives an estimate of the effective concentrations of the biopolymers in the two phases. Therefore, gelation might be achieved even if the nominal concentrations of the biopolymers used are below their critical concentration for gelation (Zasypkin *et al.*, 1997).
- Droplet size and size distribution of the included phase (Norton and Frith, 2001).
- The spatial distribution and the shape of the included phase influence the rheological properties of the mixed material (Ahmed and Jones, 1990; Kasapis, 1999; Wolf *et al.*, 2000, 2001).
- The interface between the two phases: especially the strength of the bonds between the included biopolymer phase and the continuous biopolymer phase (van Vliet and Dentener-Kikkert, 1982; van Vliet and Walstra, 1995; Plucknett and Normand, 2000; Normand *et al.*, 2001; Norton and Frith, 2001).

The mechanical properties of binary mixtures have to some extent been successfully modelled using the Takayanagi model (Takayanagi *et al.*, 1963). However, there exists a range of reinforcement theories for composite materials (for a review see, e.g. Ahmed and Jones, 1990). The Takayanagi model was originally developed for polymer blends and has been extended to take into account the partitioning of water in biopolymer–biopolymer–water systems (Clark *et al.*, 1983). The model describes the composite modulus in terms of the elastic moduli (G') and the volume fractions of the phases. Based on these parameters upper (iso-strain) and lower (iso-stress) bounds are predicted. The modulus of the composite is predicted to fall within these bounds. This extended analysis, taking the water partitioning into account, has been applied to a range of mixed biopolymer systems, gelatin: maltodextrin (Kasapis *et al.*, 1993a,b; Alevisopoulos *et al.*, 1996; Normand *et al.*, 2000), gelatin:starch (Abdulmola *et al.*, 1995), gelatin:microcrystalline cellulose (Kasapis, 1999), whey:maltodextrin (Manoj *et al.*, 1997), whey:gelatin (Walkenström and Hermanson, 1994), agarose:starch (Mohammed *et al.*, 1998a,b), gelatin:pectin (Gilsenan *et al.*, 2000).

In Fig. 3.3, long-term G' measurements of mixtures of gelatin:maltodextrin from two tie-lines are presented. It can be observed that for this system a phase inversion in biopolymer continuity occurs at around 50:50 phase volume of each phase. For the gelatin-continuous mixture, filling the elastic gelatin gel with softer maltodextrin inclusions led to an overall decrease in G', in agreement with the Takayanagi blending laws. As the phase volume of maltodextrin is increased above 50% the mixture becomes maltodextrin-continuous and the rheology is governed by the polysaccharide phase. For mixtures with roughly equal phase volumes, it is difficult to predict which polymer will control the rheology. It is also possible that a gelled included phase can percolate when dispersed and give an elastic response in a viscous continuous phase (Foster *et al.*, 1996b).

The melting behaviour of a mixed gel is dependent on the biopolymer in the continuous phase. For the gelatin-continuous and maltodextrin-continuous systems the melting temperatures were ~35 °C and 90 °C, respectively. The results presented in Fig. 3.3 were from carefully designed experiments where the systems studied were chosen from two tie-lines. For these systems the effective concentrations of the polymers in the phases are constant and it is only the relative volumes of the phases that are changing (Brown *et al.,* 1995). Interestingly, in the past most studies of the mechanical properties of mixed biopolymer systems were designed so that the nominal concentration of one of the polymers was kept constant and the concentration of the second polymer was varied (see, e.g. Kasapis *et al.*, 1993a,b; Manoj *et al.*, 1997; Mohammed *et al.*, 1998a,b). For such experiments, the effective concentrations, as well as the phase volumes, are varied. In these latter experiments, the melting temperature was used as an indicator of phase continuity. However, for this approach to be successful it is important that the effective concentrations and therefore the subsequent required gelation times are considered, so that the system subjected to melting has achieved some long-term stability. As mentioned above, the gelatin nature of the system could be explained by a percolated gelatin dispersed phase. Microscopy is considered to be the best instrument to evaluate the overall microstructure of phase separated systems.

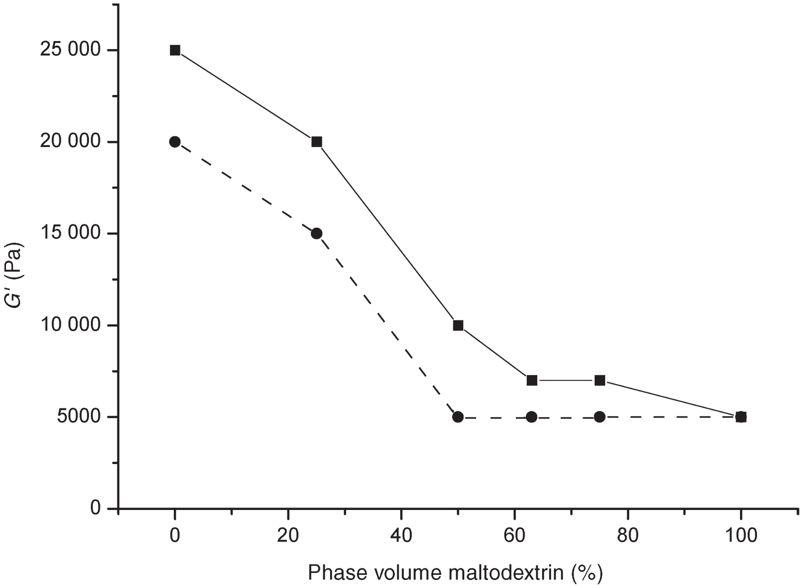

Fig. 3.3 The storage modulus (G') as a function of phase volume for gelatin:maltodextrin mixtures from two tie-lines. Tie-line 1 (dashed): 6 gelatin-rich phase (10.8% gelatin:1.4% maltodextrin) maltodextrin-rich phase (2.2% gelatin:16.3% maltodextrin) tie-line 2 (solid) 8 gelatin rich phase (12.0% gelatin:1.4% maltodextrin) maltodextrin rich phase (2.2% gelatin:17.6% maltodextrin).

3.4 Controlling biopolymer systems

To have sufficient control to design microstructures in this fashion, knowledge of the significant environmental factors, and how these physicochemical details are likely to impinge on the consequent system evolution, is crucially important. The phase behaviour of mixed biopolymer systems is dependent on conditions such as pH, ionic strength and temperature, in addition to the concentrations and properties of the individual polymers; specifically the molecular weight, charge and interaction parameters (Piculell *et al.*, 1995; Bergfeldt *et al.*, 1996; Gottschalk *et al.*, 1998). While the latter can be changed by variations in the sourcing and processing of polymeric ingredients, the former are often set by specific product issues, such as taste and microbiological stability. It is also worth noting that changes in these parameters are also likely to occur in time during the processing of products. On the face of it, the dependency of the phase separation behaviour on such a large number of time-dependent parameters makes microstructure design seem like a prohibitively complex task. However, it is possible with recourse to theoretical models to gain some valuable insights into how such systems may be controlled.

3.4.1 Theoretical frameworks

Significant theoretical work on segregative phase separation can be divided broadly into two areas: (1) that dealing with calculations of the thermodynamic properties and (2) those relating to the phase separation kinetics and ripening growth laws. It is not the intention here to give a thorough review of these areas, rather to comment on aspects of the most common theoretical framework that have proved most successful in the understanding of the behaviour of practical systems.

Models of thermodynamic properties have concentrated on calculating the binodal and spinodal boundaries. Binodal calculations are routinely based on minimising the system's free energy and equalising the chemical potentials between coexisting phases. These are most commonly performed using a mean-field model based on the Flory–Huggins (F-H) approach (Clark, 1995). Although the F-H theory was originally developed for neutral polymers, polyelectrolytes such as gelatin, pectins, alginates and carrageenans are commonly used in practice and these require additional consideration in terms of the effects of polymeric charge and counter-ions, and the inclusion of the effects of added salts. In cases where only one of the biopolymers is charged (or where both are charged but the direct interaction between them is weak), this extra complexity can typically be dealt with fairly simply within the same framework, by the addition of a term relating to the entropy of the ions (the sum of the counter-ions associated with the polymer and any added salts). Some work has been carried out with the aim of taking the electrostatic interactions specifically into account (Yu and Arons, 1994), but for most practical systems, these more complicated theories do not significantly change the qualitative learning from less involved models.

Other approaches of note include the Vrij theory. Although developed primarily for colloidal systems where the presence of polymeric compounds causes depletion flocculation of colloidal particles, it has been argued in a number of cases, particularly with mixture components such as casein miscelles, that the theory can describe observed phase separation phenomena well (de Bont *et al.*, 2002). The fundamental difference in approach here is that the property of the depleting polymer (specifically the radius of gyration and molecular weight) are subsumed into an effective interparticle potential.

Much theoretical work has also been carried out on phase separation kinetics in both the spinodal and the metastable regions (Binder and Stauffer, 1974; Rogers *et al.*, 1988; Huang *et al.*, 1998; Tanaka and Araki, 1998; Rautiainen and Sutton, 1999) using both approaches from molecular dynamics and from mean field theories. There has been further work reported focusing on the polymer area with the aim of examining how the elastic nature of polymer chains and even the presence of transient polymer networks might be expected to effect the coarsening of domain morphology (Muratov, 1998; Bhattacharya *et al.*, 1998; Tanaka, 1997). It is worth noting that some effort has also been made in outlining approaches for the coupling of phase separation and gelation (Sciortino *et al.*, 1993; Schulz and Paul, 1998), although the majority of the conclusions of work in this area have focused on network formation as an arresting mechanism for phase separation, rather than examining the role of precursor steps in driving demixing. Presently, most of the work is based on *binary* blends of liquids or polymers. However, one of the achievements of recent years has been to demonstrate successfully that the underlying physics is indeed the same in aqueous biopolymeric systems (as has been verified by the observation of universal phase separation mechanisms and system growth exponents; Tromp *et al.*, 1995; Williams *et al.*, 2001; Lorén *et al.*, 2001b; Butler 2002a,b).

In spite of these successes in describing the kinetics of structure evolution, it is fair to say that theories of phase diagram calculation have struggled to achieve quantitative results consistent with experiment in aqueous biopolymeric systems. Indeed the question of the direct analysis of tie-line data within a F-H framework has been extensively discussed (Clark, 2000) and it has been concluded therein and in work on Monte-Carlo simulations (Sariban and Binder, 1988) that success is indeed likely to be of a qualitative nature. Further complications such as those of sample polydispersity and subsequent fractionation of components upon phase separation make theoretical approaches more difficult. It is also worth pointing out that systems used to impart desired textural properties to food products are ultimately in the gelled state, a fact that complicates the predictability of models and also limits the number of techniques able to measure the local concentrations of the biopolymers.

However, despite the difficulties in obtaining quantitative data, the value of these models and their usefulness for food texture design should not be underestimated. Such approaches provide a framework to answer questions such as, ‘What is likely to happen to the phase sense of my system if I halve the concentration of polymer A?’ and ‘What is the likely outcome of adding extra salt to my system?’ The models also provide an insight into which polymeric parameters are important to standardise between polymer suppliers in order to ensure that phase separation functionality is preserved.

3.4.2 Important parameters and behaviour rationalisation within such frameworks

As discussed above, the models that provide an indication of phase behaviour have as their input a number of parameters. These are the molecular weights and charges of the two polymers (the latter used to assess the number of counter-ions present, and are dependent on pH), the amount of added salt, and a set of three interaction parameters that determine how each polymer interacts with solvent and how they interact with each other. These are commonly referred to as χ-parameters. Any physical changes in the system conditions that alter these features will also affect the position of the thermodynamic equilibrium and thereby possibly the mechanism by which phase separation proceeds. The effects of changes in polymeric and environmental parameters on the phase behaviour are now discussed further and general rules applicable for product development are outlined and shown schematically in Fig. 3.4.

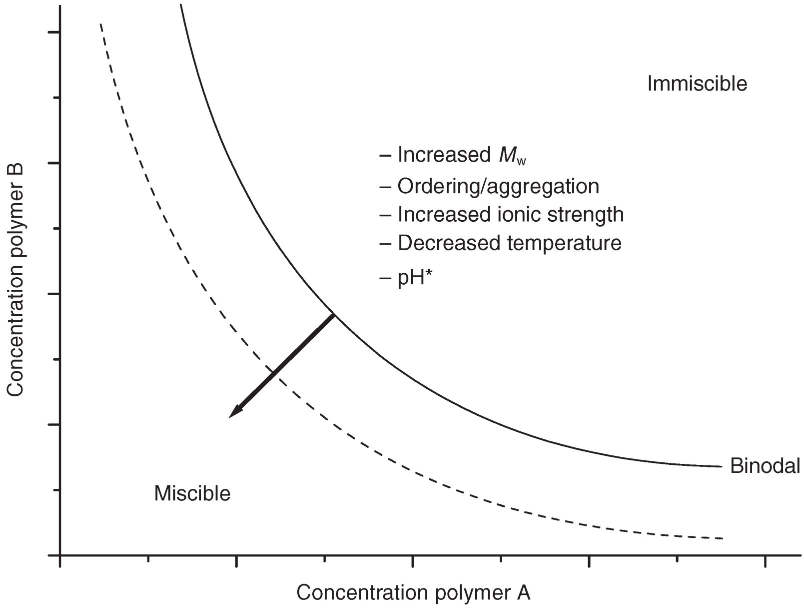

Fig. 3.4 Schematic phase diagram showing the trend for increased immiscibility observed for biopolymer mixtures as certain polymer and physicochemical attributes are changed. (*pH can change the charge density of the polymer; see section on 'Ionic strength').

Molecular weight

Typically as the molecular weights of the components are increased the phase boundary moves towards lower concentrations, i.e. the phase-separated region increases. The drive for larger molecular weight entities to demix is increased as the entropy gain of mixing is further reduced. Similarly, this effect is also found as the polymers become stiffer.

Temperature

Decreasing the temperature results in a larger region of incompatibility as the overall entropy of mixing contribution is reduced. However, the temperature dependence of the interaction parameters can also prove important and should not be overlooked (Lorén *et al.,* 2001b). Additionally, decreased temperature often also induces molecular ordering and subsequent aggregation and gelation. The effect of aggregation and network formation will be discussed further below.

Ionic strength

The tendency for two uncharged polymers to segregate is higher than for charged polymers. The addition of salt screens the electrostatic effect of the ionic polymers. When mixing an uncharged polymer with a charged one, the tendency for phase separation is low at low salt concentrations. This is due to large entropic cost of the counter-ions being restricted to one phase in a separated system. In order to minimise this effect and promote phase separation of an uncharged:charged polymer mixture, additional salt has to be added. Addition of salt will decrease the relative entropic loss of the counter-ions associated with segregating the polymers (Picullel *et al.*, 1995; Harding *et al.*, 1995).

pH
In a similar manner to ionic strength the pH will influence the charge density of biopolymers and hence also influence their phase separation behaviour by modifying the number of counter-ions that must be associated with the biopolymer to retain electrical neutrality (Lorén *et al.*, 1999). Care must be taken when considering indirect effects of pH changes such as conformational changes or the introduction of aggregation (particularly with proteins). Such changes in molecular assembly are expected to influence the phase behaviour greatly, as detailed below.

Conformational ordering, aggregation and network formation
In general, the ordering and aggregation of the biopolymeric components enhance the thermodynamic drive towards phase separation (Elicabe *et al.*, 1998). It is worth stressing that not only do ordering, aggregation and network formation affect the location of the binodal, but the ability of the system to reach its equilibrium state is also affected, i.e. the mobility of the polymers is impeded by aggregation and network formation.

From a modelling point of view, a detailed calculation of how the binodal moves with ordering is elusive. This is because the way in which the effective molecular weight and interaction parameters will change with ordering is difficult to predict or measure. However, by changing the molecular weight within the F-H model, it was possible to demonstrate from data in a gelatin–maltodextrin system that the effective molecular weight increase of gelatin alone was not the only driving force behind ordering induced phase separation (Lorén *et al.*, 2001b), changes in the χ-parameters also played a role. Therefore to predict theoretically the local concentrations in a final microstructural state is by no means trivial and depends crucially on estimates of the kinetics of ordering, demixing and gelling.

Shear
Experimental data exist suggesting that subjecting a mixed polymeric system to mechanical shear causes both shifts in the binodal position and in ripening kinetics. The former has been carried out on polymer blends where shear has been used to induce de-mixing, by the flow-induced change in the orientation of molecules (Soontaranun *et al.*, 1996; Higgins *et al.*, 2000). The latterly mentioned modification of ripening kinetics has been examined in an aqueous biopolymer mixture and found to play a significant role in the generation of the phase morphology (Brown *et al.*, 1995; Foster *et al.*, 1996b; Butler, 2002a,b). Shear can also modify the phase continuity for sheared-cooled systems. It has been shown that for mixtures where one of the components forms a gel during shear cooling, the other component will form the continuous phase (Foster *et al.*, 1996b).

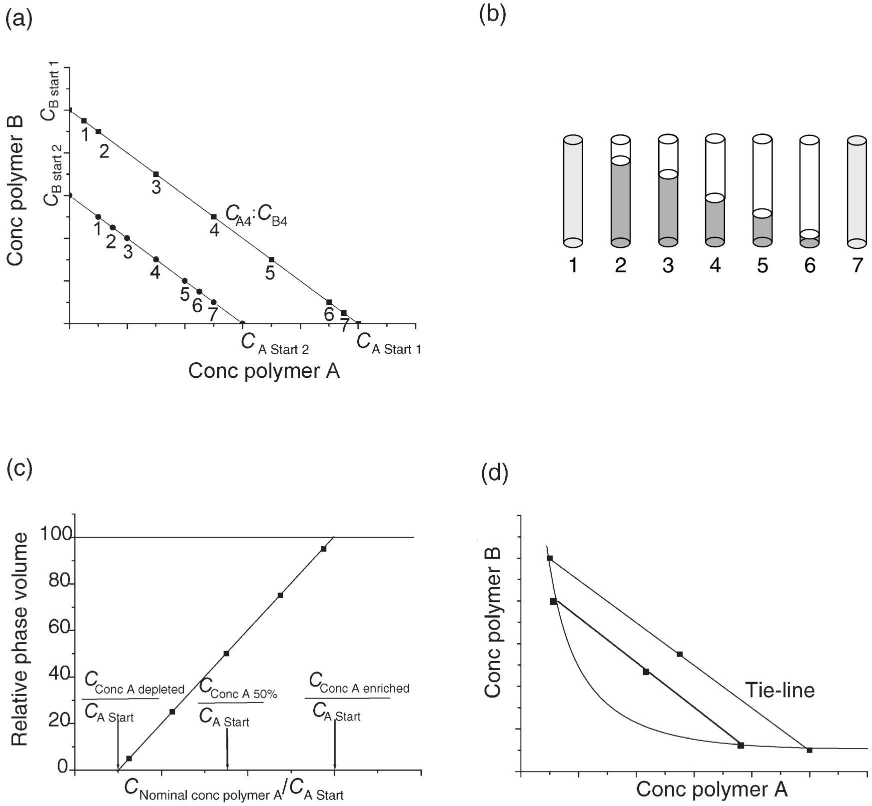

Fig. 3.5 Phase volume method.

3.4.3 Measurement

Measurement of phase separation can be done in both a qualitative and quantitative manner. To serve the purposes of microstructure and consequent texture design, qualitative understanding and measurement are often adequate. Therefore a knowledge of phase/state diagrams and how they can be used to control phase volumes of two incompatible phases is all that is necessary. The reader is directed to an article by Tolstoguzov and co-workers, in which the creation of phase diagrams by use of the bulk phase separation of mixtures along a series of 'secant' lines is described (Polyakov *et al.*, 1980). This procedure is shown schematically in Fig. 3.5.

(a) A series of stock concentrations of the individual biopolymer solutions are mixed with different stock concentrations of another biopolymer, in varying proportions.

(b) The samples are observed with time at a given temperature, or after centrifugation, to follow bulk phase separation. Knowing the mixing ratios, the experimenter knows the nominal concentrations of each biopolymer in the mixture. Observations are made as to whether bulk phase separation has occurred in each mixture and the relative phase volumes of each phase are noted.

(c) Plotting the relative phase volumes of a given phase against the ratio of nominal concentration:starting concentration, for each mixture, extrapolation to 0% and 100% phase volume can be made for each biopolymer phase, providing the actual concentration of the given biopolymer in each phase, i.e. the one in which it is both rich and poor. Carrying out this analysis for each phase, on a given secant then provides the composition of each biopolymer in each phase.

(d) Analysing different secants in this way provides a number of points through which the binodal can be drawn for that biopolymer mixture, under the conditions that it has been measured. Knowing the binodal and the phase volumes of each mixture within it, tentative tie-lines can be drawn through each individual point. This is then still very qualitative, as the confirmation of the relative phase concentrations is still to be determined, to validate the approach.

Quantification can be carried out using analytical chemistry and more sophisticated techniques, e.g. high-resolution ^{1}H nuclear magnetic resonance (NMR). Indeed the phase diagram of LBG : casein in Fig. 3.1 was verified using analytical chemistry techniques (Schorsch *et al.*, 1999a,b). However, the techniques described above serve only to describe the phase concentration behaviour under certain experimental conditions. This chapter has shown that it is dangerous to assume that this will be maintained as conditions are changed, i.e. a decrease in temperature, increase in shear, change in salt/pH environment, and also that before bulk phase separation is achieved, there are a number of ripening processes taking place, which, depending on the mechanism of phase separation of the mixtures, might influence either phase volume or phase concentration. Therefore it is imperative to be able to measure phase concentrations during or after environmental changes, or as a function of time after mixing. Early work in this area employed the use of Fourier transform infrared (FTIR) microscopy (Durrani and Donald, 1995; Sun *et al.*, 1996), and more recent work, using Raman microscopy (Normand *et al.*, 2000; Pudney *et al.*, 2002, 2003), has expanded on this work, showing both capability and potential to elucidate this understanding further. Confocal scanning laser microscopy (CLSM) (using fluorescent stains) has also been used to establish the effective polymer concentrations in phase separated systems (Blonk *et al.*, 1995). In a recent European Union-funded project (FLAIR-FLOW CT96 1015), polarimetry and differential scanning calorimetry have also been used to determine the phase concentrations of mixtures trapped, by gelation, under certain cooling regimes. Therefore, the developments that are currently being made in this area

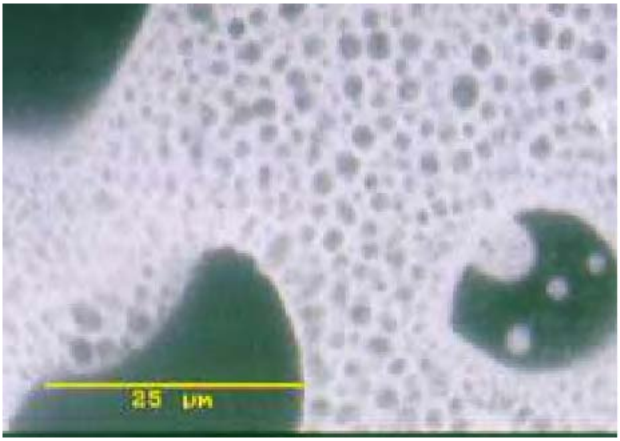

Fig. 3.6 CLSM micrograph of a fruit pastille, showing a phase separated microstructure.

of research are not only quantitative, but also are relevant to understanding thermodynamic incompatibility of biopolymer mixtures under 'real process' conditions, on 'real' microstructures.

3.4.4 Application

Throughout this chapter 'model' binary biopolymer systems have been discussed, which may lead the more applied reader to question whether the theory really holds up in practice, in creating 'real' food systems. Although such 'real' systems may be multi-component, the authors note that the theory that has been discussed can be relevant. The importance of understanding the phase behaviour of the ingredients, i.e. taking resulting phase volume and effective concentration into account, when developing food product is exemplified below.

Analysis of confectionary food systems, such as fruit gums, shows that although the products are high-solids systems, and often contain modified starch, gelatin and gum arabic, the microstructure of such products is phase separated, as can be seen in Fig. 3.6. It is therefore of great importance to know how to create such microstructures, and, maybe of even greater importance, how such microstructures can be controlled to create new textural attributes, using the same process and the same ingredients, only in different proportions. Whitehouse and co-workers (1996) showed that this phenomenon is possible when visualising microstructures of oxidised waxy maize starch and gelatin, in high-sugar solids systems. More recently, DeMars and Ziegler (2001) followed a similar approach using high methoxy pectin and gelatin mixtures in the creation of 'gummy' gels. This latter study also showed that the microstructures created go some way to explaining the sensory properties of the mixtures, when compared in free choice profiling.

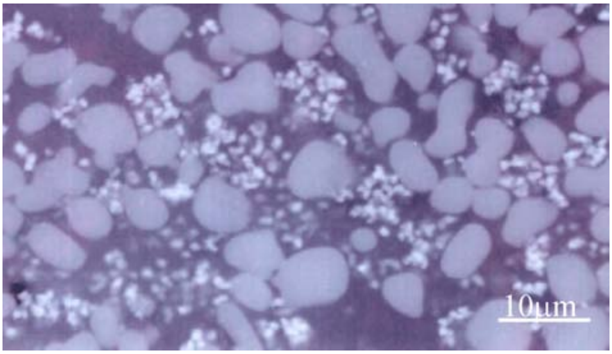

Fig. 3.7 CLSM micrograph showing a sheared cooled gelatin:maltodextrin system.

However, neither of these studies has taken the systematic approach to actually understand the importance of microstructure, outlined in this chapter. Both studies vary one or both of the biopolymer concentrations. Therefore all combinations studied have different relative phase volumes of the two phases and, equally importantly, different final biopolymer concentrations in the two phases. This therefore is an area of study still open to increased understanding.

Another example of how control of phase separation can create a microstructure that provides consumer-relevant attributes is the creation of zero fat spreads (Brown *et al.*, 1993). Here the product was simply a mixture of gelatin and maltodextrin. Knowing the relevant phase volumes of each phase, along with the impact of the process used, the product was optimised to provide both acceptable spreading and in-mouth behaviour. Work by Brown and co-workers (1995) and Foster (1996b) later explained the background to the impact of shearing and cooling on the microstructures of these systems. A typical engineered low-fat spread microstructure is shown in Fig. 3.7. The microstructure consists of included gelatin particles in a matrix of gelled maltodextrin.

3.5 Future trends

In this chapter the framework of how to take advantage of phase separation when designing microstructures with desired rheological properties has been outlined. However, it should be clear that recommendations are valid for fairly simple systems of segregated aqueous biopolymers. It is our belief that in the future, it will be important to extend our knowledge from binary biopolymer systems to multi-component systems in order to constructively design food products consisting of multiple biopolymer phases. This involves dealing with the step change in structural complexity, going from binary polymer systems to multi-components systems. The number of morphological possibilities is vastly increased going from a two to a three biopolymer system. In order to investigate such complex systems, non-invasive measuring techniques are essential. It is our belief that rapid developments within 3D-microscopy and image analysis will strongly support future developments in this area. In addition to more sophisticated microscopy techniques, FTIR and Raman microscopy will present the possibility of measuring effective polymer concentration in different phases. In line with new time-resolved measuring techniques being developed, it will also be possible to investigate dynamic non-equilibrium systems and structural ripening processes of multi-phase systems. This will allow the development of improved mechanical models, which in addition to biopolymer concentrations and phase volumes also incorporate structural and physical parameters such as droplet size distribution, droplet shape, phase continuity and debonding energies.

With an improved knowledge of the structural and textural functionality of ingredients, the product developer of the future will be well equipped to design high-quality foods meeting ever more demanding consumer needs.

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4

The structure and texture of starch-based foods

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4.1 Introduction

Starch is a biopolymer that is produced by plants and serves as a store of carbon and energy. Unlike other plant polysaccharides, such as cellulose and pectic substances, starch has no structural function in the plant. Starch becomes a structuring agent in food owing to its transformation during food processing. Since the thickening and gel-forming properties of starch arise during hydrothermal processing, it is considered a secondary texturogen (Escher, 1987). The present chapter focuses on the role of starch as texturogen in food systems such as sauces, fillings, dessert products with a gel-like texture and purees. These types of semi-solid food product typically contain a large amount of water and are easily deformed by external stresses. Given the multi-component nature of food, starch exhibits a diversity of interactions with other food components. The present chapter aims at presenting the processing–structure–rheology relationships of starch in food from a polymeric and colloidal point of view, taking into account the different levels of structural hierarchy.

4.2 The rheological properties of starch

Starch is a polymer and, as such, the principles of polymer science apply to it. On the other hand, many properties of starch arise from the complex architecture of the native starch granules. Thermal processing of starch in the presence of water leads to systems that are colloidal in nature, and exhibit a diverse range of rheological

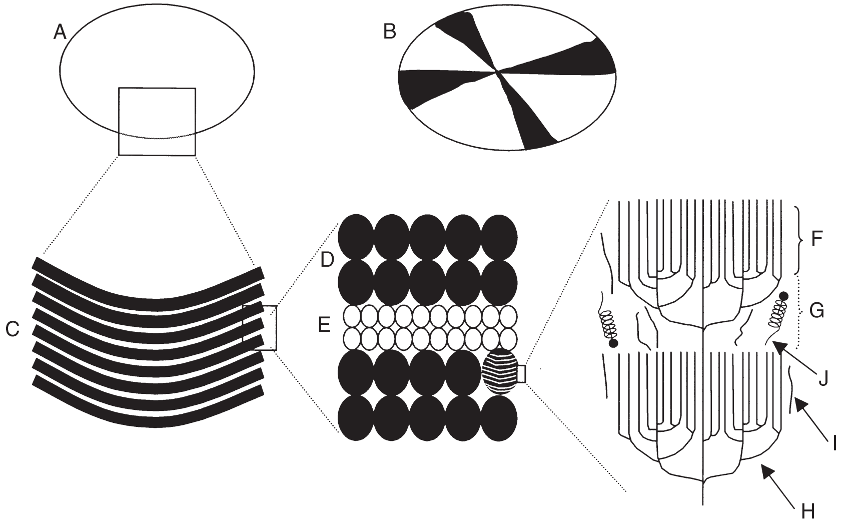

Fig. 4.1 Schematic presentation of the structural hierarchy in native starch. The starch granules (A), which are birefringent under polarised light (B), represent the lowest level of starch organisation. The granules exhibit growth rings (C) which consist of alternating hard semi-crystalline (D) and soft amorphous shells (E). The semi-crystalline shell is organised in blocklets composed of stacks of crystalline (F) and amorphous lamellae (G). Amylopectin (H) is responsible for starch crystallinity. Amylose (I) and amylose-lipid complexes (J) are not ordered in native starch and form amorphous domains.

properties varying from simple viscous fluids to highly elastic gels. This diversity is one key reason for the widespread technological utility of starch.

4.2.1 Starch: a partly crystalline biopolymer

The elucidation of the granular structure of native starch at the micrometre level developed parallel to optical microscopy, and the granular structure of starch was known long before its polymeric nature was established. The progress in physicochemical methods in the past hundred years has contributed to a detailed picture of native starch structure, taking into account different levels of organisation. The following description of the structure of native starch is based on reviews of French (1984), Blanshard (1987), Donald *et al.* (1997), Gallant *et al.* (1997) and Buléon *et al.* (1998).

Starch is synthesised in the form of microscopic granules. The shape and size distribution depend on the botanical origin of starch, the typical size range being 1–100 μm. A peculiarity of starch is that it is composed of two main α-D-glucans, the branched amylopectin and the linear amylose. Most common starches are composed of 25% amylose and 75% amylopectin. However, the proportion of amylopectin may range from 95% in low amylose or waxy starches to a few per cent in some high amylose starches. A schematic presentation of the organisation of starch granules is presented in Fig. 4.1. The starch granules, which represent the

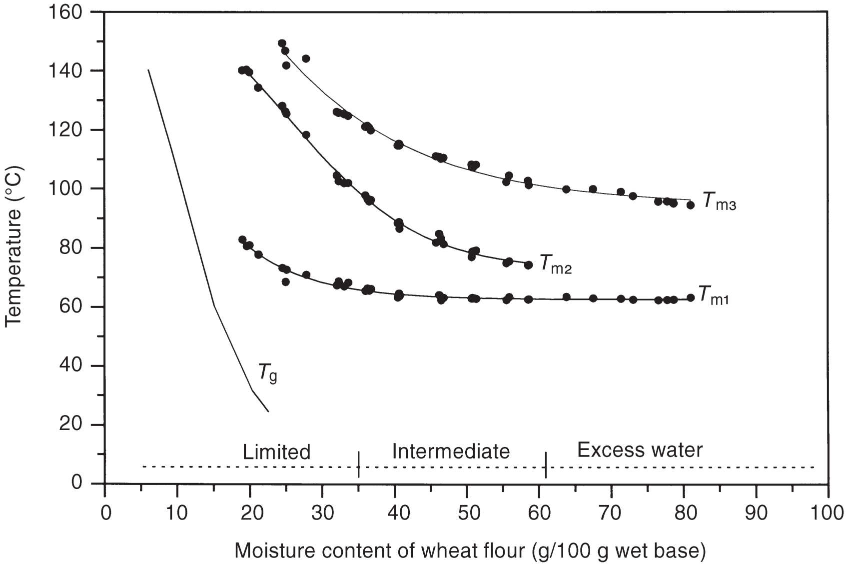

Fig. 4.2 Starch–water state diagram of wheat starch. The state diagram is based on DSC measurements of Zeleznak and Hoseney (1987) (T_g) and Zweifel *et al.* (2000) (T_{m1}, T_{m2} and T_{m3}). T_g corresponds to the glass transition of the amorphous domains of starch and T_{m1} and T_{m2} reflect the melting of native amylopectin crystallites. T_{m3} corresponds to the melting of endogenous amylose–lipid complexes in wheat starch.

lowest level of organisation, are birefringent under polarised light indicating a radial organisation of the polymers within the granules.

The next level of structure is formed by the so-called growth rings, a layered concentric shell structure that consists of alternating hard semi-crystalline and soft amorphous shells. The growth rings are generally between 120 and 400 nm in size. Little is known regarding the organisation of starch polymers in the amorphous shell, and research has concentrated on the elucidation of the structure of the semi-crystalline shells. The latter also consist of a concentric shell structure of alternating amorphous and crystalline lamellae, the distance between two crystalline lamellae being approximately 10 nm. Both the crystalline and the amorphous lamellae are organised into spherical structures termed blocklets, which range in diameter from 20 to 500 nm. The crystallinity is associated with the branched amylopectin, while the branch points of amylopectin and the amylose are not ordered (amorphous lamellae). The crystalline organisation of starch is based upon six-fold double helices, and A- and B-type crystalline packing can be distinguished by X-ray diffraction (Imberty *et al.*, 1987, 1988).

Starch can be considered a partly crystalline polymer as it is composed of amorphous and crystalline structures. This type of polymer exhibits two major transitions, a glass-to-rubber transition for the amorphous phase at the glass transition temperature T_g, and crystal melting transitions at the temperature T_m (Slade and Levine, 1991; Biliaderis, 1998). A state diagram of starch presenting T_g

and the multiple melting transitions of starch T_{m1}, T_{m2} and T_{m3} as a function of moisture content is shown in Fig. 4.2. Water acts as a plasticiser and diluent for starch as it depresses T_g and T_m. Starch exhibits a weak glass transition which extends over a rather broad temperature range since granular starch presents a range of non-ordered domains with different mobilities. Amorphous phases of amylopectin that are 'locked-in' between crystallites have a severely restricted mobility, i.e. a lower T_g compared with bulk amorphous amylose (Biliaderis *et al.*, 1986). The fact that amorphous and crystalline domains are interconnected in starch also has an influence on the melting of starch crystallites. At excess water conditions, melting of amylopectin is characterised by a single endothermic transition T_{m1}. At intermediate water conditions, the order–disorder transition of amylopectin crystallites yields a two-stage melting process as observed with differential scanning colorimetry (DSC), while at limited moisture conditions, the T_{m2} transition dominates (Garcia *et al.*, 1996). Finally, starches with endogenous lipids, such as cereal starches, reveal an additional melting transition at T_{m3} which is attributed to the melting of endogenous amylose–lipid complexes.

4.2.2 Structural changes during hydrothermal processing

A characteristic of semi-solid food is that water is a major component. The structural and textural properties of starch-based food are to a large extent controlled by the interaction of starch polymers and water. Starch presents several structural features which enable the interaction with water, the most effective factor being the 1→6 carbon linkages found in amylopectin. Furthermore, α-linkages create an improvement over β-linkages, although they do not confer true solubility (Glass, 1986). The overall solubility of starch in water is rather poor, the solubility decreasing in the order amylopectin > amylose > amylose–lipid complexes. As the temperature increases, the thermodynamic quality of water as a solvent increases (Benczédi *et al.*, 1998), which means that it becomes easier to break the hydrogen bonds that keep starch self-associated at high temperatures. Thus, heating of starch in the presence of water promotes swelling and solubilisation of starch. This process leads to a strong increase in viscosity and is known as gelatinisation in the starch literature.

The gelatinisation process has been the topic of numerous publications since it is the basis for the thickening effect of starch. Earlier studies focused on the influence of the time–temperature conditions, starch concentration and botanical origin on the rheological properties of the resulting starch dispersions and gels (Ott and Hester, 1965; Bagley and Christianson, 1982; Eliasson, 1986a; Doublier, 1987; Doublier *et al.*, 1987; Ellis *et al.*, 1989). More recent studies present details of the structural changes of starch in the micrometre and nanometre range upon heating in presence of water (Cameron and Donald, 1993; Fisher *et al.*, 1997; Atkin *et al.*, 1998 a,b). Swelling of starch requires plasticisation of the amorphous domains and melting of the starch crystallites which act as physical cross-links. The melting temperature of amylopectin crystallites and of amylose–lipid complexes decrease as more water becomes available (Fig. 4.2). Marked swelling of starch starts at around 60 °C, but in the initial swelling phase the release of soluble material is relatively small. Heating to 100 °C under excess water conditions leads to a complete loss of order as detected by birefringence, X-ray diffraction and DSC. The extent of granule swelling is influenced by the botanical origin, the swelling capacity increasing in the following order: high amylose starches < cereal starches < tuber starches. The strong swelling capacity of potato starch is probably linked to the absence of endogenous amylose–lipid complexes and to the esterified phosphate groups which make the starch–water interaction more favourable. A complete loss of starch crystallinity by heating in excess water does not necessarily lead to a complete dissolution of starch. The remaining structures resemble the original starch granules and are, therefore, also termed 'ghosts'. These supramolecular starch structures show neither signs of birefringence nor X-ray crystallinity and are mainly composed of amylopectin. Different methods have been established to determine the extent of starch swelling based on centrifugation (Leach *et al.*, 1959), filtration (Bagley and Christianson, 1982) and dye exclusion techniques (Evans and Haisman, 1979).

The gelatinisation of starch may also be viewed as a phase separation process, since starch is composed of two thermodynamically immiscible polymers (Kalichevsky and Ring, 1987). The phase separation of amylose and amylopectin, which occurs even at moderate concentrations, is promoted by an asymmetry in the affinity of the two polymers to the common solvent (Hsu and Praunitz, 1974). During heating of starch in water, amylose and amylopectin phase separate and the result is a microstructure with two phases, where each polymer is enriched in one of the two phases. Amylose, which has a higher mobility than amylopectin, diffuses out of the granules, but in the centre of swollen starch granules an amylose-rich phase may also be found. Amylose/amylopectin phase separation has been documented for different food systems using microscopy (Svegmark and Hermansson, 1991; Hug-Iten *et al.*, 1999). Amperometric iodine titration has been used as a semi-quantitative method to follow the extent of amylose leaching into the intergranular space (Conde-Petit *et al.*, 1998).

4.2.3 Structure–rheology relationships of aqueous starch dispersions

The thickening and gelling properties of starch are primarily determined by the microstructure of the system which, in turn, is influenced by the processing conditions and the botanical origin of starch. Starch dispersions are colloidal systems, where the swollen starch granules form the dispersed phase and the soluble material, in particular amylose, forms the continuous phase. The viscosity of starch dispersions is closely related to the volume fraction and the deformability of the dispersed particles (swollen starch granules). Furthermore, the viscosity of the continuous phase (soluble starch) and the interaction between the phases influence the rheological properties of the systems. The swelling and disintegration state of starch granules in relation to viscosity and leached amylose is presented in Fig. 4.3 for potato starch. Increased starch swelling leads to higher viscosity, but the disintegration of the supramolecular structure is accompanied by an exponential

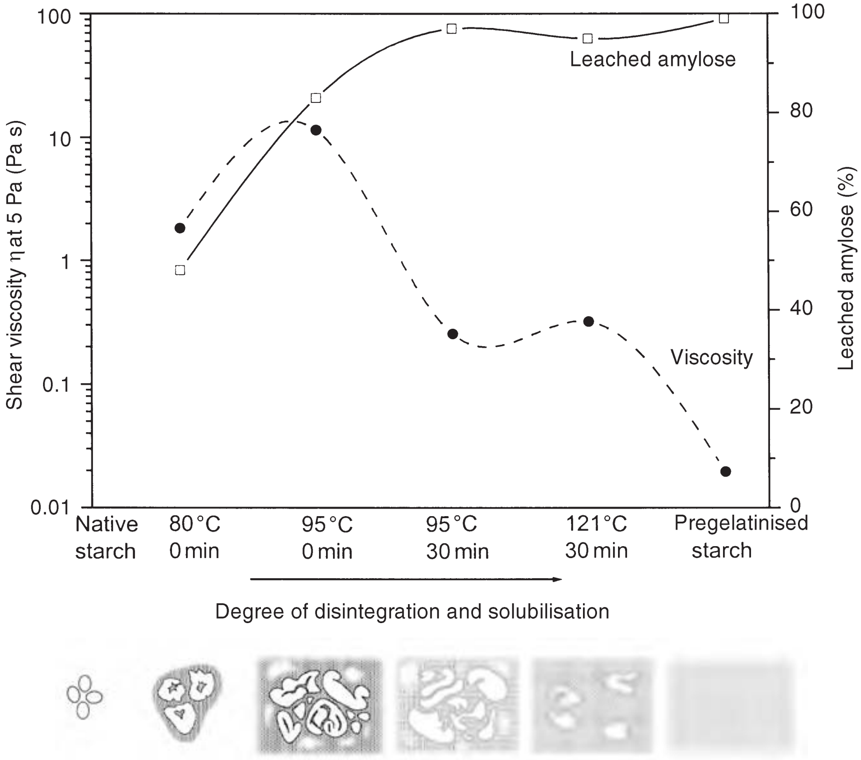

Fig. 4.3 Schematic presentation of the microstructure of potato starch dispersions (3 g/100 g) obtained under different preparation conditions in relation to shear viscosity at 25 °C and the amount of leached amylose as determined by iodine titration (adapted from Conde-Petit *et al.*, 1998).

viscosity decrease. It is noticeable that the viscosity of potato starch covers three orders of magnitude depending on the extent of starch granule swelling and disintegration. The broad viscosity range is typical for starches with a strong swelling capacity paired with a susceptibility to granule disintegration by shear (Svegmark and Hermansson, 1990). Starches with a lower swelling capacity, such as cereal starches, show a similar behaviour, though less pronounced.

In general, as the starch concentration increases, swelling and solubilisation of starch is reduced owing to the limited amount of water, and the differences between starches become less accentuated. On the other hand, a complete dissolution of the granular structure requires treatments at temperatures above 100 °C in combination with mechanical energy input (Dintzis and Bagley, 1995). For practical applications of starch as thickener, extensive swelling of starch is generally desired as it results in a high yield in terms of viscosity. However, extensive swelling makes starch granules susceptible to loss of granular integrity by mechanical energy, elongational flow being more effective than shear flow.

A widely exploited method of controlling swelling and disintegration of starch granules, and thus the viscosity of the dispersion, is through the chemical modification of starch. A further method of overcoming the limitations of unmodified starch is physical modification. More recently, the demand for natural ingredients has encouraged the development of physically modified starches in the granular state (Croghan and Mason, 1998). By analogy with synthetic polymers, starch may be annealed, that is treated between T_g and T_m, preferentially under limited water conditions, to increase the molecular order of starch polymers. Although the structural changes induced by annealing are not understood in detail, it is well documented that annealing increases the thermal stability and better preserves the granular integrity of starch during processing (Sair and Fetzer, 1944; Hoover and Vasanthan, 1994; Jacobs and Delcour, 1998; Conde-Petit, 2001).

Besides the microstructure, time-dependent changes of the starch polymers govern the rheological properties of starch dispersions. During cooling and ageing of starch dispersions, the tendency to minimise the starch–water interaction is the driving force for the creation of supramolecular structures which, in turn, determine macroscopic properties such as viscosity and gelation. The gelation of starch can be viewed as a passage to a state of lower free energy. It is one aspect of the spontaneous changes that occur upon ageing of starch dispersions, also known as starch retrogradation (Hoover, 1995; Parker and Ring, 2001). Both starch polymers have the tendency to form gels, although the gelation of amylose proceeds much faster than that of amylopectin. Amylose belongs to the polymers where gelation has been studied in detail (Gidley and Bulpin, 1989; Miles *et al.*, 1985a; Doublier and Choplin, 1989; Goodfellow and Wilson, 1990). Gelation of amylose involves phase separation and the formation of helices, interchain double helices acting as junction zones. Amylose networks are formed by filaments that result from the association of amylose chains into clusters of 10 to 15 nm in a B-type crystalline array (Leloup *et al.*, 1992; Putaux *et al.*, 2000). In contrast to amylose, the coil-to-helix transition of the side chains of amylopectin does not lead to gelation, but the subsequent crystallisation of the amylopectin side chains induces network formation (Miles *et al.*, 1985b; Ring *et al.*, 1987). Starch gels fall into the category of mixed polymer gels or composites, where the swollen amylopectin-rich granules are embedded in an amylose network.

4.3 Starch in multi-component food systems

In food, starch is always present in combination with other non-starch components. The textural properties of foods based on plant material rich in starch are to a large extent influenced by polymeric material other than starch, and by the interactions between the different ingredients. On the other hand, starch is often selectively combined with non-starch hydrocolloids in industrial food preparation to obtain products with enhanced textural properties. Finally, specific interactions between starch and small molecules, such as emulsifiers and flavour compounds, may lead to dramatic textural changes. In the following, some aspects of the interactions between starch and other food components will be presented. Given the complexity of real food systems, it is not surprising to find that most studies focus on rather simple model systems, for instance ternary systems composed of two polymers and water, or aqueous starch dispersions with one complexing ligand.

4.3.1 Starch inclusion complexation

Starch, in particular the linear fraction amylose, can specifically interact with small molecules. In the presence of suitable ligands, amylose forms a single helix including the ligand in the helical cavity. A well-known interaction is the starch–iodine inclusion complex, which develops a blue colour and is used for analytical purposes. Other suitable ligands are fatty acids, emulsifiers, alcohols, aldehydes, and different flavour compounds such as terpenes and lactones. The formation of single amylose helices as induced by a suitable ligand yield the typical V-type X-ray diffraction pattern. Fatty acids or emulsifiers are accommodated in such a way that the hydrophobic chain is positioned inside the helix and the polar part of the molecule stands out (Godet *et al.*, 1993). Linear ligands generally induce a helix with six glucose residues per turn, and for bulky ligands such as naphtol, a helix with eight glucose units per turn was found. For certain complexing ligands the position of the ligand in relation to the helix remains uncertain, and an entrapment between helices is also conceivable (Helbert, 1994). Quantitative aspects of the amylose–ligand interaction have been investigated by Robinson and Solms (1984), Karkalas and Raphaelides (1986) and Rutschmann and Solms (1990). The formation of helical inclusion complexes is primarily a property of amylose, but a complexation with the linear side chains of amylopectin is also considered by several authors (Gudmunsson and Eliasson, 1990; Huang and White, 1993; Nuessli *et al.*, 2000; Heinemann *et al.*, 2003).

The formation of starch inclusion complexes influences all properties related to structure, including the rheological properties. The coil to helix transition of amylose promotes intra- and intermolecular aggregation of helical domains, which lead to the creation of supramolecular structures such as fine-stranded networks and spherulitic crystallisation (Heinemann *et al.*, 2003). Besides added ligands, native lipid material present in starch contributes to the formation of crystalline aggregates (Fanta *et al.*, 2002). In dilute systems, amylose complexation leads to crystallisation followed by bulk phase separation. Above a critical starch concentration, which is around a few per cent of starch, amylose aggregation may lead to the formation of an interconnected network. At the macroscopic level this is manifested by viscosity increase and gelation. The resulting gel is kinetically stabilised in an out of equilibrium state.

Many formulations of starch-based food include emulsifiers, and the influence of complexing emulsifiers on the rheology of aqueous starch systems has been the topic of several studies (Eliasson, 1986b; Evans, 1986; Raphaelides, 1993; Hoover and Hadziyev, 1981; Conde-Petit and Escher, 1995). In general, emulsifiers reduce the extent of starch swelling and solubilisation modifying the rheology of the system, their effect being linked to the formation of starch inclusion complexes.

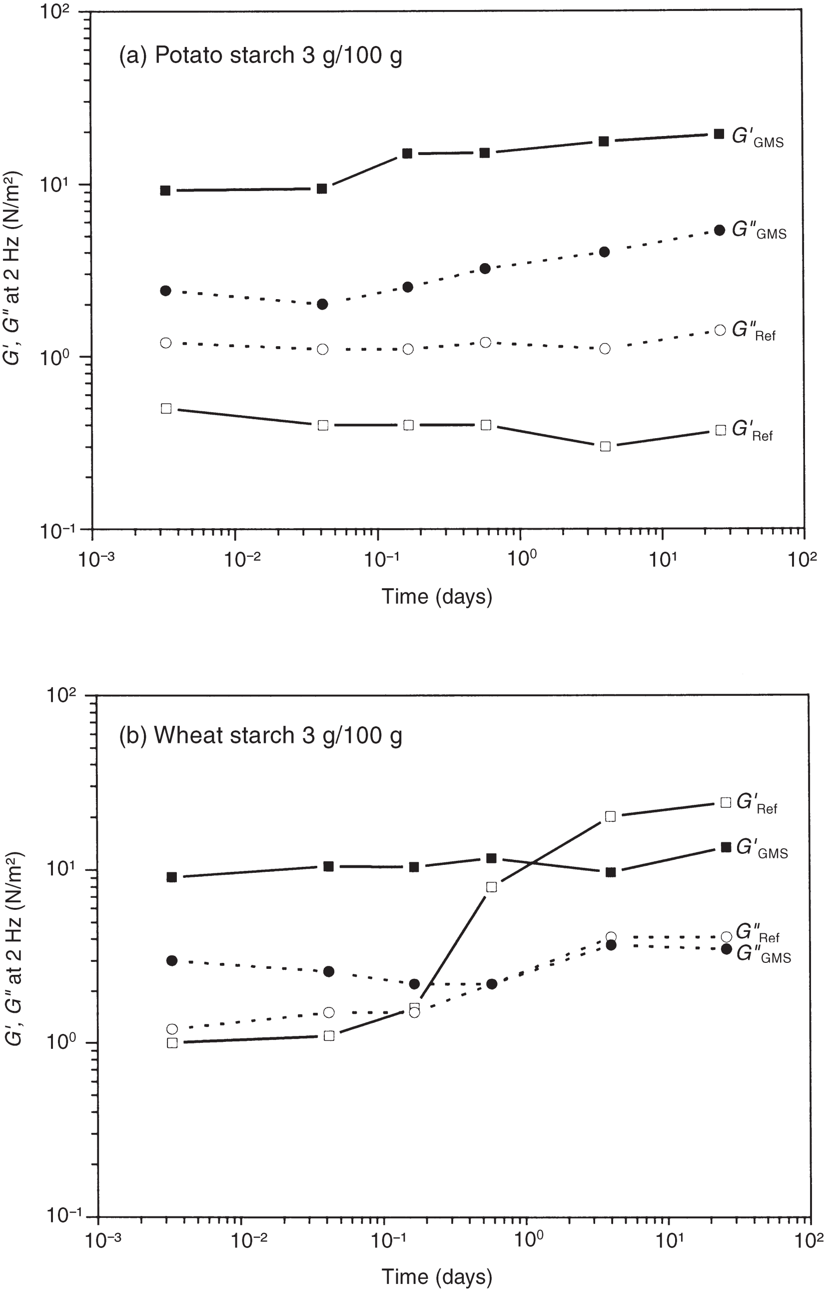

Fig. 4.4 Influence of starch complexation with glycerol monostearate (GMS) on the storage modulus G' and the loss modulus G'' of potato (a) and wheat (b) starch dispersions at 25 °C (data from Conde-Petit, 1992).

To a certain extent, the formation of starch–emulsifier complexes contributes to the stabilisation of the granular structure by increasing the rigidity of starch granules. On the other hand, the gelation of starch dispersions can be induced by starch–emulsifier complexation, provided that processing leads to extensive leaching of amylose and that emulsifiers are added at concentrations that allow a saturation of the amylose fraction. The complexation-induced gelation is manifested by a strong increase of the storage modulus as shown in Fig. 4.4 for potato and wheat starch dispersions. Note that potato starch dispersions at low concentrations show only minor changes upon ageing, whereas wheat starch dispersions exhibit spontaneous gelation (retrogradation). The complexation-induced gelation is a rapid process compared with the spontaneous gelation. It is likely that the presence of endogenous amylose–lipid complexes in wheat starch contributes to the spontaneous gelation, the small complexed amylose fraction acting as nuclei. The amylose–lipid complexes melt at around 100 °C with a variation of ±15 °C, the thermal stability of complexes depending on the size of the ligand, the degree of polymerisation of amylose and the crystallisation conditions, in particular temperature, time and moisture conditions (Biliaderis and Galloway, 1989).

4.3.2 Interaction with polymeric food ingredients

The combination of starch with other hydrocolloids that dissolve or disperse in water giving a thickening or gelling effect is a common practice as it presents advantages in terms of processing and product texture. Several publications have focused on the rheological properties of starch dispersions and gels in the presence of a non-starch hydrocolloid (Sajjan and Rao, 1987; Alloncle and Doublier, 1991; Annable *et al.*, 1994; Appleqvist and Debet, 1997; Conde-Petit *et al.*, 1997; Closs *et al.*, 1999). In general, the addition of a small amount of non-starch hydrocolloid increases the yield since the viscoelasticity of the mixture is enhanced. The admixture of a biopolymer to colloidal starch dispersions allows the generation of a broad range of structures and textures, and increases the stability of high-moisture food systems. The described effects are in general due to unspecific interaction of the polymers, that is to thermodynamic immiscibility, and specific interactions, as described for instance between starch and ι-carrageenan (Eidam *et al.*, 1995), are exceptions. Equilibrium phase diagrams of model systems have been established to determine the conditions of polymer miscibility and solvent (water) partitioning between the phases. The phase behaviour of amylopectin–gelatin mixtures has been studied at temperatures above and below the gel point (Durrani *et al.*, 1993; Durrani and Donald, 1994). Closs *et al.* (1999) established phase diagrams for amylopectin–galactomannan systems.

Since starch is already a mixture of two polymers, the addition of a further polymer yields a multiphase system with three phases that are rich in amylopectin, amylose and the added polymer, respectively. Polymer composition and concentration, processing conditions and time play a crucial role for the phase-separated morphology of multi-component systems. Heating of native starch suspensions in the presence of a small amount of dispersed non-starch hydrocolloid, for instance galactomannans or xanthan, leads to a concentration of the added hydrocolloid in the continuous phase which, in turn, results in a large increase in viscoelasticity (Alloncle and Doublier, 1991). Another application is the incorporation of starch into protein matrices based on meat, fish, milk or vegetable proteins. Heating of starch in a protein matrix leads to a composite gel microstructure, the swollen starch granules acting as fillers that alter the rheological properties of the composite material (Brownsey *et al.*, 1989; Aguilera and Rojas, 1996; Kong *et al.*, 1999; Olsson *et al.*, 2000; Lee, 2002). The mechanical properties of such composite materials can, to a reasonable approximation, be derived from the properties of the individual phases, taking into account the respective volume fractions (Clark *et al.*, 1983). An extension of this approach has been experimentally and theoretically studied with maltodextrin–gelatin gels (Kasapis *et al.*, 1993) and agarose–waxy maize starch gels (Mohammed *et al.*, 1998). Finally, a general property of composite materials is that their mechanical properties are also influenced by interactions between the components at the interface. The extent of polymer interdiffusion in the interfacial zone determines whether the failure occurs across or along the dispersed particles.

The microstructure of mixed polymer systems is controlled by the kinetics of the phase separation process which is driven by the tendency to minimise the interfacial area. In multiphase starch systems phase coarsening may result in large accumulation of amylose. Figure 4.5 shows the microstructure of a sauce béchamel composed of potato starch, xanthan and milk. Staining with iodine allows the identification of the phases rich in amylose and amylopectin, respectively. The combination of immiscible polymers and the granular nature of starch is responsible for the formation of an amylose-rich phase within the swollen starch granules. High-temperature and high-shear processing conditions favour the destructurisation of starch granules, leading to a more homogeneous distribution of amylose in the system (Conde-Petit *et al.*, 2001). The phase separation process in polymer mixtures can be almost arrested upon gelation of one of the components leading to the formation of metastable microstructures. A system containing starch and a non-gelling polymer will show macroscopic gelation provided that amylose is present in the continuous phase, which means that a substantial amount of amylose needs to be released from the granules. On the other hand, if the temperature–time conditions favour phase separation over kinetic arrest caused by gelation, coarsening of the amylose phase may proceed to the point where phase inversion occurs and macroscopic gelation is prevented. It is, therefore, not surprising to find that the textural outcome of the admixture of a non-gelling biopolymer to starch dispersions covers the range from accelerated gelation to suppression of gelation.

4.3.3 Cellular food systems

As outlined in the previous sections, the rheological properties of starch in multi-component systems are much more related to starch microstructure and polymer segregation phenomena than to starch concentration. This is particularly pronounced for plant material rich in starch, where processing leads to limited disintegration of

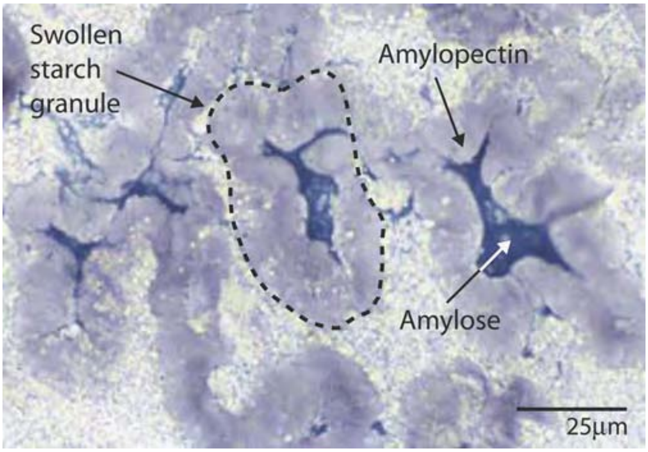

Fig. 4.5 Light micrographs of a sauce béchamel composed of potato starch (2 g/100 g), xanthan (0.2 g/100 g) and milk heated at 95 °C for 30 min at low shear in a scraped surface heat exchanger (adapted from Conde-Petit, 2001).

the plant cells. An example is potato (*Solanum tuberosum*), where processing into mashed potatoes yields a product where starch is largely encapsulated in the parenchymatic cells. During industrial processing of dry mashed potato flakes by drum drying, precautions need to be taken to prevent an extensive destructurisation of the parenchymatic cells and by this an excessive liberation of starch. A precooking step at 50–70 °C followed by cooling before the final cooking reduces the amount of extracellular starch in reconstituted flakes (Reeve and Notter, 1959). Two mechanisms are thought to account for this effect: the firming of the cell walls by the activation of the pectin–methyesterase followed by a strengthening of the cell walls through Ca-bridges (Ng and Waldron, 1997), and an increase of order of the partially gelatinised starch fraction during the cooling step (retrogradation) (Hellendorn, 1971).

The textural properties of mashed potatoes are closely linked to the presence of single cells and cell aggregates and a rather small amount of free starch, in particular amylose in the continuous phase (Escher *et al.*, 1979; Escher, 1993; Alvarez and Canet, 1999). An excessive liberation of amylose may be compensated by the complexation of amylose with added emulsifiers (Hoover and Hadziyev, 1981). Mashed potato can be viewed as a concentrated cell suspension in a viscoelastic fluid. The product behaves as a viscoplastic material that shows little or no deformation up to a certain level of stress, but flows readily above this yield stress (Schweingruber *et al.*, 1979). Small amplitude oscillatory measurements reveal that at low deformation the elastic properties greatly exceed the viscous properties, but the limit of the linear viscoelasticity is quite low, indicating a high sensitivity of the structure to the applied deformation (Alvarez and Canet, 1999). Because of this particular rheological behaviour, potato flakes are used as a binding agent in systems where a high water binding capacity without the development of a gel-texture is desired.

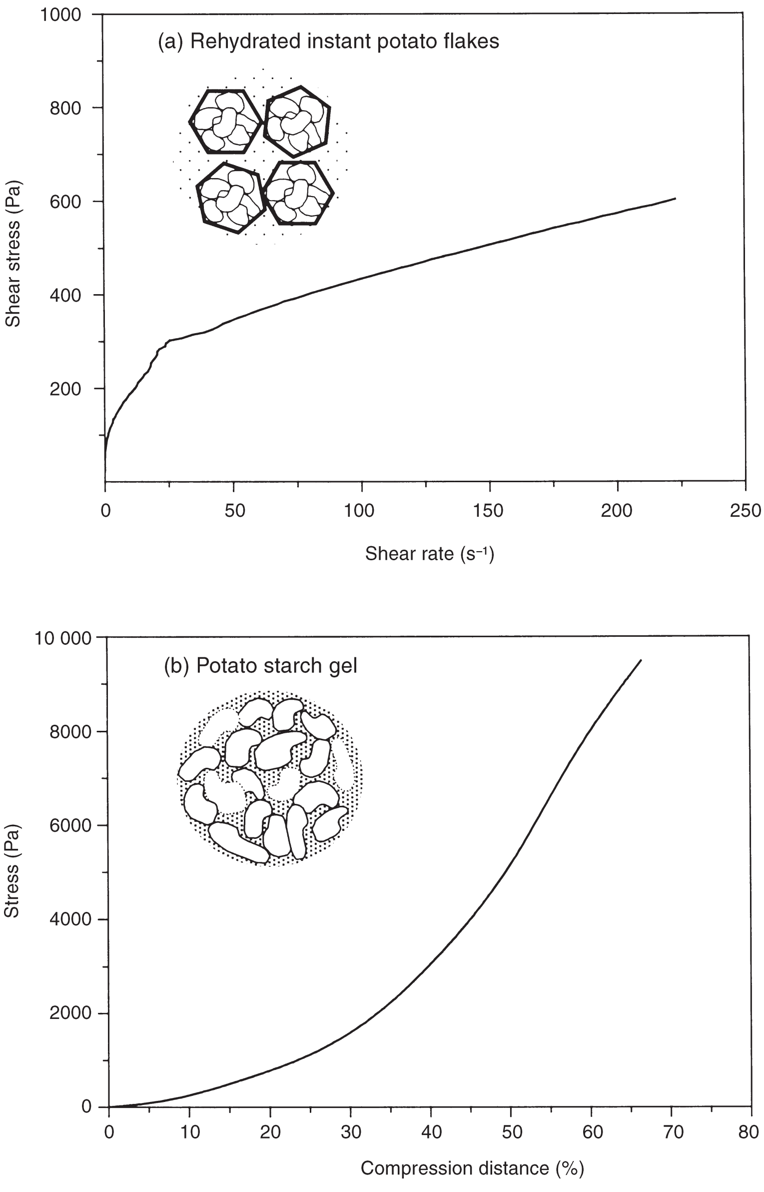

Fig. 4.6 Flow behaviour of rehydrated instant potato flakes (a) and stress-deformation curve of an aged (24 h) potato starch gel (b). Both systems have a dry matter content of 12.5 g/100 g. The microstructure of mashed potatoes can be described as a cell suspension where starch is largely contained within the cells (a). In the starch gel the swollen starch granules are interconnected by a continuous amylose network (b). Note the different stress scales (Lamberti and Conde-Petit, unpublished).

To illustrate the influence of starch compartmentalisation in high-moisture systems on their rheological behaviour, mashed potato is compared to a pure starch system at similar starch concentration in Fig. 4.6. While mashed potato shows a plastic flow behaviour, heating of isolated, native potato starch at a concentration of 12.5% results in a firm and deformable gel which does not fracture up to a deformation of 60%. The rheological behaviour reflects differences in the connectivity of the starch phase. In pure starch gels, an interconnected network is formed during ageing where leached amylose promotes the adhesion between the starch granules. In a suspension of starch-rich cells, the structural strength of the continuous phase is weak as it contains a small amount of amylose, and a starch network is formed only within the cells. This purée-type texture can also be generated with other plant material rich in starch, for instance sweet potato (Gross and Rao, 1977) and grain legumes (Würsch *et al.*, 1986), the textural outcome being strongly influenced by the processing conditions. A broad range of structures and textures can be generated by controlling the extent of cell wall disintegration, cell-to-cell adhesion and starch transformation.

4.3.4 Starch structure at different levels of organisation

The uniqueness of starch is given by the combination of the two polymers, amylose and amylopectin, and their packing in partly crystalline granules. The broad technological functionality is linked to the diversity of starch structures as induced by processing. High-moisture starch systems are far from equilibrium and the combination of entropic loss due to aggregation, and enthalpic decrease as a result of crystallisation, yield supramolecular starch structures. The structural features of starch in food cover a size range of more than six orders of magnitude. An overview of the starch structures at different length scales and the methods for assessing their respective structures is presented in Fig. 4.7. The polymers amylose and amylopectin represent the macromolecular level in the nanometer range. Radii of gyration in the order of 150–200 nm have been reported for different starches by Bello-Pérez *et al.* (1998) in a dilute regime, but large variations in the lower and upper limit are found in the literature (Yoo and Jane, 2002). As the starch concentration increases, condensed polymer structures are created by the tendency of starch polymers to associate and create ordered structures. Small regions of local order in the nanometre range develop by alignment of double or single helices. The association of amylopectin or amylose through double helices yield B-type crystalline structures, while packing of single helices with included ligands lead to V-type patterns as detected by X-ray diffraction. Phase-separated structures resulting from thermodynamic and kinetic processes are a further level of structure, which show the largest variability in terms of size and morphology. For instance, small amylopectin units of 400 nm in size, which are part of the granular structure of starch, are released during gelatinisation (Atkin *et al.*, 1998a). On the other hand, large phase separated domains of amylose may be found within swollen starch granules, covering a size range of 1–50 μm. A key factor of starch in food which largely determines the rheological and textural properties are the starch granules at

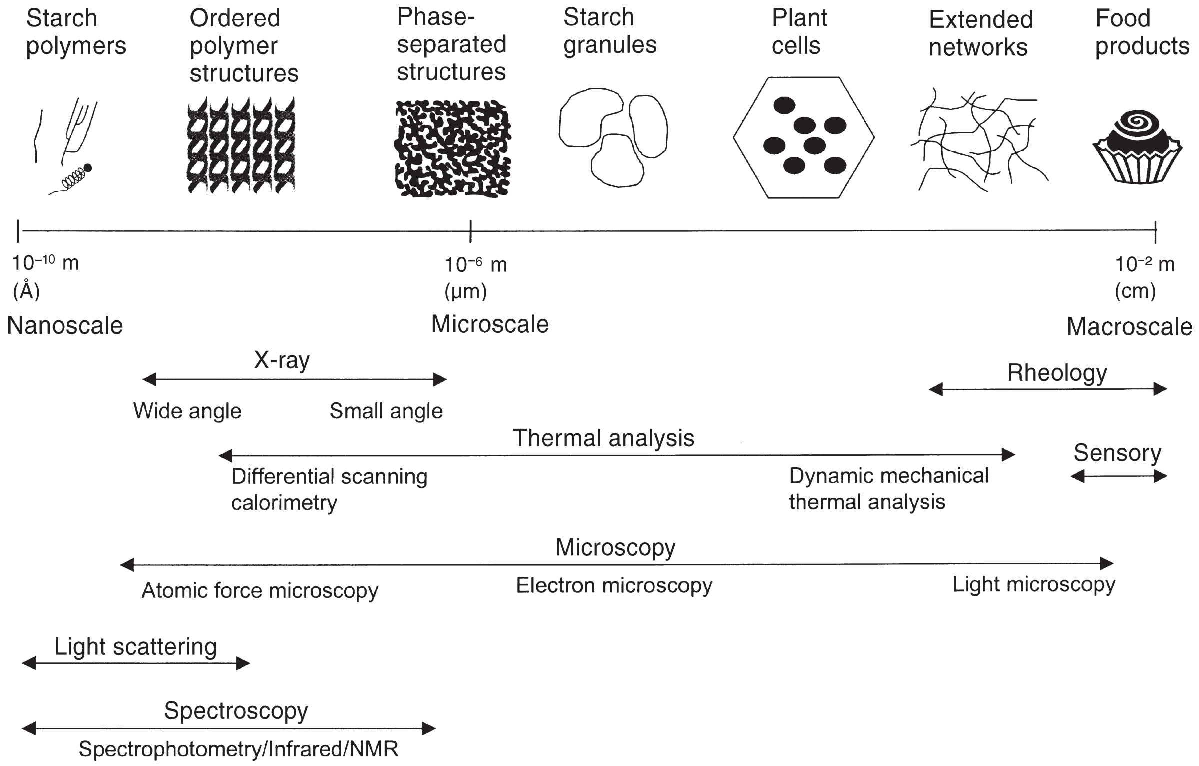

Fig. 4.7 Structural levels of starch in food ranging from nano- to macroscale, and typical characterisation techniques sensitive to the different length scales.

different stages of swelling and disintegration covering a size range between 1 and 100 µm. The morphology of swollen starch varies from almost spherical to strongly distorted and folded structures. The starch-containing cells that maintain their integrity during processing constitute a further important structural element in many plant-based food systems, the cells having a diameter of around 200 µm. Finally, continuous starch phases may extend to the macroscopic level and contribute to the gelation of food systems. Starch gels can be described as fractal-like networks that are held together by physical cross-links and contain a large volume of water as solvent. The properties of the bulk will depend on contributions from the different structural elements, and how they interact with one another. The manipulation of one or several structural levels provides different routes for controlling the texture of starch-based food.

4.4 Future trends

Many factors have contributed to important developments regarding the application of starch as a thickening and gelling agent, and the understanding of the role of starch in the textural properties of starch-based food. One key factor has been the application of concepts from polymer and material science to starch. This trend is bound to continue. Theoretical and experimental progress in polymer and biomaterial science will contribute to increase the knowledge of the processing–structure–rheology relationships of starch in food. Besides food, starch is becoming increasingly attractive as a renewable and biodegradable biopolymer for non-food applications. Progress in structure analysis, such as microstructure mapping by X-ray diffraction techniques (Buléon *et al.*, 1997), microscopy (Dürrenberger *et al.*, 2001) and optical microrheology techniques (Schurtenberger *et al.*, 2001) are promising tools for a more detailed characterisation of starch. A better understanding of starch in multi-component and multi-phase food systems will contribute to the well-directed formation of different microstructures, and by this to the generation of tailored textures. For the food technologist, the challenge lies in finding a balance between thermodynamics and kinetics by the selection of ingredients and by controlling temperature, time and flow conditions during processing. However, this approach is limited by our poor understanding of the links between rheological and sensory properties of semi-solid food. In the past, more emphasis has been placed on the instrumental characterisation of food texture, and comparatively little effort has been made regarding the relationships between well-defined physical parameters and sensory perception. Early studies have focused on the oral evaluation of liquid food (Szczesniak and Farkas, 1962; Shama *et al.*, 1973; Sherman, 1982), but little progress has been made in the past 20 years, in particular regarding the texture of biopolymer gels. Our knowledge of oral processes, for instance the breakdown of starch gels with a composite structure during mastication, is still incomplete. In connection with instrumental texture assessment this means that mechanical testing should not be limited to small deformation measurements, but that large deformation testing and the investigation of failure mechanisms need to be considered. In the future, consumers' demand for products with healthy and natural ingredients will continue to replace chemically modified starches by physical modifications. A recent development is genetic modification of plants and, as a result, the production of 'biologically modified starch' by manipulation of the biosynthesis pathway (Slattery *et al.*, 2000). This approach can be used to control starch properties such as amylose/amylopectin ratios, length of the amylopectin side chains and size of the starch granules. Although it is possible to design starch composition and structure by metabolic engineering, one has to keep in mind that starch deposition in the plant is a self-organisation process driven by thermodynamics. Thus, growing conditions, primarily temperature and humidity, have an influence on the extent of polymer order in the starch granules (Tester, 1997). Besides the starch modifications themselves, starch-degrading enzymes offer the possibility of tailoring the structure in high-moisture starch systems, for instance of minimising time-dependent changes of texture (Würsch and Gumy, 1994). Interestingly, new generations of amylases that are successfully applied for the stabilisation of bread texture have not yet been considered as texture modifiers in other starch-based food systems.

4.5 Sources of further information and advice

The main difficulty associated with obtaining information on starch is the abundance rather than the lack of information. The intense activity in starch research is confirmed by a literature search on starch: a screening from 1969 to 2002 in *Food Science and Technology Abstracts (FSTA)* resulted in 24 000 citations for the keyword *starch*. The following books can be recommended for a comprehensive coverage of starch: the books of Seidemann (1965), Banks and Greenwood (1975) and Whisler *et al.* (1984) continue to be standard reference material, although they do not include the latest developments. Up-to-date reviews include the book edited by Galliard (1987) and the proceedings of the starch conferences held in 1996 and 2000 in Cambridge (Frazier *et al.*, 1997; Barsby *et al.*, 2001). On the other hand, the following books are recommended for the reader interested in fundamental aspects of polymer physics: Flory (1953), Utraki (1991), Evans and Wennerström (1994) and Gedde (1995). The present chapter considers native and physically modified starches, but starches where chemical modifications are introduced to alter the nature of the interaction between the polysaccharide chains are not discussed. For details of chemically modified starches the reader is referred to an overview edited by Wurzburg (1986). More specific information on starch is found in several scientific journals. The journal *Starch/Stärke* is entirely dedicated to starch, and the following journals regularly include contributions on starch: *Carbohydrate Polymers, Carbohydrate Research, Cereal Chemistry, Food Hydrocolloids, Journal of Cereal Science* and *Macromolecules.*

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5

Biopolymer systems for low-fat foods

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5.1 Introduction

The purpose of many food processing operations is to create microstructures that give the product the desired properties. Often, foods are multi-phase materials structured either by nature (e.g. plant or animal tissues) or by people. The artificial structured foods use texturing processes such as, for example, emulsification processes (e.g. production of margarine, ice cream, mayonnaise), foaming (e.g. production of whipped cream), dough kneading, etc. The final product has a complex microstructure held together by binding forces between the various phases. Many features are texture-related, including mouth coating, mouthfeel and dryness. Destruction of this texture during mastication is the usual key to final product quality and appreciation by the consumer.

In addition to important properties such as stability and flavour, the control of the microstructure of artificial structured foods is often the quality-determining factor. For instance, a product such as margarine is typically a three-phase system: a dispersed aqueous phase containing proteins and salts as solutes and a continuous oil phase in which a solid fat phase is present. The fat crystals form a network of plate-like crystals holding oil phase and water phase droplets as dispersed phase. In addition, the water droplets might be surrounded by a shell of crystals that stabilize the droplets through the so-called Pickering stabilization mechanism (e.g. Heertje and Lewis, 1993).

The market need for zero or low-fat food products has become increasingly important and so new ingredients and knowledge are needed in order to manipulate these types of product that have high sensory quality. Examples of such foods are margarines, spreads, salad dressings, dairy and bakery products. Why is the production of zero or low-fat products such a challenge? For many industrial food designers, the primary difference in low-fat products is texture. In bakery products, for example, removing the fat gives cakes and breads a drier, firmer crumb. Structure in salad dressings changes significantly with different fat levels, and the product may no longer adhere to lettuce acceptably when fat is removed. Eliminating fat also alters flavour; both the flavour that is imparted by the fat itself and the fat-soluble flavours that the fat is helping to deliver.

Fat mimics or fat substitutes are often different biopolymers used to compensate for a lower fat content and to provide an oily texture and high sensory quality. Biopolymers make up the backbone structure in many traditional sold and semi-solid foods such as meat, fish, dairy and cereal products. However, when considering the importance of biopolymers in structure engineering, one has to focus on the potential of biopolymers to assist in generating new tailor-made structures with specific properties of the aqueous phase, interfaces and the overall behaviour of the multi-phase systems. Some of these biopolymers are celluloses, starches, alginates, caseinates, gelatins and dextrans. Often, combinations of several biopolymers are needed to ensure good organoleptic quality. These biopolymers and mixtures of biopolymers are not only used in the food industry but find applications in a wide variety of industries such as the pharmaceutical and the personal care business to generate specific flow behaviour, texture, appearance and, where required, tactile and oral properties to products. As a consequence of this extensive use, many academic and industrial scientists have investigated their behaviour.

In this chapter, we will limit the dicussion to biphasic mixtures of biopolymers since they are often used in the food industry to impart specific textural properties. These are especially popular whenever one – or both – of the components can be transformed in a gelled state. The types of textures obtained will depend on the way in which the biopolymers can interact. These interactions can, for instance, be governed by thermodynamic incompatibility, interpenetrating networks (especially when one or both of the biopolymers is in the gelled state) or coupled networks (Walstra, 1983). The most common form of interaction is biopolymer demixing caused by thermodynamic incompatibility, but this is very much dependent on the solution conditions, i.e. temperature, pH and ionic strength (Walstra, 1993).

This chapter focuses on two aspects. First of all, since the development of structure is a determining factor in the use of biopolymer mixtures, different methods – both indirect and direct – to study the structure of such materials will be discussed. Since food emulsions of interest mainly originate during processing, methods following structure development during flow will receive the main attention. Secondly, different studies that examine the structure evolution in biopolymer mixtures during flow conditions will be reviewed. Some models to characterize structure evolution will be addressed.

5.2 Measuring the structure of biopolymer systems

A full understanding of the properties of biopolymer mixtures will require detailed information about their composition, structure, dynamics and morphology at several length scales, ranging from the molecular to the macroscopic. Different approaches can be followed, depending on the degree of information that needs to be extracted. Basically, these different techniques can be classified into two broad categories. The first group can be called *direct imaging* techniques. It includes different classes of experimental methods that lead to direct pictures of the system under investigation at various levels of resolution. The other, *indirect*, techniques discussed in this contribution encompass different rheological and scattering techniques aimed at the collection of morphological information about the mixture. Although these can also be used post-mortem (or off-line), they have the undoubtable advantage that they can be used *in situ*, during the development of structure during flow.

The next section presents some applications of a range of techniques in the study of biopolymer mixtures. Before proceeding, it needs to be stressed that this is not an isolated problem in material science. The problem discussed in this chapter is in essence the familiar structure–property-processing route that is the heart of so many materials' processing problems. Hence, in this chapter, a link will be made with a different – though very similar – material class, i.e. mixtures of synthetic polymer components.

5.2.1 Direct imaging techniques

The use of various microscopy techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) has been quite popular in synthetic polymer blend literature. They have been applied extensively to study the effect of varying processing conditions on the resulting morphology. Also in the study of biopolymer mixtures, SEM has proven to be extremely useful (see, e.g. Abeysekara and Robards, 1995). It is perhaps the most commonly used microscopy technique by food scientists (see examples in, e.g., Lewis, 1988; Schmidt and Buchheim, 1992). However, a conventional electron microscope operates under high vacuum. This makes it necessary to remove or freeze the water in hydrated samples. Thus, special – often difficult and expensive – preparation procedures are necessary in order to study food structures by electron microcopy (Hermansson and Langton, 1993). In addition, electron microscopy techniques are typical post-mortem techniques: it is still a challenge to incorporate flow fields inside a such an instrument.

A step closer to real-time observation is the use of a simple optical microscope. The best resolution obtained by standard optical microscopy is of the order of 1 μm but such details are usually not visible because of low contrast between the object and the background. To increase image contrast significantly and to exploit fully the optical microscopy, special methods such as phase contrast or differential interference contrast with digital processors have been introduced. The explosive growth in the power of laboratory computing during the past few years has made quantitative microscopy more accessible. Video microscopies are increasingly integrated with other laboratory instruments such as rheometers and other devices (Kim *et al.*, 1996; Mather and Pearson, 1992; Madiedo and Gallegos, 2002). Simeone *et al.* (2002a) studied the time evolution of the drop size evolution of aqueous biopolymers (dextran–gelatin) in shear flow by using a parallel plate apparatus and image analysis techniques.

Another powerful method for optical sectioning 3D microscopical features is confocal laser scanning microscopy (CLSM). The CLSM method allows complex structures to be studied. Most materials, however, do not exhibit autofluorescence and therefore need to be stained with some fluorochrome, which can be excited by a laser beam. The CLSM offers good control of the image due to the very thin and adjustable focal plane and optical cutting allows different planes in the sample to be visualized (Wilson, 1990). Information on the stack of planes can be used to construct a 3D image which allows the structure to be visualized completely. In the study of immiscible polymer blends, for instance, the technique proved to be useful in identifying the continuity of phases or to monitor domain structures (Verhoogt *et al.*, 1993; Jinnai *et al.*, 1995; Nakamura *et al.*, 1996; Ribbem and Hashimoto, 1997). In the study of biopolymer mixtures the scope of CLSM has also been established (Blonk *et al.*, 1995). In addition, methods for dynamic measurements are rapidly developing. This means that special stages are being designed to follow structural changes under various flow conditions such as shear and elongational flows (Normand *et al.*, 2001; Olsson *et al.*, 2002), as well as temperature and time-induced changes (Loren *et al.*, 1999, 2001, 2002; Plucknett *et al.*, 2001). An example of such an image is given in Fig. 5.1 which shows CLSM images of a gelatin continuous mixed biopolymer gel deformed to different levels of strain (adapted from Plucknett *et al.*, 2001). In this particular case, the CLSM technique was used to examine debonding between the different phases.

5.2.2 Indirect morphology probing techniques

The non-imaging techniques may be referred to as indirect techniques. The techniques, while important in their own right, are also indispensable to complement direct imaging methods. In many cases direct imaging techniques are not able to collect time-resolved information about the structural evolution, e.g. to determine time scales of morphological changes. A wide variety of methods are available and the potential of several newly emerging methods highlights interest in examining the flow-induced structures in biopolymer mixtures. For instance, recent developments include the application of diffusing wave spectroscopy (Urban and Schurtenberger, 1998; Palmer *et al.*, 1999; Ten Grotenhuis *et al.*, 2000) in the study of emulsion stability. In addition nuclear magnetic resonance (NMR) – a technique that has been used in polymer blend research to examine polymer miscibility and the spatial dimensions of the various components – is slowly being introduced in the world of biopolymer mixtures (Williams *et al.*, 1995; Yan and Stark, 1998). In this contribution the discussion is limited to the use

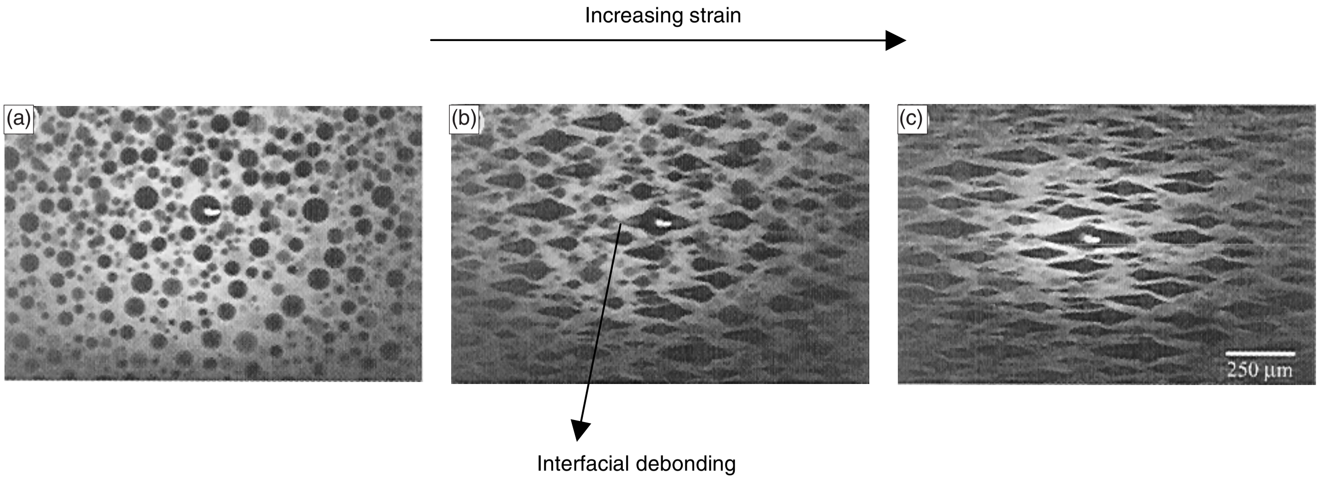

Fig. 5.1 CLSM images of the structure of a tension test conducted *in situ* on a gelatin continuous gelatin (12%)–maltodextrin (12%) sample. Specimens are deformed to a true strain of (a) undeformed; (b) 0.51 and (c) 0.82. Above a certain critical strain (here ~0.2), interfacial debonding occurs between the maltodextrin particles and the continuous gelatin matrix. This is seen by the occurrence of ‘cusp shaped voids’, which grow upon continued straining of the sample. (Adapted from Plucknett *et al.*, 2001).

of rheology and rheo-optical techniques and their use in obtaining useful morphological information on the liquid state of biopolymer mixtures.

Rheology as a morphology probe

The flow behaviour of aqueous biopolymer systems is of interest for both academic and applied sciences. The possibility of the formation of fibre-like structures in concentrated biopolymer emulsions under shear and their consequent gelation was shown many years ago for different water–protein–polysaccharide mixtures (e.g. Antonov *et al.*, 1980; Tolstoguzov *et al.*, 1982). In addition, various studies reported on the sometimes quite complex flow behaviour of two-phase biopolymer mixtures (e.g. Alves *et al.*, 1999, 2000; Antonov *et al.*, 1999). The challenge of the application of rheology in the study of biopolymer mixtures is not merely to measure and to quantify the viscosities of such mixtures. On the contrary, one would like to obtain structural information from such measurements.

A fair degree of attention has been paid to the use of rheological emulsion models to predict the morphology of synthetic polymer blends. Linear viscoelastic models have been developed from those first proposed by Oldroyd (1950, 1953, 1955), who demonstrated the presence of elastic properties of emulsions caused by interfacial tension. In particular the Palierne model has proved to be useful in this respect. The key assumption of the model is that droplet deformation remains small and hence the predictions are only valid for the description of linear viscoelastic behaviour.

In its most general form, the Palierne model can also include an effect of the interfacial tension that changes with a variation in interfacial area as for instance in systems in which surfactants are present. Such a case will not be discussed here. If one wants to take into account a distribution in droplet sizes, Palierne's model can be written as (Palierne, 1990):

$$G^* = G^*_m \frac{1 + 3\Sigma\phi_i H_i}{1 - 2\Sigma\phi_i H_i}$$

with

$$H_i = \frac{\frac{4\Gamma}{R_i}(2G^*_m + 5G^*_i) + (G^*_i - G^*_m)(16G^*_m + 19G^*_i)}{\frac{40\Gamma}{R_i}(G^*_m + G^*_i) + (2G^*_i + 3G^*_m)(16G^*_m + 19G^*_i)}$$

where G^*_m and G^*_i are the complex moduli of the matrix and of the dispersed phase respectively, taken at the same frequency as G^*, the modulus of the emulsion. ϕ_i is the volume fraction of inclusions having a radius R_i. Γ is the interfacial tension between the emulsion components. The dynamic moduli are indeed affected by the morphology of the emulsion because the size is present in the equations. The distribution of droplet sizes can be replaced by a single term involving a volume-average droplet size.

Graebling *et al.* (1993) have shown that up to a polydispersity of order 2.3, the particle size distribution can be replaced by an average size without major errors. The majority of the experimental work focuses on the response of two-phase polymeric mixtures to linear oscillatory flows (Graebling *et al.*, 1994). As this flow does not affect the structure, it can be used to study the effect of morphology on the rheological response in a non-destructive manner. The most striking characteristic of the dynamic moduli is the appearance of a pronounced elastic contribution at low frequencies, reflecting slow relaxation modes. The combination Γ/R can be used as a fitting parameter to derive the droplet sizes once the interfacial tension of the emulsion is known.

Vinckier *et al.* (1996) applied the Palierne model to determine droplet sizes of synthetic polyisobutylene-polydimethylsiloxane (PIB-PDMS) blends generated by various shear rates preceding the oscillatory flow. This approach yielded values in close agreement with direct microscopic data (Grizzuti and Bifulco, 1997). The models can be used to gain an understanding of the changes in the measured storage modulus as a function of the shear rate. Recently, the model has been applied to water-in-water emulsions that consist of phase-separated biopolymer mixtures. In contrast to the more conventional polymer blends, the individual phases have a low viscosity (often smaller than 1 Pa s) and often have negligible viscoelasticity in their non-gelled state. Secondly, the interfacial tension of such emulsion is extremely low (see Section 5.3.1), indicating that the interfacial contribution to the storage modulus will be rather small. At first glance, it might appear surprising that it is possible to measure and predict the linear viscoelastic properties for water-in-water emulsions in comparison to polymer blends. The similarity, however, can be understood by examining the time scale for the processes. Owing to the ultra-low interfacial tension of the system, the relaxation time $R\eta/\Gamma$ is of the order of 1 s for a 10 μm droplet, where η is the viscosity of the matrix material. Polymer blends have a similar relaxation time because of the high viscosity of the constituent phase. The viscosity of the biopolymer mixture components is orders of magnitude smaller but this effect is counteracted by the extremely low values of the interfacial tension which is orders of magnitude smaller than in synthetic polymer blends.

In addition, sedimentation and creaming also have to be taken into consideration in this case owing to the density difference between the phases. Notwithstanding all these drawbacks, Palierne's model has already been proved to predict adequately the droplet sizes in a variety of biopolymer mixtures. In order to minimize problems with sedimentation and creaming, the duration of the oscillatory tests was typically kept to less than 2 minutes and the frequency sweep was conducted from 100 rad/s down to 1 rad/s.

Stokes *et al.* (2001) applied the model to the industrial relevant mixture of a phase separated gelatin–maltodextrin system. Although the value of the interfacial tension was not known with sufficient accuracy, the agreement between the experiment and the predictions of the Palierne model was fair. An example of the fit of the Palierne model to the experimental results is shown in Fig. 5.2.

Simeone *et al.* (2002b) investigated the application of Palierne's model to

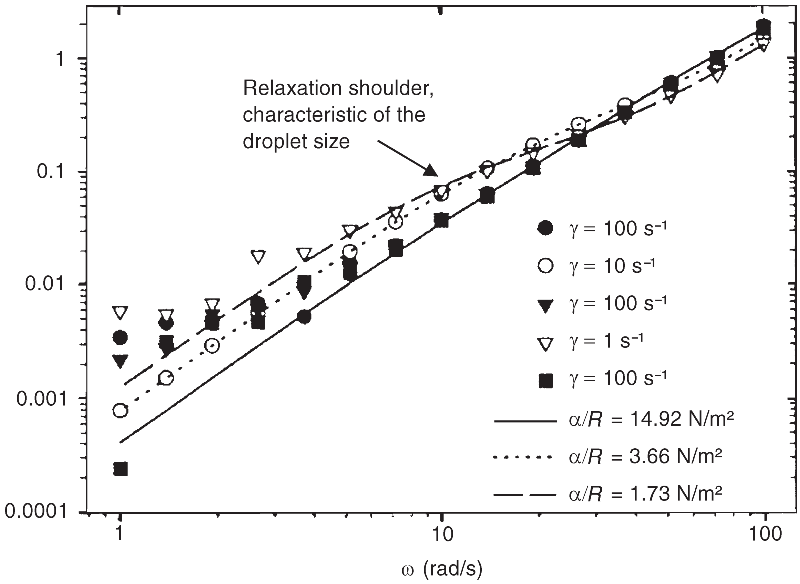

Fig. 5.2 Linear viscoelastic measurements for a 10% gelatin-rich phase. Lines indicate the predictions of the Palierne model. The arrow points towards the characteristic relaxation shoulder, which in turn is an indication of the droplet sizes present. γ is the shear rate and α is the interfacial tension. (Adapted from Stokes *et al.*, 2001).

aqueous mixtures of Na–alginate and Na–caseinate. A comparison with direct drop size measurements was performed by shearing the mixtures in a parallel plate apparatus coupled with an optical microscope. Good agreement was found for different volume fractions and different values of the viscosity ratio, i.e. the ratio of the droplet phase versus the continuous phase viscosity. A similar system was studied by Capron *et al.* (2001) who compared the droplet sizes – obtained by Palierne's analysis – to the critical capillary numbers and coalescence predictions.

The use of transient rheology as a characterization tool has become fairly widespread in the polymer blend literature. An extensive review about the methodology has been given by Tucker and Moldenaers (2002). For instance, transient step shear tests in which the shear rate is suddenly changed are able to detect deformation and break-up of the dispersed droplet phase. These types of experiment can give valuable insight into the time scales associated with the break-up of the dispersed phase. However, the methodology has not yet found widespread applications in the biopolymer world. Again it is the extremely low value of the interfacial tension that hampers a significant distinction between bulk and interfacial properties.

At higher concentrations of the dispersed phase, however, it could become possible to detect the interfacial properties. Stokes and Frith (2002) for instance considered a step shear experiment in the case of a 30% emulsion of a gelatin-rich phase in a maltodextrin-rich phase. At shear rates ranging from 1 to 100 s^{-1}, an undershoot was observed for the shear stress. As shown, for instance, by Takahashi *et al.* (1994), such an undershoot is indicative of the break-up of droplets. However, no systematic use of these sensitive rheological procedures has been applied yet in the field of biopolymer mixtures.

Rheo-optics as a morphology probe

In order to circumvent possible drawbacks of the rheological techniques such as, for instance, the rather low interfacial contribution to the rheological response of the material, a different methodology can be used. This is based on what has commonly been called 'rheo-optical' methods. These encompass various scattering methods as well as polarimetry techniques such as birefringence and dichroism methods. The 'rheo' part in the name refers to the application of these techniques under flow conditions. The applicability in the study of multiphase materials has been reviewed by Søndergaard and Lyngaee-Jørgenson (1995) and Fuller (1996).

The basic idea here is that an electromagnetic field interacts with matter giving rise to a change of state of polarization and/or a change in intensity, either due to absorption or to scattering. In the case of synthetic polymer blends, both linear conservative dichroism and small angle light scattering (SALS) have proven to be extremely useful in obtaining quantitative structural information.

Søndergaard and Lyngaee-Jørgenson used light scattering as well as light transmittance to track particle sizes during coalescence and applied the method to deduce the aspect ratio of the dispersed phase during shear flow. Yang *et al.* (1998) extended the range of the optical techniques by monitoring the evolution of the microstructure of polymer blends during flow with linear conservative dichroism in addition to SALS. It was demonstrated that dichroism was very sensitive in probing the different mechanisms for morphological changes in immiscible polymer blends such as droplet stretching and relaxation, fibril break-up and coalescence of droplets.

Some papers report on a further quantification of the methodology. Kielhorn *et al.* (2000) examined in detail the relaxation behaviour of polymer blends after cessation of shear. Van Puyvelde *et al.* (1998) and Mewis *et al.* (1998) modelled these various relaxation phenomena and applied a combination of dichroism and SALS to determine the interfacial tension in polymer blend systems. A systematic study of the break-up process, by varying relevant parameters such as viscosities and interfacial tension (Van Puyvelde *et al.*, 2000) derived a dimensionless scaling relation that allows determination of the break-up time in shear flow of droplets with known initial dimensions.

Recently, these rheo-optical methods have found applications in more realistic processing flows. Priore and Walker (2001a) applied SALS on a slit contraction flow providing *in situ* information about the overall deformation state of the blend. They showed that depending on the viscosity ratio of the blend, the morphological evolution is strongly influenced by either the transient shear or elongational component of the flow. Priore and Walker (2001b) demonstrated that models developed for simple shear flow, qualitatively, describe the coalescence and break-

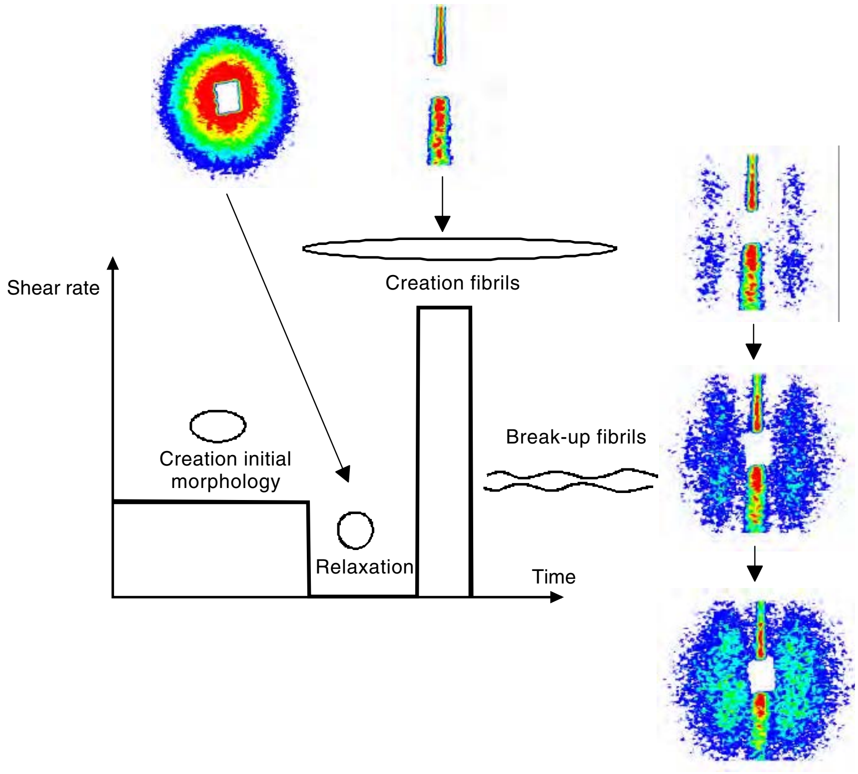

Fig. 5.3 Schematic representation of the morphological changes during the shear rate protocol used to determine the interfacial tension. Also shown is the evolution of the SALS patterns after an interrupted start-up to a shear rate of 20 s^{-1}, stopped after 1s. The sample used is a 1% gelatin-rich phase dispersed in a dextran-enriched phase at 45 °C. The initial streak perpendicular to the original flow direction corresponds to the elongated droplets. The appearance of the secondary streaks is associated with break-up by Rayleigh instabilities. (Adapted from Van Puyvelde *et al.*, 2002a).

up processes, indicating their potential for predicting these phenomena in processing flow. One step further was conducted by Hobbie *et al.* (1998) who incorporated a light scattering technique onto an extruder to monitor on-line the development of the morphology.

SALS experiments have already proven to be popular in the biopolymer mixtures community, especially to study phase separation kinetics (e.g. Tromp *et al.*, 1995; Loren and Hermansson, 2000; Butler and Heppenstall-Butler, 2001). Although a variety of biopolymer systems has been studied during quiescent phase separation, quantitative studies of the phase separation behaviour during shear are limited. Butler (2002) demonstrated by using SALS that spinodal decomposition was still the phase separation mechanism in a gelatin–maltrodextrin mixture even when shear was applied. The recent trend is the application of the rheo-optics in the structure development of liquid biopolymer mixtures. Van Puyvelde *et al.* (2002a) demonstrated that the rheo-optical methodology to measure interfacial tension, originally developed to determine the interfacial tension in synthetic polymer blends, is very suitable to measure the interfacial tension in biopolymer mixtures as well.

Figure 5.3 shows the typical scattering patterns that are obtained during the disintegration of fibrils by means of Rayleigh instabilities. It can be seen that during the disintegration process, a secondary streak emerges. The intensity of this secondary maximum grows in time. Modelling of the evolution of this pattern allows deduction of a value for the interfacial tension. Although the values are typically a few orders of magnitude lower, it was shown that the technique could accurately measure interfacial tension values as low as 10^{-6} N m^{-1}. At low shear rate conditions, Van Puyvelde *et al.* (2003) demonstrated – using SALS and polarimetry – that the biopolymeric emulsion can be regarded as a conventional emulsion. This is an important result in the sense that various structural models that are available in literature for the prediction of the morphology in these conventional emulsions can also be used for the prediction of the structure in aqueous biopolymeric emulsions. However, at high shear rate, Van Puyvelde *et al.* (2002b) demonstrated that shear flow can have a drastic effect on the phase equilibrium of the system. Using SALS and transmitted intensity measurements, it was demonstrated that a high shear flow can homogenize the structure of a gelatin–dextran system. In addition, Antonov *et al.* (submitted) used the methodology to map out the effect of shear flow on the phase diagram of the water–gelatin–dextran system.

From these observations, it is clear that *in situ* techniques to monitor the flow-induced structure development are slowly emerging in the field of biopolymer mixtures. The methodology has already proven its powerful use in the research on synthetic polymer blends. Owing to the synergy between the two material classes, it is expected that the various methods will appear more and more in the study of flow-induced structures in biopolymer mixtures.

5.3 Understanding and controlling the microstructure of biopolymer systems

In this section the relationship between interfacial tension, droplet size and rheology of phase separated biopolymer mixtures which demix to form water-in-water emulsions is examined. Inducing gelation in one or both of the phases during flow processing can produce a wide variety of microstructures (see, e.g. Wolf *et al.*, 2000). These systems can, on the one hand, be utilized to produce anisotropic microgel particles ranging from ellipsoids to threads. On the other hand, gel composites with anisotropic inclusions can be produced. The ability to control and to predict the resulting microstructures and morphology during flow processing is valuable for the design of new microstructures and hence textural characteristics. The plastics industry faces a similar problem when two immiscible polymers are blended. The resulting microstructure, generated during processing, is to a large extent responsible for the final properties of the final part. In the case of synthetic polymer mixtures, the relation between morphology development and the processing conditions has been widely investigated (see for review Tucker and Moldenaers, 2002).

For simple droplet-matrix morphologies of Newtonian components, most of the physical phenomena are relatively well understood. Recent work suggests that texture development in biopolymer mixtures, which form water-in-water emulsions, is governed by the same physical principles as conventional mixtures containing synthetic polymers (e.g. Forciniti *et al.*, 1990; Wolf *et al.*, 2001). However, some peculiarities of the water-in-water emulsions such as the limited miscibility and the extremely low values of the interfacial tension may add extra complications to the processing. In this section, emphasis is on how certain structures can be produced based on the knowledge of existing droplet models. Firstly, attention is given to the measurement of interfacial tension since it is an important and difficult to measure parameter in these systems. Secondly, different methods to generate tailor-made structures are discussed.

5.3.1 Methods to measure the low values of the interfacial tension

Very little has been published on the interfacial properties of two-phase aqueous biopolymer mixtures primarily due to the low interfacial tensions involved. An interfacial tension of ~10^{-5} N m^{-1} is generally measured, which is typically three orders of magnitude lower than normally found in oil-in-water systems. In addition, the very small density differences between the phases makes the application of the more conventional techniques such as spinning or pendant drop very difficult. Systems in which the difference is quite large – such as dextran–polyethyleneglycol – have been studied in the past (Ryden and Albertsson, 1971; Guido *et al.*, 2002). Recently, a number of researchers (Wolf *et al.*, 2000; Stokes *et al.*, 2001; Scholten *et al.*, 2002) have adopted the approach of measuring droplet deformation in a flow field to obtain the interfacial tension in a number of systems. The technique consists of following the shape relaxation of a single droplet after cessation of flow. Using this technique the difficulties associated with the small density differences are omitted. The relaxation data are then used in conjunction with models for droplet deformation (such as the Maffettone and Minale, 1998, model) to give the interfacial tension. Using this method Wolf *et al.* (2000) reported a value of 27 μN m^{-1} for a phase-separated gellan–κ-carrageenan mixture.

Stokes *et al.* (2001) measured the interfacial tension of a gelatin–maltodextran system resulting in values in the range of 50–100 μN m^{-1}, whereas Guido *et al.* (2002) obtained a value of 11 μN m^{-1} for an alginate–caseinate–water mixture. A fast and accurate methodology, based on the analysis of small angle light scattering patterns, has been applied by Van Puyvelde *et al.* (2002b) to a mixture of gelatin–dextran–water. Different methods – either the analysis of the SALS patterns during fibril break-up or the analysis of the patterns during droplet relaxation – resulted in similar values of the interfacial tension.

It is important to raise the question about the meaning of an interface in a two-phase biopolymer system in which often more than 90% solvent is present and can move unhindered between the phases. As yet, almost nothing is known about the structure of the interface between the phases mainly because the techniques for studying this require more contrast than is available in biopolymer mixtures. A second important point concerning the low interfacial tension is that of the continuity of structures. Compared with conventional oil-in-water emulsions, a change in phase continuity occurs over a small range of phase volumes (typically smaller than 1% phase volume). Based on the low values of the interfacial tension, it is expected that the phase continuity change occurs over a much larger range of phase volumes in biopolymer mixtures. Such a large range of phase continuity is indeed observed in a number of systems and even ranges as high as 10% phase volume have been observed (Wolf *et al.*, 2001). These phenomena should be taken into account when deciding the volume phase ratios used in structured products.

5.3.2 Flow processing to create tailor-made structures

Recent work has demonstrated that structure development in biopolymer mixtures is governed by the same physical principles including the role of droplet break-up and coalescence as for conventional emulsions. One interesting method applied to generate tailor-made structures is to use gelation to trap the dispersed phase in an anisotropic morphology. If the gelling biopolymer component is enriched in the droplet phase, gelled particle suspensions are formed. The rheology of such particle suspensions can be further described by conventional methods of describing relative viscosities of suspensions (e.g. by a Krieger–Dougherty relation, Wolf *et al.*, 2001). In the case of a gelling continuous phase, anisotropic gels with a capillary structure can be formed. The creation of anisotropic gel structures was pioneered by Tolstoguzov and coworkers (1982) by using extensional and orifice flow types.

Rheometric flow types have received very little attention in literature although the control of such flow types is much easier. Wolf *et al.* (2001) used controlled shear flow superimposed on gelation of the dispersed phase to form anisotropic morphologies. Cooling, at the same time as applying a shear field, is used to gel the biopolymer and to trap the structure. Interest in such structures comes from their potential use as rheology modifiers in aqueous systems, since the degree of deformation of the gelled dispersed phase will strongly influence the viscosity. Changing the flow stresses offers a broad flexibility for generating different extents of anisotropy. However, the chosen process puts some constraints on the choice of applicable biopolymer mixtures.

In terms of particle shape, predictable design of this process requires knowledge of the time–temperature dependence of the relevant material properties such as interfacial tension and viscosities. Induction of significant droplet deformation under shear flow requires a suitable viscosity ratio between the dispersed phase and continuous phase. For instance, materials that exhibit a viscosity ratio greater than 4 will not be further deformed in shear flow and will rotate only in the applied flow field.

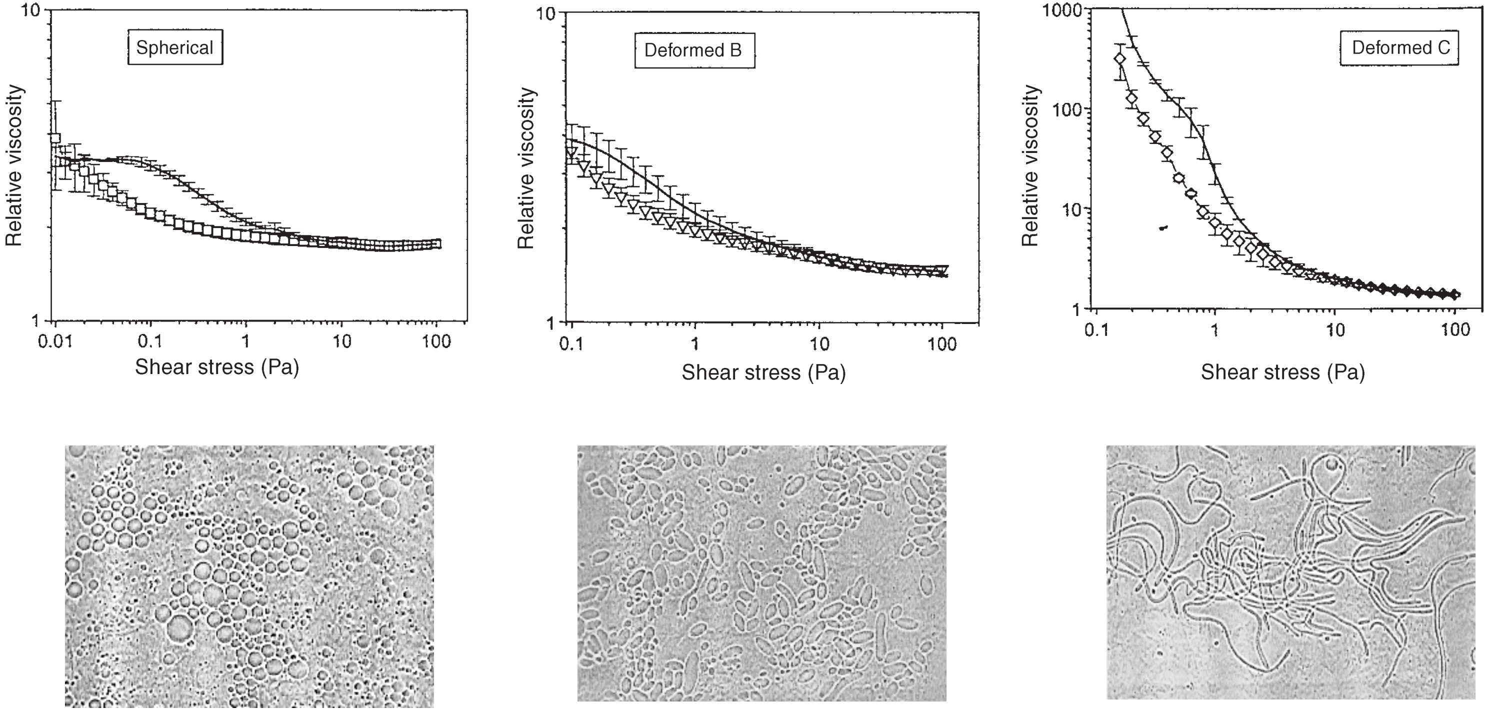

Fig. 5.4 Influence of particle shape on the relative viscosity. Curves associated with increasing shear rates are represented as lines without symbols. The down-curve results are represented as symbols. Also shown are the corresponding micrographs. The sample studied is a 1.8% gellan–1.8% κ-carrageenan mixture. The undeformed reference case is given in the curve labelled ‘spherical’. Sample A represents a sample that has been processed under a steady shear of 0.1 Pa. Sample C was processed by stepping the stress up from 0.05 Pa to 0.5 Pa prior to gelation. It is seen that the sequence of shear stress application influences the rheological results, giving higher viscosity values in the up-curve results. This illustrates the effect of the initial particle orientation by starting the measurements at high shear stress. (Adapted from Wolf *et al.*, 2000).

A suitable choice of phase viscosities is made even more difficult since as temperature conditions change during the process, both the phase behaviour and viscosity behaviour will change. Viscosity changes are particularly significant near the gelation temperature. Although these limitations are quite severe, some biopolymer mixtures still fulfill the above criteria. For instance, gelatin–guar, gellan–κ-carrageenan and gellan–sodium alginate have been shown to be adequate for this type of processing (Wolf *et al.*, 2000). Once this behaviour is established, a concentration/property relation will result that allows for the production of tailor-made structures. The experimental protocol consists of cooling the two-phase sample from 60 to 17 °C at a certain cooling rate. Throughout the cooling process, the sample can be continuously sheared. Depending on the magnitude of the applied flow rate (or shear stress), the anisotropy of the resulting particles can be different. For instance Fig. 5.4 shows micrographs of processed gelatin–κ-carrageenan mixtures, obtained at different levels of stress and hence resulting in particles with different aspect ratios.

The methodology used to process such structures is based on the observation that mixed biopolymer systems are similar to classical oil and water emulsions. Hence, models that were originally developed to describe the structure evolution in these conventional emulsions can be applied to predict the resulting microstructure in biopolymer mixtures. An example of the analogy has already been given in Section 5.2 where the Palierne model was introduced. In the case of the production of anisotropic shapes, theories to predict the deformation of dispersed droplets can be applied.

A state of the art model to predict droplet deformation is the Maffettone–Minale model which is known to give excellent results over a wide range of viscosity ratios (Maffettone and Minale, 1998). This model yields analytical expressions for the three different axes L, B and W:

$$\frac{\mathrm{L}}{2R} = \sqrt{\frac{f_1^2 + \mathrm{Ca}^2 + f_2\mathrm{Ca}\sqrt{f_1^2 + \mathrm{Ca}^2}}{(f_1^2 + \mathrm{Ca}^2)^{1/3}(f_1^2 + \mathrm{Ca}^2 - f_2^2\mathrm{Ca}^2)^{2/3}}}$$

$$\frac{\mathrm{B}}{2R} = \sqrt{\frac{f_1^2 + \mathrm{Ca}^2 - f_2\mathrm{Ca}\sqrt{f_1^2 + \mathrm{Ca}^2}}{(f_1^2 + \mathrm{Ca}^2)^{1/3}(f_1^2 + \mathrm{Ca}^2 - f_2^2\mathrm{Ca}^2)^{2/3}}}$$

$$\frac{\mathrm{W}}{2R} = \sqrt{\frac{f_1^2 + \mathrm{Ca}^2 - f_2^2\mathrm{Ca}^2}{(f_1^2 + \mathrm{Ca}^2)^{1/3}(f_1^2 + \mathrm{Ca}^2 - f_2^2\mathrm{Ca}^2)^{2/3}}}$$

in which

$$f_1 = \frac{40(p + 1)}{(2p + 3)(19p + 16)}$$

$$f_2 = \frac{5}{2p + 3}$$

Ca is the capillary number and p is the viscosity ratio.

It has been shown that the model agrees well with experimental droplet deformation data in both steady and transient flows for $p > 0.1$. From these equations, it is clear that once the parameters as a function of time are experimentally determined (viscosities, interfacial tension, etc.), the deformation of the particles can be predicted. This opens a route to tailor structures to modify the rheology of biopolymer mixtures (see Fig. 5.4).

Another methodology to create tailor-made structures was proposed by Antonov *et al.* (submitted; van Puyvelde *et al.*, 2002b). This is based on the observation that at high shear rates, an aqueous mixture of gelatin and dextran homogenizes and forms a single phase state. The experimental protocol is depicted in Fig. 5.5. After the high shear period, the flow is stopped but after some time a very small shear rate is applied. Antonov *et al.* observed that during this period, the dispersed droplets were coalescing. The methodology proposed here has potential to create small microstructures: when the sample is cooled during the period at which a small shear rate is applied (see the schematic representation in Fig. 5.5), the small gellable particles that are formed can be kinetically trapped. Hence the combination of flow-induced homogenization, phase separation and coalescence might open a new route to create very finely dispersed particles. Unfortunately, only information on the flow-induced homogenization in aqueous dextran–gelatin mixtures is known at this point. Future research should certainly include the effect of flow on the phase diagram of these biopolymer mixtures and should clarify the role of the biopolymer parameters on this subject.

5.4 Conclusions

This chapter gives an overview of the importance of structure in aqueous biopolymer mixtures. The potential of these materials for mimicking fat in food product depends largely on structure formation during processing. In order to understand the morphology development, methods suitable for studying the morphology are needed. Moreover, methods to study the structure development *in situ* and on a time-resolved basis are the key to future improvement in the processing routes. Inspiration is found in the literature on synthetic polymer blends: the synergy between the two material classes has been highlighted and the important differences, such as the low values of the interfacial tension and the partial miscibility, have been discussed. Finally, two different but recent methodologies have been proposed

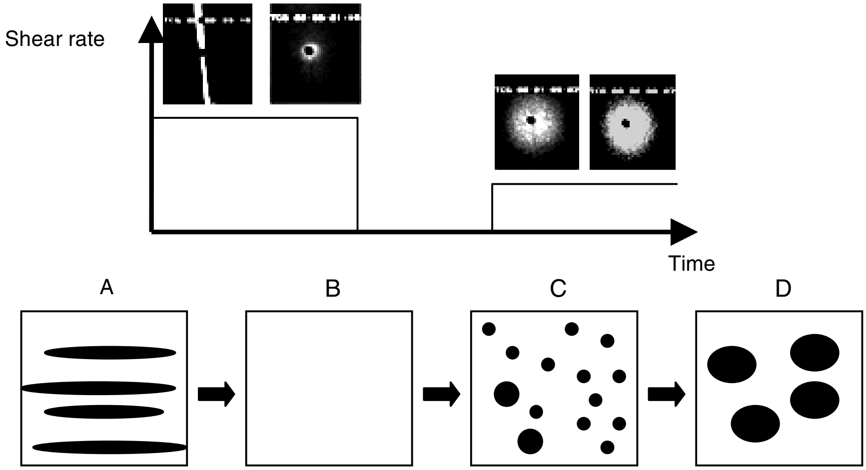

Fig. 5.5 Schematic representation of the flow history used to homogenize an emulsion containing 1% gelatin-enriched phase in a dextran-enriched matrix. The initial high shear rate is 60 s^{-1}, the corresponding SALS pattern points towards shear induced homogenization. At low values of the shear rate (i.e. 0.5 s^{-1}), the structures that have been generated during the phase separation that has occurred during the rest period coarsen.

to generate specific microstructures: a method based on the combination of gelation and flow and a method where flow-induced homogenization, phase separation and flow are combined.

Recently a lot of structural information about biopolymer mixtures has emerged. The application of models, originally used to describe structure development, are finding their way into the prediction of the microstructures in aqueous biopolymer mixtures. The use of *in situ* methods such as polarimetry and small angle light scattering experiments during flow have already proved their success in the study of synthetic polymer blends and will certainly continue to contribute to the field in the future.

5.5 Acknowledgements

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6

Introduction to food rheology and its measurement

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6.1 Introduction

Food rheology is the study of deformation and flow of foods under well-defined conditions. There are many areas (Escher, 1983; Bourne, 1992; Steffe, 1996) where rheological data are required by the food industry including:

- plant design: pumps and pipe sizing and selection, heat and mass transfer calculations, filler designs and other process engineering calculations involving extruders, mixers, coaters and homogenisers
- quality control: both of raw material and the product at different stages of the process (including ingredient functionality determination in product development and also shelf life testing)
- evaluation of sensory attributes: quantitative measurement of consumer determined quality attributes by correlating rheology measurements with sensory data
- assessment of food structure and conformation of molecular constituents.

Food rheology is often confined to the behaviour of liquid foodstuffs. However, there is an increasing tendency to consider the response of both solid and liquid materials to applied stresses and strains as being two extremes of the same science. There are in fact some foods that will exhibit either behaviour depending on the stress applied; molten chocolate, fat-based spreads, mashed potato and some salad dressings will exhibit a solid-like behaviour at low stresses and a liquid-like behaviour at high stresses (Mitchell, 1984). This tendency is increasing as more food products are developed that would be classed by the consumer as being semi-solid or semi-liquid. A more exact definition would therefore be the study of both the elastic and the plastic properties of foods. It is proposed, however, to place greater emphasis in this chapter on classical liquid rheology measurements, although elastic and viscoelastic properties will also be discussed in the context of semi-liquid foods.

There are many substantial reviews of basic rheology, a number of examples of which include Borwankar (1992), Prentice (1992), Windhab (1995), Barbosa-Cánovas *et al.* (1996) and Rielly (1997). While the objective of this chapter is to review instrumentation and measurement techniques in food rheology, it is nevertheless necessary briefly to consider some of the fundamentals. It is also necessary to justify the need for measurement given the wealth of published data already available. For a selection of these see Rao (1986), Kokini (1992), Rao and Steffe (1992), Vélez-Ruiz and Barbosa-Cánovas (1997) and the bibliography of McKenna (1990). The reason for this is as stated by Prins and Bloksma (1983): 'Rheological measurements have to be made under the same conditions as those which exist in the system studied.' In other words, there is little use in carrying out measurements on a product or extracting values from the literature, if the stresses used and their rates of application during the measurement differ from those in the process or calculation for which the measurement is required.

6.2 Relevance of rheological properties of foods

The relevance of food rheology has been summarised above into the four categories of plant design, quality control, sensory attributes, and the research and development of food structure.

6.2.1 The consumer's perception

Ultimately the food product must be eaten, so sensory attributes become most important. However, en route from the farm to the mouth the product may have to be pumped, heated, stored or subjected to other processes, and must be amenable to flow when being placed in a container/package. Equally important is its ability to flow out of the container before consumption. Indeed, it is this ability (or the occasional lack of it) that first brings the consumer into a direct and sometimes frustrating contact with rheological principles. How often has the consumer experienced the dilemma of tomato ketchup refusing to flow from its bottle and found that the application of a sharp blow to the bottle base resulted in an excess amount being deposited on the plate? This provides an excellent example of a situation in which a product has a yield stress below which it will not flow, but flows perhaps too well once the consumer unknowingly provides the stimulus that exceeds it. Not only does this example illustrate yield stress, but it also shows the relationship between force and deformation and flow!

This simple example also gives emphasis to one of the basic rules of rheological measurements, namely that the product should be tested under a range of conditions of stress and shear rate that reflect those experienced during subsequent use, whether that use be tasting, pouring, shaking, stirring or any other action that requires movement of the material.

Of course, rheological relevance does not stop when a food reaches the plate but influences the sensory perception or 'mouthfeel' of the product. Matz (1962) defines mouthfeel as the mingled experience deriving from the sensations of the skin of the mouth after ingestion of a food or beverage. It relates to density, viscosity, surface tension and other physical properties of the material being sampled. These relationships between rheology and mouthfeel have been the subject of extensive research, as reviewed in the author's bibliography on food rheology (McKenna, 1990). It will, however, be obvious that a change in the manner in which a food may move or flow in the mouth and throat will influence our perception of it as a desirable food.

6.2.2 The requirements of the processor

Rheology assists the processor in several areas. At the plant design stage, pumps, pipes, heat exchangers, stirrers, etc. need to be selected. The rate at which a liquid will flow in a pipe is highly dependent on its rheological properties (Singh and Heldman, 1993) (see Eqns 6.10 to 6.17). An alternative interpretation is that for a given flow rate of a given liquid, a particular pressure drop will be required along the length of the pipe. This, in turn, will influence the quantity delivered by the pump chosen for the system. The nature of the process itself may lead to further influences. For example, if the pipe system leads to or through a heat exchanger, the rheological properties may be changed by the heating effects, which in turn would lead to changes in the flow system. In the extreme case of a large, heat induced reduction in viscosity, the velocity of flow might increase and give a product that has had too short a residence time in the system. More seriously, the rheological changes may lead to a change in the flow (velocity) profile of the liquid in the system, to a change in the residence time distribution, and again to an underprocessed product. Of course, not all heat induced changes lead to 'thinning' of the liquid. Starch gelation and similar processes can induce the opposite effects, slow the liquid and increase the severity of the heating process.

There are, however, many other rheological problems in processing. Yield stress, as exhibited in the ketchup example above, may lead to more serious processing problems with significant economic relevance. An area where there is considerable innovation at present is coatings for enrobed food products (Hilliam, 2000). These coatings may range from chocolate enrobed confectionery to batter enrobed fish or meat products, all demand an enrobing material that exhibits a yield stress. If this yield stress is too low, the weight of enrobing liquid adhering to the sides of the product will induce a stress in excess of the yield stress, either on the vertical side of the product or on a plane parallel to this within the enrobing material, and will cause the material to flow off the product. Conversely, too high a yield stress will lead to excessive thickness of enrobing material possibly attractive to the consumer of a chocolate bar, but with adverse economic consequences for the processor.

Quality control is also an area of rheological significance for the processor. While there is the obvious need to induce the desired characteristics into the product and to test the product for these attributes, rheology can provide other quality control information by drawing on the wealth of correlations between rheological and other data that have been developed over many years. For example, Sharma and Sherman (1966) have shown that for ice cream the rheological measurements correlate with fat droplet size, the amount of air incorporation (overrun), ice crystal size and product temperature. For chocolate, information on the hardness and consequently the fat composition of the major ingredient, cocoa butter, can be deduced (Lovegren *et al.*, 1958).

The dairy industry also provides many examples of the use of rheological control techniques. While there are obvious textural related rheological attributes for both set and stirred yoghurts, the ever-increasing range of dairy-based spreads demands that the successful product should have the correct viscoelastic properties for spreadability. Soft and cream cheeses also have liquid properties that must be kept within chosen ranges and which are highly dependent on the ongoing microbiological activity, proteolysis and syneresis within the product. Holsinger *et al.* (1995) emphasise the importance of rheology in providing an insight into the influence of composition and processing on cheese texture. Less obvious is the need for rheological control of concentrated milk products during evaporation and drying; changes in the rheology will change the drop size range produced by the spray drier atomisers (McKenna, 1967), which will in turn change the particle size distribution in the finished powder, not only altering its bulk density and ease of reconstitution but also leading to increases in powder losses in the final air-powder cyclone separators. The reader is also referred to the review on rheological properties of dairy products by Vélez-Ruiz and Barbosa-Cánovas (1997).

The food ingredients sector (including dairy ingredients (especially soups and sauces), cereal ingredients and the aforementioned batters and coatings) is one in which there has been a rapid expansion in sales since the mid-1980s. This expansion is largely in response to increasing consumer demand for convenience meals, which require functional ingredients for their manufacture. The study of the rheological properties is an important component of the product development stage of many of these ingredients. Cream sauces are an example of a component of many convenience meals, but the use of fresh cream is problematical owing to its perishability and poor process stability. Such sauces can be developed from dry ingredients but to ensure the manufactured sauces have appropriate rheological characteristics they can be compared to sauces formulated from fresh ingredients. Another area, which is continually developing, is extrusion cooked ingredients, which are used in the production of snacks, coatings and convenience meals. The expansion of these products as they pass through the extruder die is dependent on the viscoelastic properties of the dough as is the flow behaviour of the paste within the screws of the system (Kokini *et al.*, 1992).

Probably the most extensively researched area of food rheology has been that of dough of various types. Typical of the many reviews of this topic are those of Bloksma (1990), Faridi and Faubion (1990) and Rasper (1993). Dough rheology is important in bread making because of its influence in determining the texture of the bread crumb produced and also on the final volume of the baked product. The use of frozen dough has become an increasingly popular alternative to conventional dough processing both within in-store bakeries and domestically. Rheological measurements have been used to predict the baking performance of such products (Kenny *et al*., 1999). High fat, microencapsulated powders are a healthy and convenient alternative to fats normally used in cereal products and rheological properties have been used to assess the impact of these powders on wheat flour doughs (O'Brien *et al*., 2000). Such has been the interest in dough rheology that a series of specialised instruments have been developed over the years to monitor these properties (e.g. farinograph and extensigraph). Unfortunately, while they are widely used, many of the properties measured are machine specific and are not the absolute properties defined in the next section.

6.3 Basic rheology

As has been stated, food rheology is concerned with the description of the mechanical properties of food materials under various deformation conditions. Under external force food materials exhibit the ability to flow, or accumulate recoverable deformations, or both. According to the extent of recoverable deformation, the basic rheology concepts can be classified into viscous flow, elastic deformation and viscoelasticity (Babosa-Cánovas *et al*., 1996).

6.3.1 Viscous flow

As has been stated, rheology is the study of deformation and flow of foods under well-defined conditions. These conditions could be defined in terms of their rate of deformation or in terms of the magnitude of the stress or the strain applied. Foods of differing internal structure and bonding will react in different manners to these applied conditions. We take as an example a system designed to apply a controlled rate of deformation to a fluid. In the simplest case the shear stress developed in the fluid is directly proportional to the rate of deformation or the rate of strain. In such cases, the liquid is said to be Newtonian and obeys the relationship:

$$\tau = \mu\dot{\gamma} \qquad [6.1]$$

where, τ is the shear stress and $\dot{\gamma}$ is the shear rate. Such a relationship is shown by line (a) of Fig. 6.1. In SI units, τ will normally be in pascals (Pa), $\dot{\gamma}$ in reciprocal seconds (s^{-1}) and μ in pascal seconds (Pa s). The constant of proportionality μ between the shear stress and the shear rate is termed the viscosity of the fluid, and from the 1663 definition of a fluid by Pascal can be viewed as a measure of its internal friction (i.e. ability to resist motion when a shearing stress is applied). The nature of this friction can be demonstrated by a hypothetical experiment shown in Fig. 6.2, in which a fluid is placed between two infinite parallel plates (Singh and Heldman, 1993). The bottom plate is stationary while the top plate

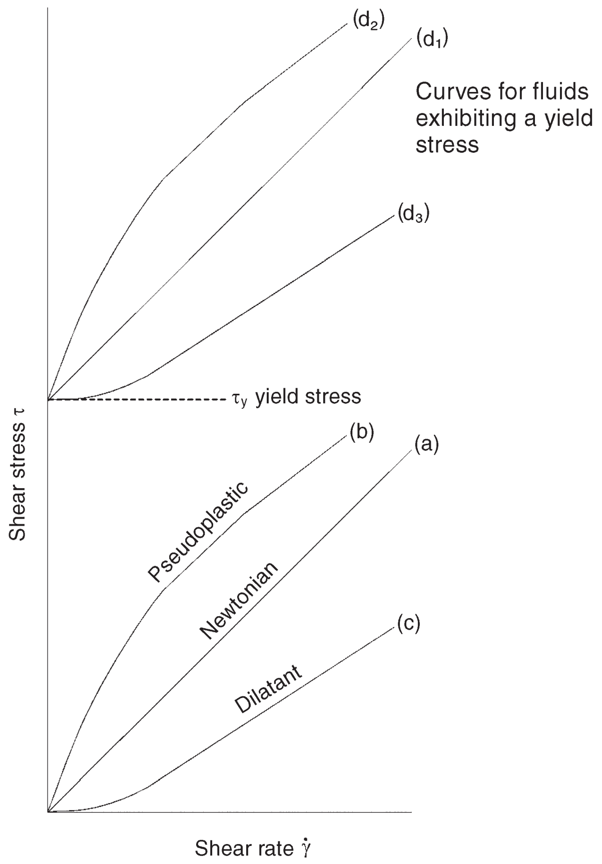

Fig. 6.1 Typical flow curves.

moves in the x-direction at a fixed velocity. A force per unit area perpendicular to the y-axis is required to maintain movement of the top plate. This is termed the shear stress and is given the symbol τ.

Force balances will demonstrate that this same shear stress is exerted between any two layers of fluid parallel to the plates. A velocity gradient or shear rate exists which is simply the difference in velocity of the two plates divided by the distance between them. In such a simple experiment it is assumed that the fluid at each plate has the same velocity as the plate, in other words that there is no slip occurring between the fluid and the surface.

One might be forgiven for thinking that inclusion of the early Pascal model of Fig. 6.2 is using too early a concept in a chapter on modern instrumentation. However, while the modelling of fluid behaviour has progressed significantly since that time, the basic principles of many instruments still use the two-surface concept, one moving and one stationary, with the fluid being characterised by force measurements at one of the surfaces. It also highlights the significance of slippage between the fluid and the instrument surfaces.

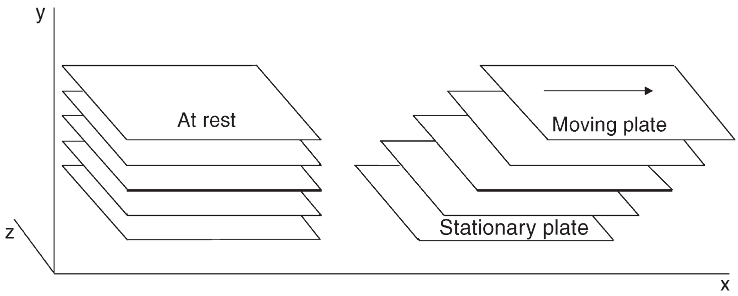

Fig. 6.2 Model system.

It is now useful to return to Fig. 6.1 and the concept of a Newtonian fluid in which there is a fixed proportionality between shear stress and the applied shear rate. Because of the relatively simple form of the flow curve, such liquids can be characterised by a single term, namely the constant of proportionality or the viscosity. More importantly, a single experiment such as the measurement of the shear stress at one surface at a single shear rate is sufficient to quantify the rheological characteristics of the fluid. However, only a few liquids follow this simple relationship (water, unconcentrated milk, vegetable oils, some dilute solutions); most foods may be classified as non-Newtonian and exhibit responses (termed flow curves) such as curves (b), (c) and (d). It will be shown that such fluids cannot be characterised by a measurement at a single shear rate as can the simple Newtonian fluid, and it is in ignoring this requirement that measurement errors are most often made in the food industry. Furthermore, there are many food liquids in which shear stress is not only determined by shear rate but is also time dependent, a factor which demands its own unique measurement system.

Many foods are termed 'pseudoplastic' and their response to an applied deformation varies with the rate of application of the deformation. Typically, plots or flow curves such as curve (b) of Fig. 6.1 represent such fluids. Because the slope of the curve decreases as shear rate increases, the term 'shear thinning' is often applied to such fluids (e.g. concentrated milk, solutions of concentrated molecules (xanthan and guar gum) and several fruit juices). Of lesser importance in the food industry are foods with curves of type (c), which are 'shear thickening' or 'dilatant'. Shear thickening behaviour of foods is only rarely observed (e.g. concentrated suspension of starch granules) and then over $\dot{\gamma}$ ranges normally not observed in practice (Van Vliet, 1999).

Rather than apply polynomial regression analysis to obtain equations for such behaviour, it has been found more convenient to plot the logarithm of shear stress against that of shear rate. For most pseudoplastic or dilatant fluids this results in a straight line and leads to the equation:

$$\tau = k\dot{\gamma}^n \qquad [6.2]$$

which is normally termed the power law equation. In this equation, n is the power law exponent and k is the apparent viscosity or consistency index. While this equation is a useful mathematical model for most foods, there is a theoretical objection to its use, namely that the dimension of k is dependent on the value of n. A Newtonian fluid would of course have an n value of 1.0 and k would equal its viscosity. For pseudoplastic fluids, n will lie between 0 and 1.0, while for dilatant liquids the value will be greater than 1. Though widely used, the power law model is not the only available, and in some cases its two-parameter equation represents an oversimplification (Launay and McKenna, 1983). Ree and Eyring (1958) proposed a three-parameter model:

$$\mu = \mu_0 + (\mu_0 - \mu_\infty)\,\frac{\sin h^{-1}\beta\dot{\gamma}}{\beta\dot{\gamma}} \qquad [6.3]$$

where μ_0 and μ_∞ are the Newtonian viscosities at zero and infinite shear rate, while β is a characteristic relaxation time. Obviously, such a model facilitates consideration of time dependent behaviour. Cross (1965) proposed a four-parameter model

$$\mu = \mu_0 + (\mu_0 - \mu_\infty)[1 + (t\,\dot{\gamma})^{1-n}] \qquad [6.4]$$

where t is another relaxation time. However, while Eqns 6.3 and 6.4 give more precise modelling of the flow curves of many foods, the widespread use of power law values in engineering equations makes Eqn 6.2 the most useful if not the most exact model. Neither do three- or four-parameter models imply a better understanding of the structure of the food in question.

Finally, one must consider the family of curves marked (d) in Fig. 6.1. Such foods exhibit a yield stress τ_y which must be exceeded before any deformation or flow can occur (i.e. these materials behave like solids under low stress and like fluids under high stress). For certain food processes (e.g. chocolate, confectionery and other coatings) the existence of a yield stress in the food is essential for application of the technology. Indeed, in the absence of rapid crystallisation or solidification of a coating, the magnitude of the yield stress will determine the thickness of the coating on a vertical surface. If the weight of coating divided by the vertical area (i.e. the shear stress exerted by the coating itself) exceeds the yield stress, then the coating will flow off the product. If not, it will neither flow nor deform and will remain to set on the product.

Equations, which describe such products mathematically, are those of Casson (1959) and Herschel-Buckley (see Charm, 1971):

$$\text{Casson:}\quad \tau^{0.5} = \tau_y^{0.5} + k'\dot{\gamma}^{0.5} \qquad [6.5]$$

$$\text{Herschel-Buckley:}\quad \tau = \tau_y + k''\dot{\gamma}^{n} \qquad [6.6]$$

where τ_y is the yield stress and k' are k'' constants. While the Casson equation is widely used (particularly in the chocolate industry, where it is generally accepted that molten chocolate can be modelled using the Casson equation), the Herschel-Buckley equation has the added attraction of merely adding a yield stress to the power law model.

Time-dependent behaviour of liquid foods is not considered in detail in this chapter and the reader is referred to texts such as Steffe (1996), Rielly (1997) and Van Vliet (1999). This is not because such aspects are unimportant for many foods but because, in steady state flow in pipes or channels in a food processing operation, little or nothing of time-dependent behaviour is observed. However, in storage of foods these properties become increasingly important as the onset of undesirable change may limit the effective shelf life of a product.

In concluding this section on basic rheological concepts, attention must be drawn to the dependence of such behaviour on temperature. As internal friction is a molecular phenomenon, anything that alters molecular movement will influence internal friction. Consequently, the rheology of most liquid foods is highly temperature dependent. In particular, the viscosity of Newtonian liquids exhibits such a dependency, as does the consistency index or apparent viscosity of power law fluids. The power law exponent is, however, relatively unaffected. No attempt will be made to quantify this phenomenon mathematically or to give a thermodynamic explanation for its existence. It is merely highlighted here to stress the importance of temperature control on the accuracy of any of the experimental rheological techniques detailed in later sections. For example, since the viscosity of water at 20 °C (293 K) will change by 2.5% per kelvin temperature change, an accuracy of 0.1% in the measurement of this viscosity will demand temperature control to within 0.04 K. Many oils will change in viscosity by 10% for each kelvin temperature change at 298 K (25 °C), thus demanding temperature control to 0.1 K for a 1% accuracy. It should be assumed that close temperature control is an essential feature of any of the measurement systems described in the following section.

6.3.2 Elastic deformation

As was stated earlier, greater emphasis will be placed on classical liquid rheology in this chapter. However, it is necessary to mention briefly elastic deformation in solids before going on to discuss the concept of viscoelasticity, which can be observed in semi-liquid fluids. Certain types of solids, known as hookean solids, display ideal elastic (or hookean) behaviour. This particular behaviour occurs when a force is applied to a solid material and the resultant response gives a straight line relationship between stress and strain (Vélez-Ruiz and Barbosa-Cánovas, 1997). This relationship is known as Hooke's law and occurs in an ideal elastic solid (also called Hooke's body).

Based on Hooke's law the following relationship (Eqn 6.7) has been established for a Hooke solid subjected to distortion by shear stresses:

$$\tau = G\gamma \qquad [6.7]$$

where G is the shear modulus (Pa), τ is the shear stress (Pa) and γ is the shear strain ($\gamma = (L'_o - L_o)/L_o$, dimensionless, where L'_o is the final length after deformation of the material and L_o is the original length before deformation) (Barbosa-Cánovas *et al.*, 1996).

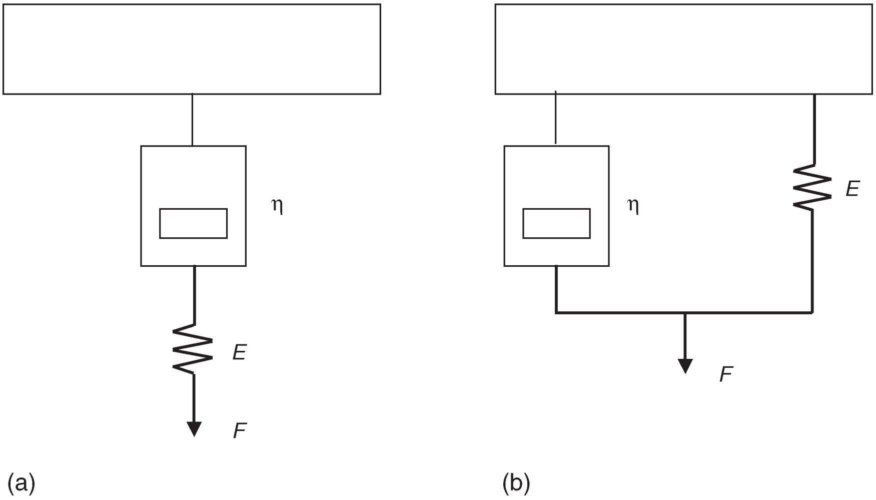

Fig. 6.3 Schematic diagram of (a) Maxwell and (b) Kelvin–Voight models.

6.3.3 Viscoelasticity

Many complex structured foodstuffs display both viscous and elastic properties and are known as viscoelastic materials. The use of this term is often restricted to solids, with the term ‘elastico-viscous’ being used to describe liquids displaying similar characteristics. However, following on from Whorlow (1992) in this chapter we will use the term viscoelastic to describe both, because it is often not possible to establish whether a material is behaving as a solid or as a liquid. Linear viscoelasticity is the simplest viscoelastic behaviour in which the ratio of stress to strain is a function of time alone and not of the strain or stress magnitude, while non-linear viscoelastic materials exhibit mechanical properties that are a function of time and the magnitude of stress used. The theoretical complexity of non-linear viscosity makes it impractical for most applications (Steffe, 1996) and in this text we will focus on viscoelasticity in its simplest linear form. Such viscoelastic behaviour may be explained using models, examples of which include the Maxwell and also the Kelvin (sometimes called the KelvinVoight) models. Both of these models use an ideal spring to represent the elasticity, while viscosity is represented by an ideal dashpot. In the Maxwell model this spring and dashpot are joined in series (Fig. 6.3a). In the Maxwell model if the strain rate is kept constant and the sample is deformed at a known rate, the buildup of stress can be calculated from:

$$\tau = \mu\gamma(1 - e^{-t/t'}) \qquad [6.8]$$

where t' is the relaxation time.

In the Kelvin model, the spring and dashpot are joined in parallel (Fig. 6.3b) and similar treatment for the Kelvin body gives rise to the following:

$$\tau = \gamma(\mu + Gt) \qquad [6.9]$$

The Maxwell and Kelvin models may be used as building blocks in parallel or tandem to construct more sophisticated models (e.g. Burgers model) but these are beyond the scope of this chapter and the reader is referred to texts such as Muller (1973), Prentice (1992) and Steffe (1996) for further information.

6.4 Measurement systems

Instrumental food rheology measurement systems can be broadly categorised into fundamental or empirical tests. Fundamental methods are conducted on a material by imposing a well-defined stress and measuring the resulting strain (or strain rate) or alternatively by imposing a well-defined strain (or strain rate) and measuring the stress developed (Barbosa-Cánovas *et al.*, 1996). Based on the geometry of the fixtures used, fundamental measurement systems can be divided into two groups: (a) capillary viscometers (Section 6.4.1) that make use of gravity (hydrostatic head) or pressurised (piston or pressurised gas) flow in capillary tubes for the measurement process; (b) rotary viscometers (Section 6.4.2) in which the sample is enclosed between rotating or oscillating surfaces. Empirical methods (Section 6.4.3) are also important in that they can give rapid results, but are arbitrary, poorly defined, have no absolute standard and are effective only for a limited number of foods. In general they measure rheologically affected phenomena from which it is possible to make a correlation to a desired variable. The main emphasis in this chapter will be on fundamental methods.

6.4.1 Capillary viscometers

Theory

Capillary viscometers are the simplest form of viscometer available from which it is possible to obtain absolute values of viscosity for Newtonian fluids and to obtain limited information on power law fluids. The basic measurement made is of the time t taken for a fixed volume V of the test fluid to pass through a length L of capillary tubing. Relative movement takes place between the axial part of the sample and that in contact with the tube walls. The driving force for fluid flow can come from gravity (as determined from the hydrostatic head difference between two liquid reservoirs in the viscometer) (glass (U tube) viscometers) but pressurised gas or a piston (high-pressure capillary viscometers) can also be used (see Fig. 6.4).

From first principles it is possible to derive an equation for the flow rate of fluid through such a tube or pipe. For Newtonian fluids, this equation is known as the Hagen–Poiseuille law (Hagen, 1839; Poiseuille, 1841) and relates the flow rate to the driving pressure for flow, with many of the variables of such a system incorporated into the constants of the equation:

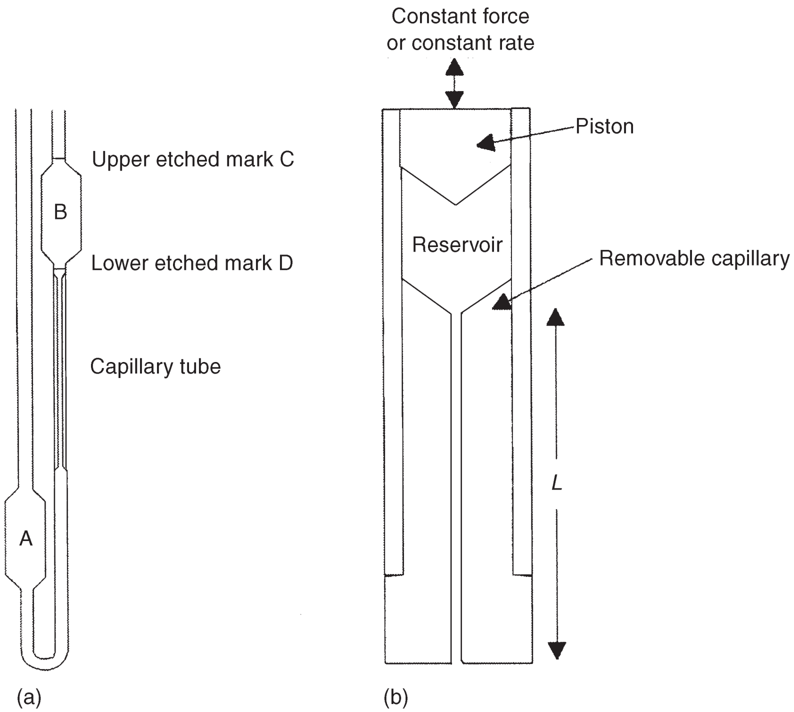

Fig. 6.4 Capillary viscometers: (a) Ostwald viscometer; (b) pressure capillary viscometer.

$$\frac{Q}{d^3} = \frac{\pi d \Delta p}{128 \mu L} \tag{6.10}$$

which can be rearranged to

$$\mu = \frac{\pi \Delta p d^4}{128 L Q} \tag{6.11}$$

where Q is the flow rate through the tube (m^3/s), d is the tube diameter (m), L is the tube length (m) and Δp is the pressure difference across the tube ($N\ m^{-2}$). For a given instrument d and L are fixed, so by measuring Q at a known Δp the coefficient of viscosity μ may be calculated. Indeed, since the volume processed in a given instrument is fixed at V, then Q may be replaced by V/t, where t is the time required for the flow. Taking the glass capillary (U tube) viscometers as an example, the driving force for flow will normally be the hydrostatic head within the system and will be equal to the product $\rho g h$, where ρ is the liquid density, g is the gravity constant) and h is the difference in liquid levels between the reservoirs of the system. For the U tube viscometers it is then possible to simplify equation 6.11 and write it in the form:

$$\mu = K\rho t \tag{6.12}$$

where ρ is the density of the fluid under test, *t* is the time taken for the fluid to flow through the capillary tube, and *K* is a constant for the instrument given by:

$$K = \frac{\pi g h d^4}{128LV} \tag{6.13}$$

This value is often supplied by the viscometer manufacturer. However, a common alternative approach is to use such capillary viscometers for comparative measurements against standard fluids of known viscosity. If the pressure difference causing flow is the same while measuring both fluids (for the glass (U tube) viscometers atmospheric pressure and gravity flow are usually applied), then the ratio of the viscosity of the food sample to that of the standard fluid will be equal to the ratio of the time required for equal volumes of the fluids to flow through the viscometer tube. Similarly, such standard fluids may be used to compute or to check the value of *K* given in Eqn 6.13. In the case of piston or gas pressure viscometers, the mean hydrostatic head due to the test fluid must be added to the measured applied pressure but the slight variation in hydrostatic head as the fluid leaves the upper bulb can usually be ignored (Whorlow, 1992).

The equations above have traditionally been used not only for viscometry but also to quantify the flow rate in a pipe system by monitoring the pressure drop along a section of the pipe. However, as the following section will demonstrate, this method should be used only as a rough estimate with food liquids as their generally non-Newtonian behaviour will demand that more complex relationships be used.

The flow of more complex fluids is governed by variations on the above equation. For laminar flow of power law fluids through a cylindrical tube under the influence of a pressure difference Δp, the following equation is obtained:

$$\frac{Q}{d^3} = \frac{\pi}{8(3 + 1/n)} \left(\frac{d\Delta p}{4kL} \right)^{1/n} \tag{6.14}$$

where *n* and *k* are the power law constants. At constant temperature, the apparent viscosity, *k* will be constant, so a plot of log *Q* versus log Δp will give a straight line of slope $1/n$, with the value of *k* being abstracted from the intercept value of the plot:

$$\log \frac{\pi d^{3+1/n}}{(4kL)^{1/n}[8(3 + 1/n)]} \tag{6.15}$$

However, with such simple equipment, facilities are seldom available to apply different pressure differences so as to obtain the points for such a plot. A more limited possibility is to take a range of viscometers of different capillary diameters but similar tube lengths and then to test the power law liquid in each using gravity flow. A plot of log *Q* versus log *d* should then give a straight line of slope $3+1/n$. Again, *k* could be abstracted from the intercept value:

$$\log \frac{\pi \Delta p^{1/n}}{(4kL)^{1/n}[8(3 + 1/n)]} \qquad [6.16]$$

A food liquid that behaves as Newtonian once its yield stress value has been exceeded (curve (d_1) in Fig. 6.1) will have a characteristic behaviour equation as follows:

$$\frac{Q}{d^3} = \frac{\pi d \Delta p}{128 \mu_p L} \left(1 - \frac{16 \tau_y L}{3 d \Delta p} + \frac{256 \tau_y L}{3 d \Delta p} \right)^4 \qquad [6.17]$$

where μ_p is the slope of the straight line plot of shear stress versus shear rate once the yield stress had been exceeded. For a more complete discussion of this equation see Leniger and Beverloo (1975); Prentice (1984) details the flow of Herschel-Buckley and Casson liquids in tubes or capillaries. As this is a more complex equation and post-yield stress linear behaviour is seldom experienced with food products, these simple viscometers cannot be recommended for examination of such products. They are, however, widely used, often in circumstances where their limitations are not fully understood. This is because they are relatively cheap and are easily available from most laboratory supply companies. Indeed, when one considers the equations involved and the multiple measurements required for all but simple Newtonian fluids, the use of such unsophisticated equipment presupposes knowledge of the basic behaviour of the fluid under test. In other words, these viscometers should be used only for known Newtonian fluids. This would confine their use to dilute solutions and vegetable oils. For other foods, they can provide only rough quality control tests.

The sizes of the food sample and of the constituents within the sample are important with viscometers of this type. As they rely on measuring the time taken for a given volume of sample to flow through the capillary tube, it is important to ensure that a homogeneous sample of the volume required can be obtained from the food. Difficulty may be experienced with foods containing large amounts of suspended solids. Indeed, suspended solids will contribute to large errors in the measured times if they are of a size that is significant when compared with the diameter of the capillary tube. Further, particles that affect laminar streamline flow within the capillary will change the time measurement. Of course, these comments are equally relevant to droplets within an emulsion as they are to solid particles. Care must also be taken to ensure that suspended particles within a food do not settle during the duration of a test. Nor should any separation occur within a food emulsion.

Examples have already been given that place general emphasis on the need for exact temperature control during measurements with this as with any type of viscometer. Prentice (1984) quotes an instance where temperature variations of ±0.12 K will alter the linearity of the flow curves obtained.

Instruments

In this section (similar to Whorlow, 1992) capillary viscometers will be classified according to the method used to apply pressure. Glass capillary (U tube) viscometers rely on a hydrostatic head to force the test fluid (generally a low-viscosity liquid) through the capillary tube, while in high-pressure capillary viscometers (generally used for more viscous liquids), air, gas or hydraulic pressure is applied or the fluid is forced through the tube by means of a piston. The distinction between pipe versus capillary type systems is also mentioned at the end of this section.

Glass capillary (U tube) viscometers

Figure 6.4a shows the simplest glass capillary viscometer available known as the Ostwald viscometer. However, there are many variations of this on the market (e.g. Cannon-Fenske, Ubbelohde), each of which would claim a special advantage and may have its own specific name applied by its manufacturers. These glass capillaries rely on a hydrostatic head to induce fluid flow through a tube.

Operation is as simple as the design of the system. A standard volume of the test food liquid is pipetted into reservoir A of the viscometer and the U-tube below it. The instrument should preferably be held exactly vertical. If not, the support fixture should be such as always to hold the instrument at the same angle from the vertical. The instrument and the test liquid must now be equilibrated at the test temperature by immersing the viscometer in a controlled temperature water bath. Earlier sections have discussed the influence of the precision of this temperature on the accuracy of the results obtained. As these are related to the temperature sensitivity of the viscosity of the test liquid and this is often unknown before the measurements are undertaken, this author recommends that ±0.1 K be taken as a target temperature variation. Equilibration may take up to 0.5 h, during which the earlier comments on sedimentation or separation become relevant. Suction is then used to raise the liquid through the capillary into reservoir B until the meniscus of the liquid is above the etched mark C. The liquid is now allowed to flow under gravity and the time is taken for the meniscus to pass between marks C and D. Generally reservoirs A and B should be of similar radius to minimise surface tension errors.

During this process the hydrostatic head will fall as the liquid level falls on the right hand side in Fig. 6.4a and rises in the left-hand leg. However, because the geometry of the system is arranged so as always to have test liquid within reservoir A with its large cross-section, the rise in the level in reservoir A will be very small. Consequently, the variation in hydrostatic head will be minimised. In addition, the shape of reservoir B is such that most of the measured flow will occur with the level central in this chamber and further reduce the variation in the head. A mean value will be quoted by the manufacturer. Examination of Eqns 6.10 and 6.14 shows that this variation has no effect on Newtonian fluid measurements, while its effect on power law fluids could be considerable if the power law exponent n varied significantly from 1.

As previously stated, variations in design of glass capillary viscometers are many in number. One common form involves bending both legs of the U-tube slightly so that the bulb of the lower reservoir A is directly below that of reservoir B. Another variation is the use of light sensors to note the passing of the meniscus across the etched marks C and D coupled to electronic timing, thereby ensuring more accurate measurement. As with most scientific instruments, corrections are necessary if a high level of accuracy is required. These include kinetic energy effects, end effects, turbulence and wall effects, effects of time-dependent properties and thermal effects. Many authors cover these corrections in some detail and the reader is referred to Lapasin and Pricl (1995) for a complete discussion.

High-pressure capillary viscometers

High-pressure capillary viscometers are also available and are constructed from glass or steel tubes. As earlier stated, these systems differ from the glass capillary viscometers mentioned above in that they rely on pressure from either compressed gas (air or nitrogen) or a piston to induce fluid flow through a tube. The gas pressure viscometers normally operate at a constant pressure whereas piston viscometers tend to operate at constant flow rate. In both the gas and piston systems the intake reservoir and capillary tube should be held in a thermostatically controlled environment for the duration of any measurements. These high-pressure systems are widely used in the plastics and lubricants industries but are less commonly used for rheology measurements on foodstuffs.

Whorlow (1992) outlines a number of different gas pressure viscometer designs. In general terms these systems consist of a straight length of capillary tube that connects two reservoirs (an intake and a receiving reservoir). The gas supply passes via a pressure regulator into an intake reservoir whereupon it forces the liquid through a capillary tube. The capillary tubes can be removed for cleaning and are interchangeable, with the possibility of being replaced by tubes of different diameter or length as required. Tubes range in diameter and length from 2.5 to 6 mm and from 25 mm up to 3 m, respectively. Other variations in design include a facility to prevent gas becoming dissolved in the test liquid, by housing the fluid in the intake reservoir within a plastic bag with pressure being applied to the outside of the bag forcing the liquid through the capillary tube.

Piston viscometers differ from a gas pressure viscometer in terms of the design of intake reservoir and also in the fact that they can be used at constant flow rates or constant pressures. The intake reservoir consists of a cylindrical barrel into which the fluid to be measured is placed. A piston head fitted with sealing rings is inserted into the barrel and is used to force the liquid through the capillary tube. Similar to the gas pressure viscometer, the intake reservoir and capillary tube are held in a thermostatically controlled environment. The reader is referred to Whorlow (1992) for a more complete description of these systems.

Pipe viscometers

Pipe and capillary viscometers differ in terms of tube diameter but there are no clearly defined sizes at which a tube should be called a capillary rather than a pipe. Commercial capillary instruments range in diameter from 0.1 to 4 mm. Pipe viscometers vary widely in diameter with some systems having diameters as small as 7 mm but values of greater than 12 mm and up to 32 mm are not uncommon in food applications (Steffe, 1996).

6.4.2 Rotary viscometers

In rotary viscometry the product is enclosed between two surfaces, one of which subsequently undergoes an applied rotary motion. The geometry of these surfaces can be in the form of concentric cylinders (or Couette viscometers) while other possibilities include a cone and plate or a pair of parallel plates. Depending on how the rotating surfaces are controlled these viscometers can be classified as rate-controlled or stress-controlled. In rate-controlled instruments the velocity of rotation of the one of the surfaces is the controlled quantity and the transmitted torque is recorded on the measuring surface, while for stress-controlled instruments a controlled torque is applied to one surface and the resultant rate of rotation is subsequently recorded (Lapasin and Pricl, 1995).

Traditionally a rheometer was designed to measure under controlled-stress or controlled-rate conditions but combined units, which offer measurement under both conditions are now available. Although we use the term viscometer in this section many instruments are generically called rheometers (versus viscometers) since they measure other properties in addition to viscosity. In the more sophisticated microcomputer-controlled systems, several operating modes are generally possible, examples of which include creep measurements, controlled stress flow and oscillatory mode.

Concentric cylinder viscometers

Theory

The concentric cylinder type is shown schematically in Fig. 6.5 and owes its development to the pioneering work of Couette (1890). These instruments consist of a cylindrical bob positioned concentrically in a hollow cylinder. In Searle-type viscometers the bob rotates while in Couette-type viscometers the cylinder can be rotated. In rate-controlled instruments the measured variable is either the torque transmitted through the liquid to the stationary cylinder or the torque required to keep the moving cylinder rotating at a given velocity. In stress-controlled systems the rate of rotation induced in the measuring surface is recorded as controlled torque (or shear stress) is applied. The shear-stress/shear-rate relationship is the same with each system of rotation. Continuous measurements may be made and time-dependent effects studied. Continuous or step variation over a wide range of torques or velocities is normally available. Because of this, a range of shear stresses or shear rates may be readily obtained, thus permitting analysis of Newtonian or non-Newtonian behaviour. However, a major disadvantage is that the liquid is not subjected to a spatially uniform shear rate even if it is a simple Newtonian liquid.

Owing to their versatility these systems are, without doubt, the most widely used in rheological measurements, and fluid behaviour within the annular gaps of these instruments has been the subject of intensive investigation. Consequently, there is a wide range of analytical equations available for assessing their results and for modifying the readings obtained to correct for a wide variety of error sources.

For Newtonian fluids, the simplest relationship is the Margules equation (1881):

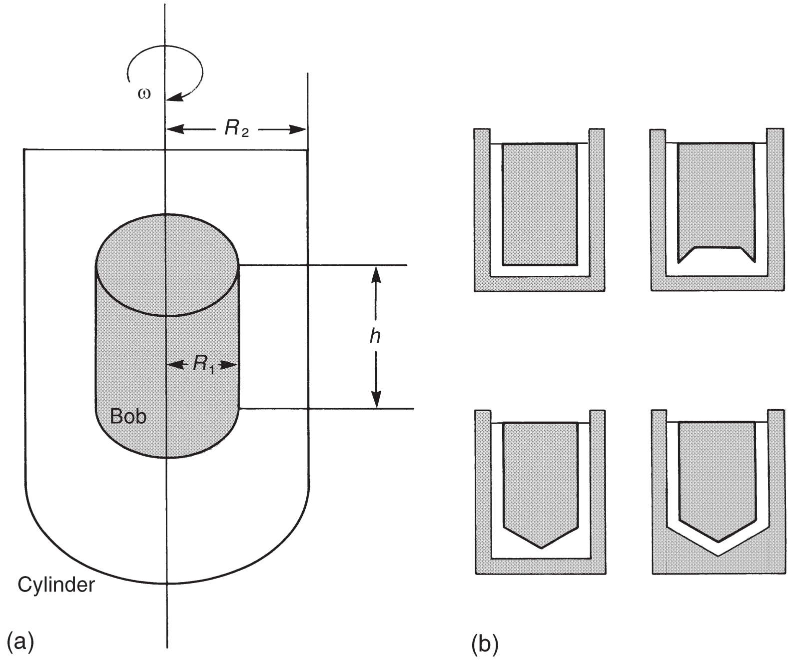

Fig. 6.5 Concentric cylinder viscometer (a) dimensions and (b) side profiles illustrating flat, angled and recessed bottoms.

$$\mu = \left(\frac{T}{4\pi h'\omega}\right)\left(\frac{1}{R_1^2} - \frac{1}{R_2^2}\right) \qquad [6.18]$$

where μ is the viscosity, T is the torque on the cup or bob (measured in rate-controlled and fixed in stress-controlled), ω is the angular velocity of the rotating cup or bob (measured in stress-controlled and fixed in rate-controlled), h' is the height of the bob, R_1 is the radius of the bob and R_2 is the radius of the cup.

For non-Newtonian materials, Van Wazer *et al.* (1963) derived the general equations for flow in the annular space between the concentric cylinders and provided solutions for Newtonian fluids, power law fluids, power law fluids with a yield value (Herschel-Buckley), Eyring model fluids and several others. For simple power law fluids the following relationship is available for shear rate $\dot{\gamma}$:

$$\dot{\gamma} = \frac{\omega(R_2^2 + R_1^2)}{(R_2^2 - R_1^2)} \cong \omega\left(\frac{R_2}{\Delta R}\right) \qquad [6.19]$$

where ΔR is the width of the gap between the cylinders, and the shear stress at the bob τ_b is calculated from the following equation:

$$\tau_b = \frac{T}{2\pi R_1^2 h'} \qquad [6.20]$$

In the case of rate-controlled systems ω will be fixed and T will vary while for stress-controlled systems T will be fixed while ω will vary. Plotting the logarithms of the values derived from equations 6.19 and 6.20 should give a straight line of slope n and intercept k. It may, however, prove more convenient to calculate from equation 6.19 (stress-controlled systems) or τ_b from equation 6.20 (rate-controlled systems). These values calculated from these equations can then be used in further calculations, for example a plot of ln ω versus ln τ_b should give a curve that fits the equation:

$$\ln \omega = (1/n)\ln \tau_b + \ln\{(n/2)(\sqrt[n]{k})[1 - (R_1/R_2)^{2/n}]\} \qquad [6.21]$$

There are a number of potential sources of error in concentric cylinder viscometers. The major ones include inertial effects, differences in shear rate distribution, edge and end effects and thermal effects (Lapasin and Pricl, 1995; Steffe, 1996). Inertial effects manifest themselves in localised circulation instabilities known as Taylor vortices. Data analysis equations developed for concentric cylinder viscometers assumed that laminar flow occurs. However, the outward movement of liquid under the influence of centrifugal force can give rise to secondary flow or Taylor vortices. These vortices occur at lower Reynolds numbers in Searle-type relative to Couette types where the rotation of the outer cylinder helps in stabilising the flow of liquid.

End effects are the most common source of error and occur due to the fact that the cylinders have finite dimensions, instead of being infinite, as the theory requires. In rate-controlled systems the torque response imposed by the bottom of the cylinder was not accounted for in the development of the fundamental theory. These end effects can however, be corrected for by taking torque readings at several different immersion depths of the cylinders in the test fluid. If T is then plotted against h, the resulting graph will intersect the h axis at a negative value h_c that corresponds to the correction to be added to h in any of the above equations. Alternatively, this may be calculated from the following equation of Oka (1960):

$$h_c/R_1 = (R_1/8e)[1 - (R_1/R_2)^2]$$

$$\{1 + (4e/R_1)\sum_{n=1}^{\infty} A_n I_2(n\pi R_1/e) + (8e/\pi R_1)\sum_{n=1}^{\infty} B_n[\sinh(K_n h)]/K_n R_1\}1 \qquad [6.22]$$

where e is the distance between the bottom of the bob and the cup, I_2 is a modified second-order Bessel function, K_n is the nth positive root of a derivation of the Navier–Stokes equation for incompressible fluids, and A_n and B_n are functions of the variables R_1/R_2, h/R_2 and e/R_2. However, if the immersion system is such that the gap between the end of the bob and either the cup or the fluid container is large, then the end effects become negligible and the difficult application of equation 6.18 is avoided. In rate-controlled systems, the end correction can also be calculated using an equivalent torque (T_e) using the method described by Steffe (1996). In addition to adjusting calculations to account for end effects, various cylinder designs have been developed to minimise the occurrence of end effects. A number of these cylinders have been designed one of which has a slightly angled bottom (Mooney Couette bob) while another has a recessed bottom and top (Fig. 6.5).

Another source of error is shear rate variation across the sample. Equation 6.19 gives a mean value for shear rate. However, for power law fluids this can be corrected by the relationship:

$$\dot{\gamma}_{\mathrm{eff/}}\dot{\gamma}_{\mathrm{meas}} = (1/n)(11/2^{1/n-1})[1 - (R_1/R_2)^2][1 + (R_1/R_2)^2]^{1/n-1}[1 - (R_1/R_2)^{2/n}]^{-1} \qquad [6.23]$$

where $\dot{\gamma}_{\mathrm{eff}}$ is the effective shear rate and $\dot{\gamma}_{\mathrm{meas}}$ is the measured value. The reader is referred to a correction table available in Prentice (1984), which obviates the need to carry out this detailed calculation.

Temperature rises can occur in concentric cylinder viscometers where some of the work done is dissipated as heat. Many viscometers have temperature control systems, which are designed to remove excess heat generated during testing. Although these temperature increases can potentially affect rheological properties it is possible to accommodate them in some substances, while for others it is possible to adjust the results appropriately to account for them (Whorlow, 1992; Lapasin and Pricl, 1995; Steffe, 1996).

Instruments

There is a large range of concentric cylinder viscometers available from many different manufacturers. All use the same basic configuration, but they vary significantly in their degree of sophistication. Systems with dial displays are still available but digital displays of rotational speed and torque are more or less standard. Many systems have their own microprocessor incorporated and have the capacity to be operated from a PC, which also serves as a data acquisition and analysis system. It is impossible to make specific recommendations in this general chapter other than to emphasise the guidelines of Prins and Bloksma (1983) to which reference has already been made in Section 6.1. However, it is essential that when selecting an instrument, consideration be given to the range of shear rates required in the case of rate-controlled or the range of stress rates required in the case of stress-controlled systems. The fluids must be subjected to the same shear or stress rates as those in the application for which the rheological characteristics are required. In particular, processors of fluids such as chocolate, which have a yield value, must select an instrument capable of accurate measurement at very low shear rates. Systems differ in the method that is used to detect torque, some are fitted with mechanical transducers (i.e. torsional bar), whereas other systems use a non-mechanical force transducer (electronic force sensor). Another feature of sophisticated modern-day systems is that many are fitted with air bearings, which lubricate and minimise the friction of the measuring shaft. The reader is referred to Ma and Barbosa-Cánovas (1995) for more information on the range of viscometers currently available.

Cone and plate viscometers

Theory

A much recommended system for rotary measurement is the cone and plate viscometer (Fig. 6.6). This consists of a cone of shallow angle, normally of less than 3 degrees (up to 5 degrees are possible but edge effects can distort the flow field) and possibly with a truncated tip, that almost touches a flat plate. The sample for assessment is placed in the intervening space and different angular velocities (in a rate-controlled instrument) or torques (in a stress-controlled one) are applied to either the cone or the plate (most commonly the cone). While in theory it is possible to rotate either the cone or the plate and measure the torque transmitted through the intervening liquid, the normal procedure is to rotate the cone and measure either the transmitted torque on the plate or the torque required to rotate the cone at a constant angular velocity. In rate-controlled instruments the velocity of rotation of the cone (or plate) is controlled and the transmitted torque on the plate (or cone) is measured, while for stress-controlled instruments the opposite situation occurs where a controlled torque is applied and the resultant rate of rotation is measured.

The major advantage of this measuring system is that the shear rate is constant at all points in the fluid. This feature is true only when small conical angles are used and makes the system particularly useful when characterising non-Newtonian fluids, since the true rate of shear may be determined without the need for detailed corrections as in the concentric cylinder type. This constant shear rate may be determined from the relationship:

$$\dot{\gamma} = \frac{\omega}{\alpha} \qquad [6.24]$$

where ω is the angular velocity (rad s^{-1}) and α is the angle of the cone (rad).

The shear stress may be calculated from the following equation where R_c is the radius of the cone:

$$\tau = \frac{3T}{2\pi R_c^3} \qquad [6.25]$$

For a Newtonian fluid these may be simply combined to give:

$$\dot{\gamma} = \frac{1.5T\alpha}{\pi\omega R_c^3} \qquad [6.26]$$

Indeed, even when measurements are performed on more complex systems, the analysis of results simply requires the substitution of the above equations for $\dot{\gamma}$ and τ into the relevant behaviour model for the fluid. Carrying out this substitution for power law (Eqn 6.2), Casson (Eqn 6.5) and Herschel-Buckley (Eqn 6.6) fluids leads to the following set of relationships:

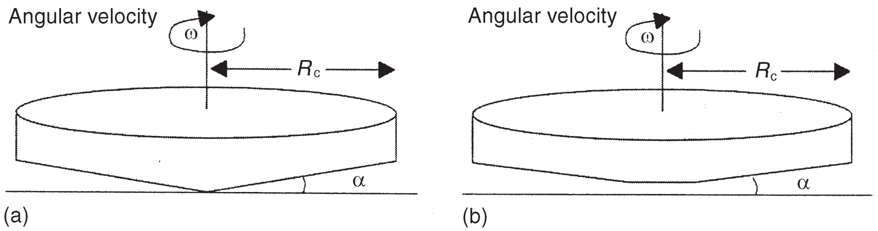

Fig. 6.6 Cone and plate viscometers: (a) normal; (b) truncated cone.

Power law: $3T/2\pi R_c^3 = k(\omega/\alpha)^n$ [6.27]

Casson: $(3T/2\pi R_c^3)^{0.5} = \tau_y^{0.5} + k'(\omega/\alpha)^{0.5}$ [6.28]

Herschel-Buckley: $3T/2\pi R_c^3) = \tau_y + k''(\omega/\alpha)^n$ [6.29]

While these instruments are much recommended, particularly for transient measurements, care must be exercised when treating any fluid containing suspended solids. The gap between the plate and the truncated cone is normally 50 μm or less. The problems and errors that would be encountered with particles of this size or larger need not be stressed. There is a general recommendation that particles should be at least ten times smaller than the size of the smallest gap between the cone and plate.

Potential sources of error in cone and plate viscometers, include inertial effects, edge and end effects and thermal effects (Whorlow, 1992; Lapasin and Pricl, 1995; Steffe, 1996). Inertial effects give rise to secondary flows, which can affect the torque and can occur in a number of different forms as illustrated by Whorlow (1992) and Lapasin and Pricl (1995). However, for sufficiently small gap angles and fluids with low Reynolds numbers the effects of secondary flow can be ignored. Edge effects manifest themselves as edge failure in thick fluids. Edge failure occurs in rate-controlled systems where as the rotation speed is increased the fluid at the edge of the cone and plate breaks up and gives rise to a sharp drop in torque. These edge effects limit the maximum shear rates that can be used in cone and plate systems. Temperature effects can also occur and the reader is referred to Whorlow (1992) and Lapasin and Pricl (1995) for more detailed information. However, Steffe (1996) claims that temperature rises caused by viscous heating are rarely a problem when testing biological materials.

Instruments

Similar to the concentric cylinder viscometers, a range of cone and plate rheometers is available. There are a number of options regarding the choice of cone including its angle and diameter. Increasing shear stress is encountered with decreasing cone diameter, while increasing shear rate is encountered with decreasing cone angle (internet diagram). As stated above, the apex of the cone is often truncated by a small amount so that it does not touch the plate and as a consequence there is a small region near the tip where the opposing faces are parallel. This truncation also prevents wear on the cone tip and also the indentation of the plate. Many cone and plate rheometers are fitted with autozero and autogap controls which allow the operator to control and standardise the gap between the cone and plate and assist in ensuring reproducible data are obtained from the system.

Parallel plate viscometers

The parallel plate viscometer is similar in operation to the cone and plate device outlined above. However, unlike the cone and plate, and concentric cylinder geometries where the gap separating the two surfaces is fixed, the parallel plate system has the advantage of flexible gaps. This is useful for materials such as coarse dispersions, which are intolerant of the narrow gaps in cone and plate and concentric cylinder viscometers. Another difference between the parallel plate and cone and plate system is the uneven distribution of shear rate, which varies from zero at the centre to a maximum ($\dot{\gamma}_{\max}$) at the outer edge (i.e. the rim) of the plate, the value for which can be calculated from the formula:

$$\dot{\gamma}_{\max} = \frac{\omega R_p}{H} \qquad [6.30]$$

where R_p is the radius of the plate and H is the separation of the two plates.

The shear stress at the outer edge of the plate can be calculated from the following equation:

$$\tau_{\max} = \frac{3T}{2\pi R_p^3}\left(1 + \frac{1}{3}\,\frac{\mathrm{dln}\,T}{\mathrm{dln}\,\dot{\gamma}_{\max}}\right) \qquad [6.31]$$

Owing to the large variation in shear rates this method is not that commonly used in steady shear measurements. The limitation of parallel plate geometry is that the shear rate has to be below 500 s^{-1}. The major sources of error associated with parallel plate viscometers are similar to those outlined for cone and plate systems. However, parallel plate systems may also be subject to slippage, although slip correction methodology, which allows for correction of this phenomenon, is available (Steffe, 1996).

Dynamic rheology

Dynamic rheology is a form of rheology which uses the same applicator geometries as those described for rotary rheometers (i.e. concentric cylinder, cone and plate and parallel plate). However, unlike rotary rheometry where the sample is subjected to an applied rotary motion, dynamic rheology is a form of rheometry where samples are subjected to small sinusoidally varying loads in which either the shear stress τ or strain γ is controlled (i.e. control stress or control strain, respectively). The magnitude of these deforming loads is small and they are chosen (e.g. by an amplitude sweep test) such that the material structure is not destroyed. Under such conditions the viscoelastic properties of the sample become evident. To illustrate dynamic rheology, imagine a slab-shaped volume between two parallel

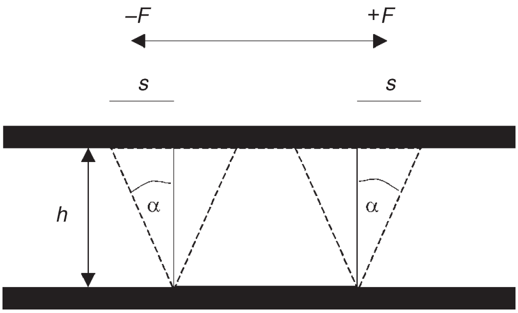

Fig. 6.7 Oscillatory strain between rectangular plates.

rectangular plates (Fig. 6.7) in which the lower plate is fixed and the upper plate is allowed to move backwards and forwards in a horizontal direction. In a control strain test the strain γ is applied by presetting the path and the volume is submitted to a force (±*F*) or shear stress. In control stress systems, the oscillating stress from the force (±*F*) means that the volume element undergoes a strain. With controlled strain instruments the strain curve as a function of time is given by:

$$\gamma = \gamma_o \sin(\omega t) \qquad [6.32]$$

where γ_o is the amplitude of the strain equal to L/h (L is the distance from centre moved by the plate and h is the distance separating the plates), ω is the frequency expressed in rad $^{-1}$s and can be calculated from $2\pi f$, where f is the frequency (Hz) (Steffe, 1996). Thus the magnitude of the strain is governed by the amplitude and frequency. Corresponding to the strain curve the strain rate can be calculated from:

$$\dot{\gamma} = \gamma_o \omega \cos(\omega t) \qquad [6.33]$$

which is the derivative of Eqn 6.32.

For controlled stress instruments the stress curve as a function of time can be calculated from:

$$\tau = \tau_o \sin(\omega t) \qquad [6.34]$$

τ_o being the stress amplitude (Pa). The measured result from a controlled strain system is a shear stress curve the equation for which is:

$$\tau = \tau_o \sin(\omega t + \delta) \qquad [6.35]$$

where δ is the phase displacement angle. In rheometers, which operate as controlled stress systems, the following equation characterises the strain curve produced by the sinusoidally varying stress input:

$$\gamma = \gamma_o \sin(\omega t + \delta) \qquad [6.36]$$

Regardless of whether a controlled stress or controlled strain system is used, in perfectly elastic substances the strain and stress waves will be in phase with each other (i.e. $\delta = 0°$) while in purely viscous fluids the strain and stress waves will be exactly 90° out of phase with each other (i.e. $\delta = 90°$). For viscoelastic substances the phase angle will lie in the range 0° < δ < 90°. From the recorded sinusoidal curve the storage modulus (G') and loss modulus (G'') can be calculated. The storage modulus represents the elastic behaviour of a sample as its magnitude represents the strain energy, which is reversibly stored in and recoverable from the substance:

$$G' = \left(\frac{\tau_o}{\gamma_o}\right)\cos\delta \tag{6.37}$$

As the name suggests the loss modulus represents the quantity of energy irreversibly given off by the substance to its environment and thus lost. This modulus characterises the viscous behaviour of the sample.

The storage modulus and loss modulus can be combined to give a single figure called the tan δ which gives a ratio between the amount of energy lost and stored per cycle and hence a relationship between the viscous and elastic portions of the sample. Tan δ can vary from zero to infinity with highest values in Newtonian fluids and lowest values in substances, which resemble hookean solids (Steffe, 1996):

$$G'' = \left(\frac{\tau_o}{\gamma_o}\right)\sin\delta \tag{6.38}$$

$$\tan\delta = \frac{G''}{G'} \tag{6.39}$$

Dynamic testing is not the only method that can be used to gather information on the viscoelastic properties of substances. Other non-oscillatory methods are available including step strain (stress relaxation), creep and recovery and startup flow (stress overshoot), which differ from dynamic testing in that the sample, is subjected to a constant load (shear stress τ or shear rate $\dot{\gamma}$). These methods are widely used and the reader is referred to Whorlow (1992) and Steffe (1996) for further detailed discussion on their theory and application.

6.4.3 Empirical rheology methods

The emphasis of this chapter is on fundamental versus empirical measurements. However, empirical measurement methods are widely used in areas such as quality control, correlation to sensory analysis results and even as official identification standards. They are suitable for foods with non-homogeneous complex structures where measurement by fundamental means is not possible but empirical methods can be used to obtain an index of product rheology. Empirical methods include dough testing equipment (farinograph, mixograph, extensigraph, alveograph), cone penetrometers, Warner-Bratzler shear devices, Bostwick consistometers, Adams consistometers, Zhan viscometers, viscoamylographs, rapid visco analysers, falling ball viscometers, Hoeppler viscometers, compression extrusion cells, Kramer shear cells and texture profile analysis systems, each of which are outlined in detail by Steffe (1996). Some of these methods are more suited to the measurement of solids and as stated earlier, they measure rheologically affected phenomena from which it is possible to make a correlation to a desired variable.

To illustrate empirical systems we will take an example of rotary viscometers in which cylinders, bars or agitator paddles rotate in a test fluid. Analysis of such systems is difficult because of their geometric complexities. Consequently, their use depends on the existence of empirical relationships, which relate measured variables, normally the torque required to rotate the instrument at a known speed, to the rheological characteristics. However, it must be stressed that many such instruments provide correlations with Newtonian viscosity only and consequently may have limited use when one is considering the more complex fluids normally found in the food industry.

Two instruments are worthy of mention because of their widespread use in the food industry. In the Brookfield Synchro-Lectric Viscometer a series of cylindrical spindles and horizontal disks are rotated at fixed speeds while the torque required to overcome the viscous drag of the fluid is recorded. Conversion tables are available to convert this into Newtonian viscosity. It is, however, very difficult to estimate accurately the shear rates being used and consequently its use for non-Newtonian fluids is limited. Some work has, however, been carried out (Mitschka, 1982) to enable calculation of some of the basic power law (Eqn 6.2) variables from Brookfield readings. In repetitive quality control use, many processors find its rugged simplicity useful and happily use its readings for comparative purposes. For more precise rheological evaluations, an optional attachment converts it into the more useful concentric cylinder geometry.

The Brabender Viscocorder measures the torque imparted to a paddle by the viscous drag of the test fluid in a rotating cup. Various forms of the instrument are available and a version capable of heating the test fluid in the rotating cup has found widespread use in the starch industry. Again it is difficult to relate the results obtained to the fundamental rheological properties, and the instrument, while widely used in quality control in the baking industry, has not seen extensive use in other areas. Details of both the Brookfield and the Brabender instruments are widely available in reviews including those of Matz (1962) and Sherman (1970).

6.5 On-line measurement systems

On-line systems are finding ever increasing applications in process control. An excellent comprehensive review of the instruments available was published by Cheng *et al.* (1984) of the Warren Springs Laboratory, UK, while a more recent review has been published by Davidson *et al*. (1989) and also Steffe (1996). The reader is also referred to Chapter 7 for a comprehensive up to date review of this area.

Many of the instruments available are modifications of those outlined in the previous sections of this chapter. In general, all share the same limitations and difficulty or ease of use as their laboratory counterparts. In particular, they must operate at a shear rate or range of shear rates that correspond to those experienced (at the relevant stage of processing or consumption) in the system being controlled. They also have the additional requirements of hygienic design and suitability for cleaning in place.

Rotary systems may incorporate a chamber in the process line through which all or a bypass stream of the process fluid may flow. Within this chamber may be fitted the cylinder and cup of a concentric cylinder Couette viscometer. In some instruments the measuring chamber itself is also the outer cylinder within which a concentrically mounted bob rotates (Fig. 6.8a). A second variation is the tank-mounted system (Fig. 6.8b). In addition to these geometrically well-defined systems, there is a range of systems which continuously monitor the torque required to rotate blades and paddles at fixed speeds. As with the laboratory systems, these must rely on empirical correlations to convert the torque/speed readings into rheological parameters. Alternatively, process specific target and limit values are set for the torque/speed readings without conversion.

On-line capillary instruments are also available. As the entire process stream cannot be sent through a small capillary, these always use a bypass stream through which some of the process fluid is accurately metered (normally by a gear pump) through a capillary tube. Pressure sensors at each end provide the only measurements required for their operation. Capillary tubes may of course be changed for others of different diameter to give a variation in shear rate, but this is an intermittent rather than a continuous variation. Cleaning-in-place difficulties may also be experienced with these narrow-bore capillaries. As they are subject to all the same limitations as their laboratory counterparts, their use tends to be limited to Newtonian liquids of low viscosity.

In addition to these adaptations of laboratory systems, other systems include vibrational techniques in which the power to keep spherical bobs or rods vibrating at fixed amplitudes within the liquid food is measured and correlated with rheological parameters. An ultrasonic wave source can potentially be incorporated into an on-line system and the velocity and attenuation of the propagated waves can be related to the rheological properties of a sample (Lee *et al.*, 1992). Other systems use falling cylinders within the fluid coupled to magnetic measuring systems. However, shear rates within such instruments are not readily available, thus limiting their use to quality control systems. Hot wire methods have been suggested (Sato *et al.*, 1990) using the temperatures in the fluid surrounding sheathed and heated wires as rheological correlates. This system requires development to overcome any potential hygiene problems and to ensure the degree of robustness necessary for continuous industrial use.

6.6 Instrument selection

Guidance on the choice of correct instrument is a very subjective area in which authors will generally have a personal preference for a particular instrument or for a particular manufacturer. Therefore, the intention in this section is not to express

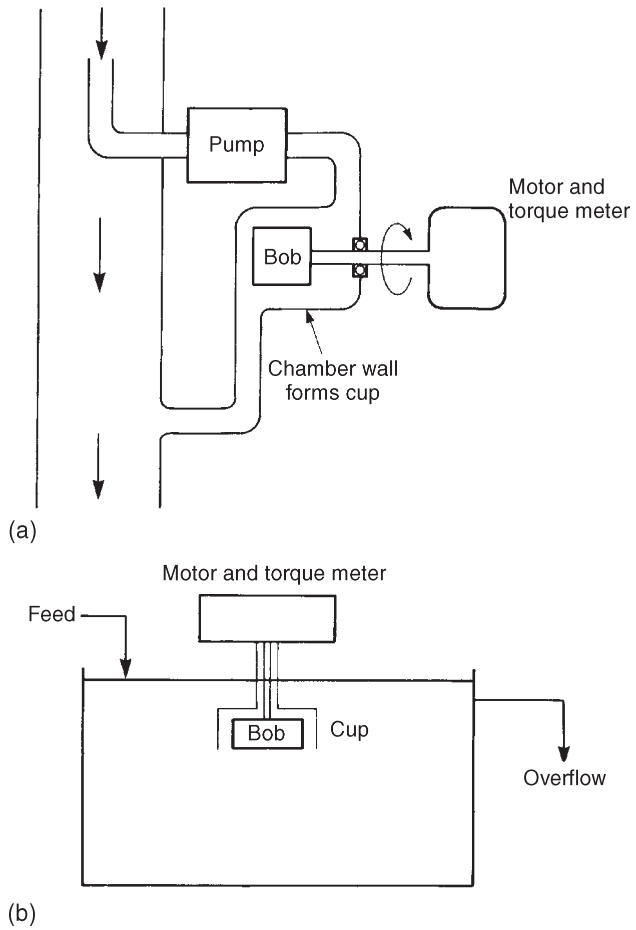

Fig. 6.8 Rotary viscometers: (a) on-line; (b) tank mounted in-line.

such preferences but merely to give broad guidelines. For occasional monitoring of the viscosity of a Newtonian food, a capillary viscometer is the cheapest and often quite a suitable selection. It must be emphasised, however, that such behaviour is rather rare in foodstuffs and is rarer still in food liquids that require frequent rheological checking. Rotary viscometers should therefore be regarded as the effective minimum equipment requirement. This is particularly true for all fluids exhibiting non-Newtonian behaviour. Temperature control of the test sample is essential with fluid types. Launay and McKenna (1983) recommend a control limit of ±0.1 K. It should also be noted that viscous heating of the test sample at high shear rates can lead to significant underestimation of the viscosity.

There are many different instrument manufacturers producing both concentric cylinder and cone and plate viscometers. They will differ in their sensitivity, degree of variation of shear rate available, and consequently price. There is little published on comparative measurements using the various instruments available. However, the COST90 collaborative European study of food rheology as reported by Prentice and Huber (1983) is of interest in their presentation of results on the same samples from eight laboratories using many different viscometer types of different degrees of sophistication. On the test Newtonian liquids used, namely sucrose solutions, a cooking oil and mineral oils, agreement between the various instruments is remarkably close. For non-Newtonian liquids the agreement is not as good. Indeed, at low shear rates many of the flow curves diverged. Consequently, it is important to select an instrument with shear rates covering the range of interest. Launay and McKenna (1983) stress this, as even with the simplest of non-Newtonian models, the power law fluids, this behaviour may not be exhibited over the entire range of shear rates.

A similar caution should be noted with liquids exhibiting a yield stress. The report by Prentice and Huber (1983) on the COST90 study cites values for a chocolate melt using the different viscometers. The measured yield stress (found by extrapolating the stress/shear-rate curve to zero shear rate) varied by more than a factor of three between the lowest and highest values, with a similar variation in the constant k' of the Casson equation (Eqn 6.28). Only those instruments capable of applying very low shear rates gave good agreement between instruments. This again emphasises the importance of choosing an instrument with an appropriate shear rate range.

Other foods tested exhibited shear thinning together with a yield stress. This was particularly true for an apple sauce, which prompts the question of how to measure the rheological properties of such a suspension, which may be subject to settling or other separation during shearing in the viscometer. Launay and McKenna (1983) recommend that for such a material with time-dependent foods, care should be taken to ensure that standardised methods are employed, such as varying shear rate in the same manner in each test, allowing equilibrium to be established or standardising the reading time, and so on.

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7

In-line and on-line rheology measurement of food

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7.1 Introduction

The rheological characteristics of a food product influence many aspects of the fluid performance during processing (pumpability, droplet break-up in spray drying, emulsion formation, flow into molds, foamability, etc.) and of the finished product quality (texture, flavour release, stability, appearance). Knowledge of the rheological properties of materials during processing is important for process design, process control and optimisation, and the production of a consistent quality of product.

7.1.1 How do we currently measure?

Traditional off-line rheological measurements, from flow cups and Bostwick consistometers to computer-controlled capillary and controlled-rate viscometers, have in many cases adequately served the food industry for decades. What then, is the justification for moving away from these tried and tested techniques?

7.1.2 Where are we going and why?

The increasing competitiveness within the food industry has driven a conscious move from traditional factory Quality Assurance laboratories to rapid, real-time response on quality and process control which necessitates near-line or in-line measurement techniques. The incorporation of a relevant in-line measurement into the production control procedures, be it in a feed-forward or feed-back control loop, provides continuous monitoring of a key process parameter with the option of an automated action. Correctly governed this can provide enhanced process control, thus, consistent controlled product quality, thereby significantly reducing the incidence of rework or waste at the finished product stage. Thus, real-time response and process relevance are the primary justifications for using in-line rheometers and viscometers in food production.

7.1.3 In-line, on-line or near-line

Within this chapter the terms in-line, on-line and near-line are defined as follows:[1]

- An in-line measurement is performed directly in the process line.
- An on-line measurement is performed in a bypass loop from the main process line and may be returned to the main process line after measurement.
- A near-line measurement is performed on a sample taken from the process and the sample is discarded after measurement.

7.1.4 Why is it difficult to relate off-line measurements to the process?

The majority of food materials, being primarily suspensions, emulsions, gels, foams, or frequently a combination of these structures, display highly complex rheological behaviour. This behaviour encompasses time-, shear-, temperature-, and pressure-dependence, each of which severely restricts the the validity of off-line rheological measurements to provide information pertinent to the processing conditions. An off-line analysis provides a retrospective snapshot of the sample rheology, which may not reflect the sample behaviour a minute before or after the sampling time.

Meaningful interpretation of off-line analysis in terms of the process, becomes considerably complicated when dealing with complex food systems, for example:

- A 50% total solids milk concentrate will flow readily into a sample container from the process line, but frequently undergoes rapid gelation thereafter, even with controlled temperature, thus it is not tested under relevant process conditions.
- Chocolate displays marked dependence on shear history, requiring time-consuming sample preparation prior to measurement to iradicate the sampling and pre-shear conditions, thus the measurement is not real-time.
- Sampling an emulsion system through a valve can result in high shear exposure for the sample sometimes irreversibly altering its structure. Thus the sample properties measured off-line may be a consequence of the sampling and are not representative of the sample under processing conditions.

Although the process relevance of off-line measurements is often limited by the material and the type of process information sought, plus the often fragile heterogeneous samples, modern laboratory rheometry can provide access to a wide temperature and shear rate range, high pressure, dynamic low strain measurements, normal stress measurement, creep experiments and extensional rheology, therefore providing an in-depth characterisation of a food system. For research and finished product analysis modern laboratory rheometers offer an important source of information. However, to obtain real-time knowledge of fluid perfomance during processing, both in-line and on-line rheometry is extremely desirable.

7.2 Requirements of an in-line or on-line sensor

The food processing environment demands that a process sensor must satisfy the following requirements.

7.2.1 No hygiene risk

The sensor must clean-in-place using the standard process CIP procedures. Flow dead zones must be avoided and the construction material must be food grade. For food production the hygene criterion must be satisfied or the sensor should not be considered.

7.2.2 Physically robust and stable

The sensor must require little maintenance, operating at high temperatures, high pressures and providing a strong, reliable output signal, unaffected by plant noise (mechanical vibration or electrical interference). If a sensor with a low-level output signal is operated at a point distant from the control room, signal amplification should be close to the sensor to avoid signal loss due to electrical interference.

7.2.3 Simple to operate

The sensor should be operator independent, providing a readily interpretable output directly to the factory programmable logic controller (PLC).

7.2.4 Real-time

The measurement, signal processing and signal usage should be real-time. If a continuous process is operating at 10 000 kg h^{-1} then during every minute after a product is seen to be out of specification 167 kg of rework or waste are produced. For a batch process where, for example, viscosity is used as an indication of the onset of gelation, an entire batch may be lost if response time is too long. Thus, a measurement to signal usage time (i.e. corrective action) in the order of seconds is necessary to meet the requirement of real-time.

7.2.5 Sensitive, usable

The sensor must be sufficiently sensitive to provide a usable signal for process control, be it feed-forward or feed-back, thus satisfying the real-time measurement and usage critieria. Thus the sensor must be able reliably to detect incremental changes significantly smaller than those which require corrective action.

7.2.6 Non-destructive

The measurement should not in any way perturb the process or the quality of the product.

7.3 In-line rheometry

Over the last three decades an extensive literature has developed detailing the application of in-line rheometers within the chemical and polymer industries, yet scant information is available pertaining to the food industry.

Given the relatively recent awakening of the food industry to the possibilities of in-line process measurements, the paucity of reported applications probably finds its root cause in the high complexity of the food materials being processed and fairly reflects the low degree of usage of in-line rheological measurements.

Food materials are generally non-Newtonian in nature, that is, the viscosity is dependent on the shear rate. The majority of food materials, being shear-thinning, display three clear regions of behaviour (Fig. 7.1):

1. A low-shear Newtonian region, where the apparent viscosity (η_o) is quasi-independent of shear rate;
2. A shear-thinning region, where apparent viscosity decreases with increasing shear rate;
3. The high-shear Newtonian region, where apparent viscosity (η_∞) is quasi-independent of shear rate;

In its simplest form (a linear relationship between log viscosity and log shear rate, depicted by the dotted line on Fig. 7.1) the shear-thinning region may be represented by the power law model,[2]

$$n = K(\dot{\gamma})^{n-1} \qquad [7.1]$$

where n is the power law exponent ($n = 1$ for a Newtonian fluid, $n < 1$ for a shear-thinning fluid and $n > 1$ for shear-thickening behaviour), K is the consistency index and $\dot{\gamma}$ denotes the shear rate.

Steffe[3] lists power law indices for a range of food products from $n = 1$ for raw milks and creams, $n = 0.574$ for molten chocolate, $n \approx 0.5$–0.6 for mayonnaise, $n = 0.3$–0.4 for mustards, $n = 0.2$–0.3 for many fruit purees to $n < 0.1$ for apple pulp. The consistency index, K, has units which depend on the value of n (Pa s^n) and must be compared with caution.

In addition to the shear dependence of food materials, the force to be overcome to initiate viscous flow (yield stress) must be considered. Extensive debate[4]

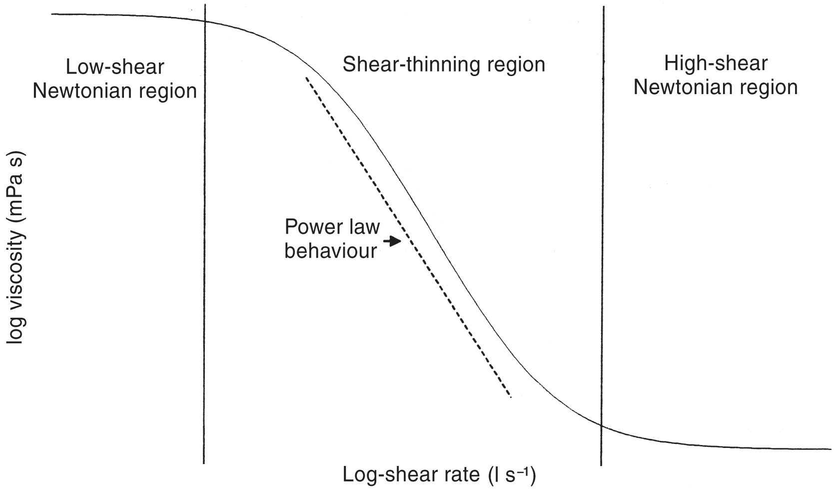

Fig. 7.1 Typical viscosity flow curves of shear-thinning food products.

concerning the existence of yield stress has pragmatically concluded that yield stress is an engineering reality and is a useful quantity for process design and product quality control. The consequences of overlooking yield stress in process design can result in dead zones during mixing or underspecified pumps unable to cope with start-up if a line stops when filled with product. Yield stress fluids commonly exhibit wall slip; that is, a generally low particle concentration fluid layer at the pipe wall which is not representative of the bulk material, and a plug-like unsheared region across the centre of the pipe. The shear is concentrated in this slip layer and a low viscosity is measured, which is not representative of bulk sample properties.

The model of Herschel-Buckley[5] (1926) shown in Fig. 7.2 conveniently represents the behaviour of many materials which, although displaying shear-thinning behaviour, require that a finite force be exceeded to induce flow, hence can be said to exhibit a yield stress (σ_y).

$$\sigma = \sigma_y + K\dot{\gamma}^n \qquad [7.2]$$

The selection of in-line viscometers is considerably influenced by the material properties under the shear rate of interest. If a particular process stage has a shear rate that falls in the high-shear Newtonian region, with viscosity quasi-independent of shear rate, a single point viscosity measurement may well be sufficient. If however, the area of interest lies within the shear-thinning region of the flow curve, viscosity data at several shear rates may be necessary. If the low-shear rate region is of interest, for example determining yield stress in chocolate, then a range of shear rates may be employed to approximate yield stress by extrapolation or a very low shear rate measurement must be designed.

Steffe[3] provides typical shear rates for a range of common processes from extrusion (10^0–10^3 s^{-1}) to mixing and stirring (10^1–10^3 s^{-1}), pipe flow (10^0–10^3 s^{-1})

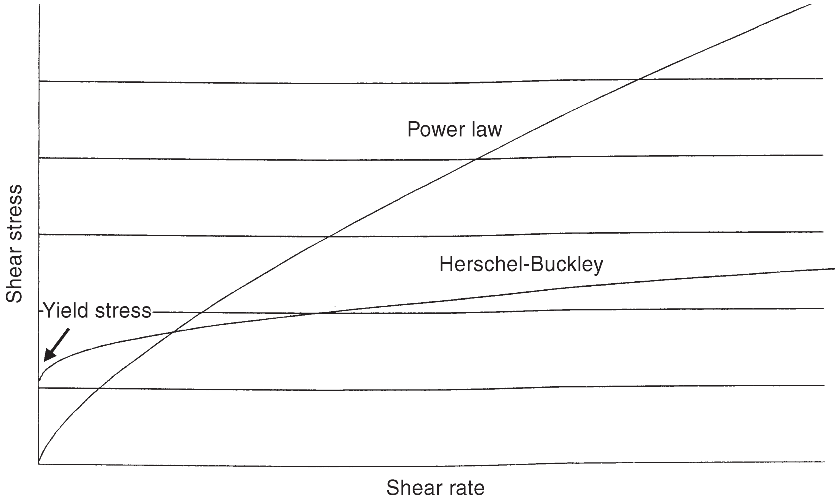

Fig. 7.2 Power law and Herschel-Buckley materials.

and spraying (10^3–10^5 s^{-1}). Thus an a priori basic knowledge of fluid mechanics, covering at least the shear rates encountered within the process, combined with a laboratory characterisation of the flow properties, is required to guide the selection of a relevant process measurement.

7.4 In-line viscosity measurements

The in-line characterisation of process fluids provides a complex and challenging area of research. Recent developments, however, provide some encouragement for the application of such instruments to food processing. A short review of the more common, commercially available in-line/on-line viscosity measurement techniques follows. This is sub-divided according to the principle of operation, into the following categories:

1. Near-line measurement techniques,
2. Capillary or tube viscometers, including slit viscometers and velocity profile measurement,
3. Rotational viscometers,
4. Vibrational viscometers.

7.4.1 Traditional near-line measurements

In addition and for completeness it is necessary to consider some of the less frequently employed in-line/near-line devices.

Efflux or flow cup

Typical near-line devices are efflux cup or nozzle viscometers, which provide rapid information, and are extremely robust and simple to operate. The flow time of a prescribed mass or volume through a nozzle or capillary is frequently sufficient for a rough viscosity assessment. For Newtonian fluids the flow time may be correlated to the kinematic viscosity.[6] When particulate matter is present blocking of the nozzle is an issue for particle diameters greater than 0.1 capillary/nozzle diameter. Owing to the extensional flow during contraction to a capillary or nozzle, the shear viscosity does not always dominate the measurement. Thus for systems such as textured yoghurts this can provide an extremely simple measure of extensional properties.

Bostwick consistometer

The operation of Bostwick consistometer-type equipment finds wide usage as a simple, robust and rapid, quality-control tool for purees, pastes and yoghurts. The distance that a sample flows under its own weight in a designated time (generally 30 s)[7,8] is measured. Investigations of the relationship between the Bostwick measurement and viscosity[9–11] suggested a limited correlation, but McCarthy and Seymour[10] provided an experimental and theoretical relationship between kinematic viscosity and extent of flow for Newtonian fluids.

Empirical devices

Computerised falling ball viscometers and bubble time viscometers can provide precision measurements of viscosity off-line for Newtonian fluids, whilst the falling piston viscometer can be employed in-line to give consistently reproducible data. Its applicability is limited to Newtonian fluids unless sophisticated displacement sensors are available. Float viscometers[6] are used throughout the chemical industry but require precise control of flow rate to enable a viscosity parameter to be determined. Again this may be practically achieved only for Newtonian fluids. In addition to these traditional robust techniques, rotational viscometers are becoming more and more common in the production environment for quality control of ingredients, and finished products as well as processing quality tests, e.g. after batch-mixing processes.

7.5 Capillary (or tube) viscometers

The simplicity of the capillary viscometer has resulted in its wide usage within the process industries[3,11–13] for applications as diverse as iron ore slurries,[14] foams,[15] distilled water and glycerol[16,17] polymer solutions.[18] In the food industry reported applications include starch solutions,[19] ice cream, milk and cream,[19] apple sauce and honey,[20] and recently pizza sauce viscosity.

7.5.1 Operating principle

The measured pressure drop resulting from fully developed laminar flow of a fluid through a straight section of tubing of a known length and at a known flow rate is employed to calculate viscosity.

As an on-line instrument the capillary viscometer is cheap, requiring only a reliable pressure drop measurement and a reliable flow rate measurement to provide valuable process information.

7.5.2 Restrictions

The use of pressure drop measurements requires a fully developed laminar flow (thus the Reynolds numbers < 2100). If entry effects are to be neglected the ratio of tube length to tube diameter must be large (>90)[3,21] and pressure transducers must be placed at least 10 pipe diameters from pipe contractions, expansions or elbows. In practice the pressure drop must be corrected for energy loss per unit mass in the fluid caused by wall friction, which is commonly expressed in terms of the Fanning and Moody friction factors.[22] If pressure drop is measured in a long straight pipe the influence on viscosity of temperature fluctuations must be accounted for.

7.5.3 Newtonian fluids

In the simplest case, that is for Newtonian fluids in fully developed laminar flow, the Poiseuille–Hagen equation may be employed to determine viscosity (μ) directly from the pressure drop (ΔP) created by a volumetric flow rate of liquid (Q), along a straight pipe of length (L), and radius (R), such that:

$$\mu = \frac{\pi(\Delta P)R^4}{8LQ} \tag{7.3}$$

and the velocity profile across the pipe (see Fig. 7.3), assuming no slip at the wall, hence velocity (u) is zero at $R = r$, is given by:

$$\mu = \frac{\Delta P}{4L\mu}(R^2 - r^2) \tag{7.4}$$

For a measurable pressure drop along a straight section of pipe the viscosity of a Newtonian fluid may be calculated using Eqn 7.4, thus providing a simple in-line viscometer, and as such it is highly recommended. However, for non-Newtonian fluids (such as represented in Fig. 7.1) the situation becomes more complicated.

7.5.4 Non-Newtonian fluids

Utilising the simplest case of the power law model (Eqn 7.1) the velocity profile of a non-Newtonian fluid is dependent on the shear-thinning behaviour indicated by the power law exponent n. As n decreases from 1 to 0 the velocity profile tends to 'flatten', with the fluid moving like a plug across the pipe radius, thus with near constant velocity.

Significant shear occurs only at the region close to the wall where a thin layer of material undergoes shear and at the wall the velocity reaches zero. This condition is represented by the graph $n = 0.2$ in Fig. 7.4.

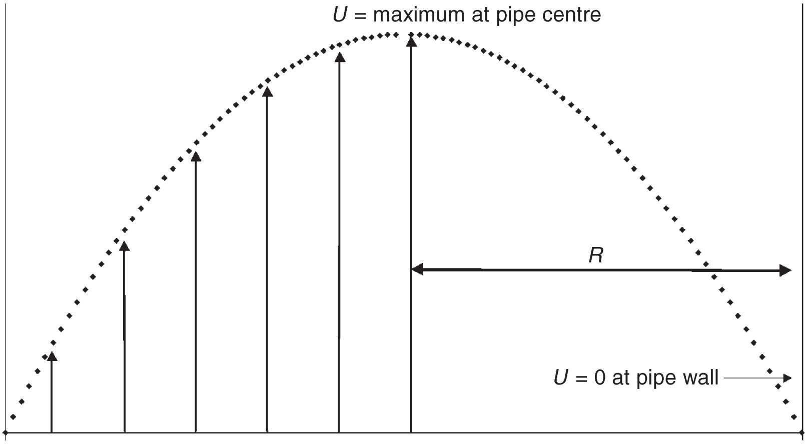

Fig. 7.3 Velocity profile of a Newtonian fluid flowing through a pipe of radius R, in fully developed laminar flow conditions.

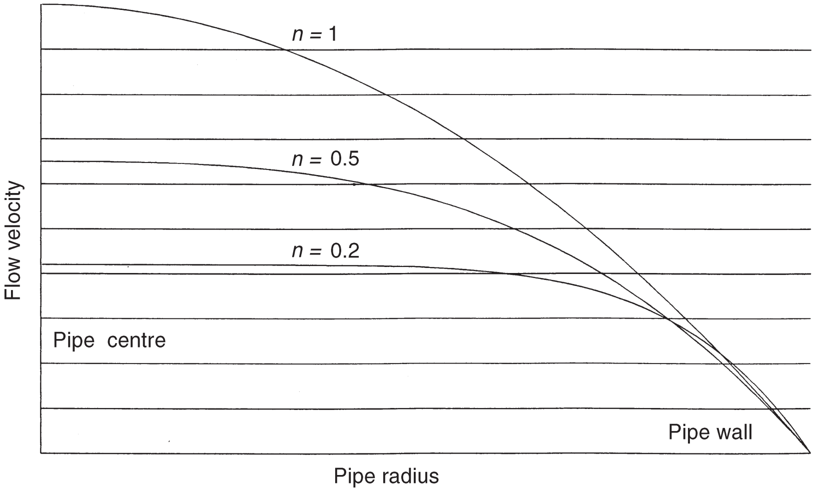

Fig. 7.4 Velocity profiles for shear-thinning fluids; comparison with the Newtonian case (n = 1).

For a power law fluid the velocity profile may be calculated assuming a no wall slip condition using Eqn 7.4:

$$U = \left[\frac{\Delta P}{2LK}\right]^{1/n}\left[\frac{n}{n+1}\right][a^{(n+1)/n} - r^{(n+1)/n}] \qquad [7.5]$$

Therefore if the power law index is known, the velocity profile in the pipe may be calculated. However, the key point of interest is to characterise the fluid behaviour in terms of a viscosity across a range of shear rates. The shear rate and shear stress information required to produce this flow curve are contained in the velocity profile and the pressure drop measurement when the flow rate is known. The shear rate is obtained by differentiating the velocity as a function of radial position. To access this data confidently the pressure drop measurement must be combined with techniques that provide real-time knowledge of the velocity profile in the pipe. Several candidate techniques are discussed in Sections 7.5.5 to 7.5.7. However, for non-slip conditions the pressure drop and flow rate data may provide the power law constants.[3,18] The gradient of natural logarithmic plots of apparent wall shear rate ($\dot{\gamma}_w$) against shear stress (σ_w) yields n' (for a power-law fluid $n' = n$) and the intercept gives k'. For a power law fluid a straight line is obtained. To produce this plot the measurements must be performed across a range of flow rates, thus invalidating this technique under normal processing conditions.

The wall shear rate in the tube is given by $32Q/\pi D^3$ and the shear stress by $D\Delta P/4L$, where D is the tube diameter, Q is the flow rate and ΔP the pressure drop. Thus:

$$n' = \frac{\mathrm{d}\ln(\sigma_w)}{\mathrm{d}\ln(\dot{\gamma}_w)} = n \qquad [7.6]$$

and

$$k' = K\left[\frac{3n+1}{4n}\right]^n \qquad [7.7]$$

Similar analyses may be performed for known rheological models (Casson, Bingham, Herschel-Buckley, etc.).[3]

Thus for fully developed laminar flow conditions, in the absence of wall slip, the power law parameters n and k may be determined directly from pressure drop and flow rate measurements. For a yield stress material no flow occurs for stress values lower than the yield stress, hence a plug develops across the pipe. Yield stress is commonly associated with wall slip thus significantly complicating the analysis of pressure drop data.

7.5.5 Magnetic resonance imaging (MRI)

By passing a non-metallic pipe through a nuclear magnetic resonance imaging system (similar to a medical body scanner) a velocity profile of the fluid within the pipe can be determined and employed to provide viscosity flow curves consistent with those obtained using conventional off-line rheometers. The measurement can be performed on time scales of seconds and is non-invasive and non-destructive. However, the time required to process the signal is significant hence the real-time requirements of Section 7.2.4 are not met. Wall slip may be investigated at the pipe wall and yield stress determined from the ‘zero-shear’ plug-like behaviour at the centre of the pipe.

MRI employs two magnetic field gradients. One in the direction of flow to measure the positions of protons at two different times and then, knowing time and displacement, velocity is calculated. The second field is applied perpendicular to the flow and provides the spatial location of the displacement as a function of tube radius. Pulsed gradient spin echo (PGSE) techniques spatially resolve fluid displacements.

In addition to the determination of viscosity, additional compositional and structural quality-related attributes may be derived from the signal. Using ceramic screws MRI is used to image flow profiles in extruders[23,24] and in complex flow profiles such as converging flows and conventional rheometrical cone and plate measurement geometries.[25] Currently MRI is a valuable research tool, but the cost and portability of the equipment severely limit its application as a routine process measurement technique.

7.5.6 Ultrasonic velocity profiling (UVP)

The velocity profile is determined from ultrasonic pulsed echo Doppler measurements.[26–29] An ultrasonic impulse of known frequency is emitted into the fluid flow and reflects from a moving body (particle or droplet) within that flow field. Owing to the Doppler effect the frequency of the reflected pulse is shifted in proportion to the fluid velocity. From the time between sending and receiving the pulse, and knowledge of the speed of sound in the fluid, the radial spatial position of the particle within the flow may be determined. The technique relies on the presence of reflective solid surfaces within the fluid and is thus suited to food suspensions such as chocolate,[29] or starch, with a particularly interesting application to fat crystallisation processes. It is not suitable for fluids with entrapped gas bubbles. Again the spatial resolution can be sufficient to provide information pertaining to yield stress, but wall slip measurement can be hampered by the radial resolution which gives a first point approximately 2 mm from the wall. The technique has been explored to map converging flow fields.[29]

Instruments including user friendly software are expected to become commercially and although intolerant of gas bubbles, it shows considerable promise for many food systems. Currently the actual measurement time scales are short, but data processing time is an issue if the real-time measurement criteria of Section 7.2.4 are to be met.

7.5.7 Laser Doppler anemometry

The employment of laser Doppler anemometry (LDA) to research flowstreams of air and water[30] now spans three decades, but only recently have its applications to food been explored.[31,32] LDA has been successfully employed to measure velocity profiles in twin screw extruders,[33–35] and for shear rate distributions during dough mixing.[36,37] In the latter a Brabender Farinograph was used as a model mixing system and the velocity profile and hence shear rate distributions measured using LDA. Corn syrup and Carbopol were employed as model transluscent materials to replace turbid dough mixtures. The application of LDA has clear application to improve understanding and subsequent design of processing stages via non-invasive measurement of fluid velocities.

7.5.8 Slit die viscometers

A slit viscometer may be represented as flow between two parallel square plates (a squared orifice) of a width which, if edge effects are to be neglected is at least ten times greater than the plate seperation. The viscosity is determined from the pressure drop across the slit for a known flow rate. To eliminate entry and exit effects the length of the slit should be as great as possible. Wall-flush pressure transducers distributed along the length of the slit should measure a constant pressure drop per unit length for the required fully developed flow conditions. The Rabinowitsch–Mooney equation is employed for non-Newtonian fluids.[3] Many multi-phase food systems exhibit wall slip which manifests itself in a lubrication fluid layer under high shear at the wall and a low sheared region towards the centre of the die. Thus the measurement may be dominated by the slip layer and does not reflect the bulk properties of the material.

Slit viscometers are frequently employed when considering extrusion rheology.[38,39] They are readily fitted to the outlet of the extruder and provide a continuous measure of rheological properties. However, the variable output flow rates often associated with food extruders severely complicate data interpretation and with the extruder outlet being the point of product forming in many cases, the slit die cannot be used as a continuous rheology measurement during processing. In addition, the physical requirements of the device (small gap, large length) will increase the pressure in the extruder, potentially changing the nature and structure of the material. Considerable literature details the studies of rice starch, dough and corn meal rheology at the extruder outlet as a function of processing parameters, such as temperature, die temperature, screw speed and mass flow rate, as well as moisture content and enzyme addition.[40–46] Benfer and Onken[47] describe a routine process control slit viscometer for continuous monitoring of fermentation broth viscosity which was then related to biomass concentration. Ouriev,[29] in combination with UVP, continuously monitored chocolate mass at the exit of a temperer as an in-line control of viscosity consistency after crystallisation.

The procedure for determining power law parameters from pressure drop and flow rate are the same as those described in Section 7.5.3, but geometrical considerations require that wall shear rate is given by $6Q/wh^2$ and the shear stress by $\Delta Ph/2L$, where w, h and L are the width, height and length of the slit, respectively.

7.6 Rotational viscometers

7.6.1 Operating principle

As with standard laboratory rotational viscometers, the torque resulting from the rotation of a spindle at a known rate of rotation when immersed in a fluid is measured. For defined measuring geometries the shear stress and shear rate may be determined and employed to calculate the viscosity. For systems with variable speed motors a range of shear rates may be accessed to provide on-line flow curve measurements. Rotational process viscometers are usually mounted in a bypass loop and thus are considered as on-line.

7.6.2 Description

In-line and on-line rotational viscometers are produced in various guises with parallel plate[48] and concentric cylinder[49] geometries available, however, the most widely used are concentric cylinder or bob-in-cup-type geometries. As for off-line rotational viscometers the fluid is sheared between two concentric cylinders (see Fig. 7.5). The conversion of the torque and rotation rate to viscosity assumes that the flow is laminar and that there is no secondary flow. Secondary flows will occur at high rotation rates with low-viscosity fluids.

From the torque, M, at the surface of the rotating inner cylinder the shear stress, σ, may be calculated:

$$\sigma = \frac{M}{2\pi a^2 L}$$

and for a Newtonian fluid the shear rate is determined as:

$$\dot{\gamma} = \frac{2\Omega}{[1 - a/A]}$$

and the ratio of shear stress to shear rate yields the viscosity.

When dealing with non-Newtonian fluids the situation is complicated. The shear rate across the gap can be approximated as constant for $a/A > 0.97$.[2] To maintain this condition a small gap is necessary, which in practice may lead to blockages and cleaning problems. The shear rate can be estimated as:

$$\dot{\gamma} = \frac{\Omega a}{[A - a]}$$

but, if the relationship between shear rate and viscosity is known, for example, to fit the power law model then the shear rate is determined as follows:

$$\dot{\gamma} = \frac{2\Omega}{n[1 - (a/A)^{2/n}]}$$

However this demands an a priori knowledge of material behaviour.

A potential problem of in-line rotational viscometers is sensitivity. The reservoir must be sealed to avoid hygene risk, but the friction on the inner rotating cylinder caused by this seal can reduce the sensitivity of the measurement particularly for low-viscosity fluids. A second consideration must be the influence of entry flow to the reservoir on the torque response and the flow field. Nonetheless, with knowledge of these limitations, rotational instruments can provide valuable information about fluid process viscosity.

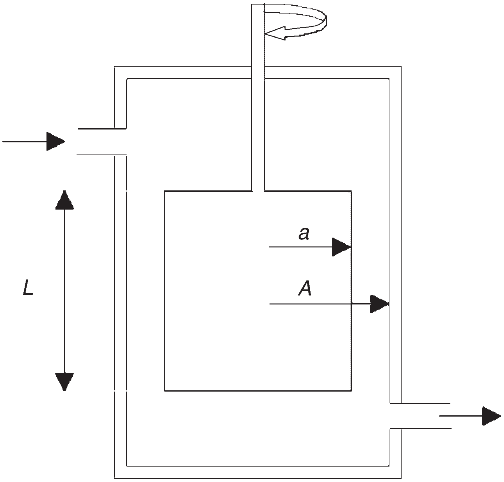

Fig. 7.5 Concentric cylinder rotational viscometer.

7.6.3 Applications

A range of home-made rotational viscometers are reported in the literature from companies as diverse as de Beers Diamond Research Laboratory who developed the Debex single bobbin in-line viscometer for monitoring mining slurries,[50] to the so-called Mars home-made viscometer, developed within the confectionery division for chocolate viscosity control and used since 1966. These two instruments have one common goal, the continuous in-line monitoring of process viscosity for non-Newtonian suspensions. The Mars viscometer is employed for process control during cocoa butter addition to chocolate and as a guide to plant operators who are equipped with information on how to adjust the viscosity until it is in the specified acceptance range.[51] The instrument has a rotating inner cylinder and fixed outer cylinder and operates at a shear rate 49 s^{-1}, for in-house historical reasons. The system is water jacketed to control temperature at 46 °C and operates in a bypass loop with a constant flow rate of 5.46 kg min^{-1}. The instrument is said to be vibration stable, free of hygene problems and undergoes weekly control. Varying the shear rate, and hence the rotation rate of the inner bob, would provide a more complete characterisation of chocolate behaviour with the possibility of obtaining a low shear rate measurement from which a yield stress measurement may be inferred.

Perhaps the most commonly employed rotational process viscometers are those manufactured by Brookfield which successfully provide rapid on-line data for a range of industrial applications. The STT-100 hygienic model (Fig. 7.6) is aimed at food production and the company application lists suggest a range of food installations covering sauces, purees, chocolate, starches and gelling agents.

Kawatra and Bakshi[49] compared results obtained for a Brookfield viscometer

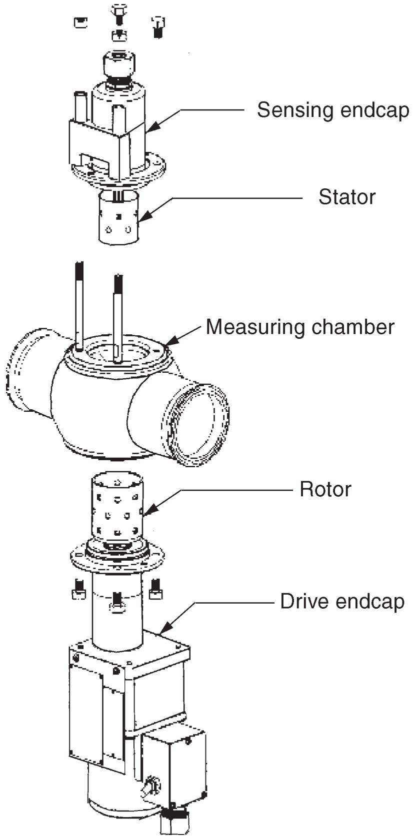

Fig. 7.6 Brookfield STT-100 in-line viscometer. Reproduced with kind permission of R. Bates, Brookfield Engineering, USA.

with co-axial cylinders and a Nametre corporation vibrating sphere viscometer. Good agreement was reported for the Newtonian fluids and solids suspensions employed. Potential was identified for the use of vibrational viscometers as a routine plant monitoring device (discussed in the next section).

7.7 Vibrational viscometers

7.7.1 Operating principle

The operating principle is the measurement of the amplitude, or phase shift, of oscillation of a probe immersed in a fluid.

7.7.2 Description

Vibrational viscometers are generally high-frequency (10^2–10^6 Hz) instruments, which undergo damping due to the fluid as it flows past a probe. They operate as surface loading instruments, that is, there is no reflection of waves from vessel walls and the propagated waves attenuate rapidly in the fluid layer surrounding the probe surface. In itself, this can present problems if density gradients or fouling occurs on the probe surface, as this will prevent bulk viscosity measurement. A metal probe is driven in torsional oscillation at its resonant frequency. For a high Q (low damping) resonator the resonant bandwidth is narrow providing a natural filter to normal plant vibrations. The presence of the fluid surrounding the probe damps this response either by simple analysis of the displacement, or by analysing the frequency difference in response to a given phase shift between excitation force and velocity near the resonant frequency.[52]

Vibrational viscometers are generally robust, simple to install, do not require additional flow loops or pumps and provide continuous real-time data (updated every 1–10 s). They are relatively small and can be installed directly in the main process line or in mixing vessels, holding tanks or fermenters. They offer negligible disruption to the process, they can generally be cleaned in place, can be manufactured from food-grade materials, and can be tolerant of extreme temperatures and pressures.

High-frequency oscillating probes provide opportunities to measure very low viscosity fluids, indeed using in-line viscometers developed at ETH Zürich, heating and cooling viscosity curves for water have been measured and agree well with physical data tables. In addition these instruments are sensitive to small changes in total solids and can provide repeatable viscosity solids loading curves consistent with standard laboratory data. The rapid measurement provides real process monitoring opportunities and even a single point measurement may be employed to indicate process instability or changes in product consistency. However, the presence of gas bubbles on the measuring surface has been found to increase markedly the viscosity derived from the damping. The fluid boundary layer surrounding the probe requires adequate renewal for real-time representative measurements and the relationship between the boundary layer properties and the bulk rheological properties of a multi-phase system may be difficult to interpret.

Efforts to derive fluid elasticity from the shift in the resonant frequency of the probe are complicated by the temperature dependence of the resonant response of steel. A precise correction for the influence of temperature is thus required to derive elastic parameters with these instruments. These are generally single point measurement devices and hence the information available is limited. However, where only a high shear parameter is required, i.e. a value in the upper Newtonian region, or if the change in material properties owing to sedimentation, phase seperation or gelation in a storage vessel or fermenter are required, this may be adequate.

A considerable problem with high-frequency measurements is relating the results to the more familiar off-line viscosity results. For Newtonian fluids this does not present a problem, but for non-Newtonian fluids where the shear rate is critical, understanding the relationship between a measurement at high frequency

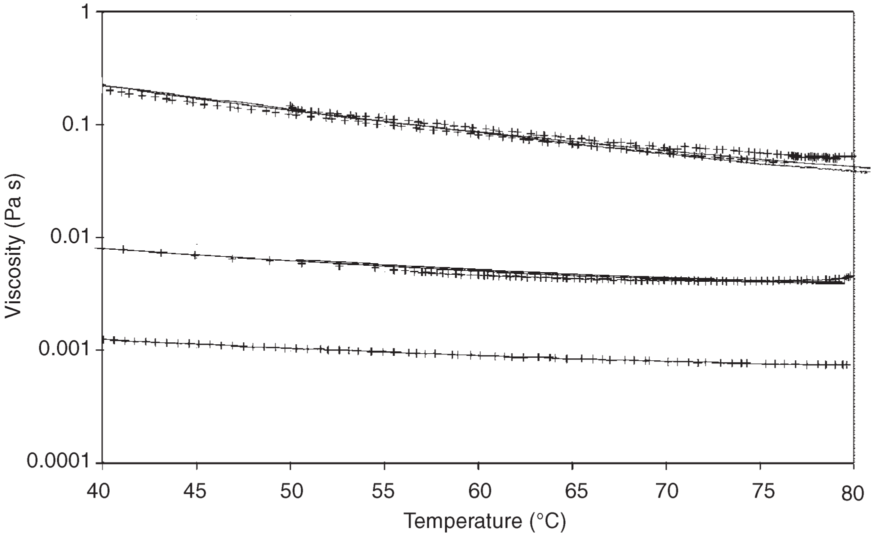

Fig. 7.7 Comparison of in-line Hydramotion PXL/7viscosity (continuous trace) with off-line Haake RS150 controlled stress rheometer at 300 s^{-1} (crosses) for a food suspension.

of a narrow fluid layer and what is measured off-line is difficult. At high shear rates suspension and emulsion behaviour is dominated by the continuous phase and this is frequently Newtonian. The high-frequency measurement corresponds to the off-line measurement at high shear (Fig. 7.7). The same in-line instrument was employed for each measurement for solids content from 12 to 55% with a viscosity range of 0.8 to 200 mPa s. A selection of the commercially available instruments are mentioned below.

Nametre vibrating sphere viscometer

The Nametre vibrating sphere viscometer now finds wide usage throughout the process industry.[53–55] The power required to maintain a constant vibration amplitude in the presence of a fluid is related to a product of the density and the viscosity. Generally the viscosity change caused by composition is considerably greater than the change in density. The findings of Kawatra and Bakshi suggested that the instrument was tolerant to the presence of suspended particles (up to 70% wt/wt) and gives a rapid and reproducible measurement, which correctly reflects the process behaviour, therefore identifying a potential for process control. The operating range for the sphere is listed in the specification as 10.0 mPa s to 100 Pa s, although the range may be extended up to 1000 Pa s and as low as 0.1 mPa s by employing the other four instruments in their range.

ETH Zürich resonating tube viscometer

Developed at the Institute for Mechanics, ETH Zürich, the resonating tube viscometer offers considerable potential for food systems. The flow-through oscillating tube viscometer employs a pipe section as the resonating measurement surface, thus presenting a conventional pipe surface to the measured fluid. In this sense it is totally non-invasive and attractive for food process monitoring. The tube and probe versions of this instrument operate with patented phase-locked-loop control to maintain precisely the relative phase of the resonator providing an optimal frequency resolution of 0.01 Hz for a resonant frequency in the kHz range. Instruments have been tested on materials ranging from water to bitumen with good results and are now undergoing commercialisation. Comparison of the in-line measurements with off-line T-bar Brookfield viscometer measurements reveal a good correlation for complex food emulsions (Fig. 7.8). This provides a reference between existing routine measurements and the in-line device and thus facilitates the changeover from off-line to in-line measurement.

Solartron Type 7827 viscometer

A combined instrument for the determination of density, viscosity and temperature the Solartron Type 7827 has been developed by Schlumberger. Presently process data is not available, but the manufacturers suggest the food and drink industry as a primary target market.

Hydramotion in-line viscometers

This range of instruments has found wide application in process industries and offers potential for food systems (see Fig. 7.7). The instruments are extremely robust and simple to install, require little or no maintenance, and give rapid data feedback to a control unit or into the process control processor. The product range provides for extreme temperatures (–40 to 400 °C) and pressures (300 bar) exceeding those in food production and viscosity ranges from 0.1 mPa s to 1000 000 Pa s.

7.8 High-frequency rheometry

Wave propagation and ultrasonic measurement provide considerable promise for in-line measurement of rheological properties. Two wave propagation techniques, the so-called pulse-resonance rheometer[56] and the virtual gap rheometer,[57,58] exploit wave velocity and attenuation in a viscoelastic material. They offer considerable promise as in-line devices particularly for gel matrices and for monitoring texture development.

Ultrasonic waves have been exploited to determine physical properties in food systems and rheological characteristics in the polymer industry.[59] These two types of measurement, being non-destructive, are ideally suited to food process measurement. Additionally, they offer the opportunity to determine viscoelastic properties of food systems directly in-line and with the aid of frequency multiplexing structural information across a wide range of frequencies may be accessed in real-time.

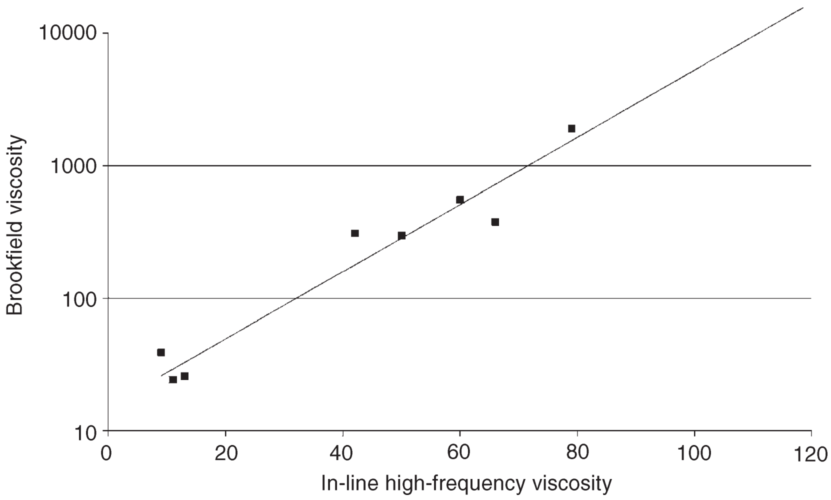

Fig. 7.8 In-line viscosity ETH against off-line Brookfield rotational viscosity (T-Bar) for a concentrated non-Newtonian food emulsion.

7.9 Summary

The author makes no pretence that the techniques discussed herein represent the complete spectrum of available in-line rheological techniques, but offers an introduction to an established area which has been growing rapidly. The current interest and developments in in-line rheometry suggest that it is entirely feasible to envisage a completely different chapter in the very near future. Advances in ultrasonic measurements, microsensors and imaging techniques will surely provide the future of in-line process measurement.

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Part II

Product development

8

Engineering food emulsions

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8.1 Introduction

Fine-disperse emulsions can be produced in different ways as shown in Fig. 8.1. Mechanical processes are those most frequently applied and have been the subject of a publication (Schubert, 1998) which also provides a list of essential references. Rotor-stator systems with a variety of uses are capable of producing emulsions both continuously and discontinuously. High-pressure systems, frequently referred to as high-pressure homogenizers, are used to continuously produce fine-disperse emulsions. Here, mechanical energy which is essential for droplet disruption, in all mechanical emulsification processes, is applied as pressure difference. A simple aperture shown in Fig. 8.1 has been found to be very efficient (Stang, 1998). Results obtained so far from a current research project in which the aperture has been modified and optimized have shown this system to be very promising. Fine-disperse emulsions can also be produced by ultrasound. Fundamental studies on the use of ultrasound in continuous emulsifying processes are available (Behrend *et al.*, 2000; Behrend and Schubert, 2001). These papers also provide further references. The method is mainly employed in laboratories and less on an industrial scale.

Besides the processes mentioned above which are based on droplet disruption, emulsions may also be produced by membranes (see Fig. 8.1). In this case, the disperse phase is forced through the micropores of a membrane. The droplets formed at the pore outlets are detached by the continuous phase passing by. The method developed by Nakashima and Shimizu (1991) in Japan offers a great many possibilities for gently producing very fine droplets and narrow droplet-size

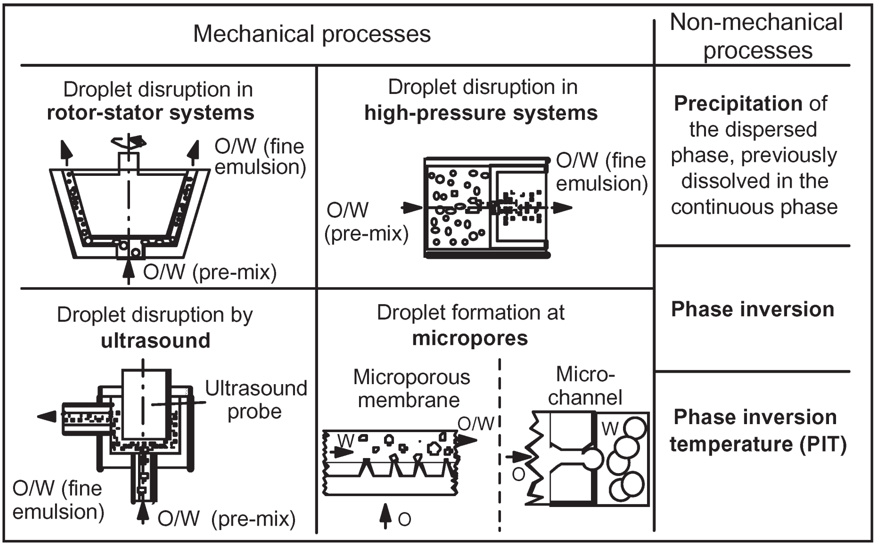

Fig. 8.1 Schematic of the systems producing fine-disperse emulsions.

distributions. Studies have shown that pre-mixes forced through membranes are a means of achieving high throughputs, effecting phase inversions and producing multiple emulsions (Suzuki, 2000; Altenbach-Rehm *et al.*, 2002). Membrane emulsification has been a subject of intense studies at the authors' institute (Schröder, 1999; Schröder and Schubert, 1999; Vladisavljevic *et al.*, 2001). Microchannel emulsification is a novel process that produces monodisperse emulsions (Nakajima, 2000; Nakajima and Kobajyashi, 2001).

Apart from high-pressure and membrane or microchannel emulsification, emulsions may either be produced batchwise or continuously. In the case of continuous emulsification, ingredients are usually dosed separately and pre-mixed in a stirrer; the coarse-disperse raw emulsion is then fed into the droplet disruption machine for fine emulsification. The energy required for the raw emulsion is negligible compared with that required for fine emulsification. Continuous processes have better utilization of energy, but discontinuous processes can usually produce a narrower distribution of droplet sizes.

There are also several non-mechanical emulsifying processes applied to produce specific products, for example, in the chemical industry. One of these is characterized by precipitation of the disperse phase which was previously dissolved in the external phase. Changes in the phase behaviour of substances to be emulsified prompted by variation of temperature or composition or by mechanical stress are used to achieve the desirable state of the system. Another process of interest is the phase-inversion temperature (PIT) method, reported upon in an earlier paper, which also refers to the pertinent literature (see Schubert, 1998).

Emulsions are most frequently produced in a continuous mode by droplet disruption. The principles that prevail differ depending on the type of mechanism

Table 8.1 Maximum droplet size x_{max} in continuous emulsification for various types of mechanical stress, modified according to Walstra and Smulders (1998). For explanations, see text. A_i = constant value, η_d and η_c = viscosity of the disperse and continuous phase, respectively, ρ_c = density of the continuous phase, γ_L = interface tension

	Turbulent inertial forces	Turbulent shear forces	Laminar shear or elongational forces
Re_{flow}	> 2000	> 2000	< 1000
$Re_{droplet}$	> 1	< 1	< 1
Rotor-stator mill, ultrasound homogenizer	$x_{max} \propto P_v^{-0.4}$ $x_{max} \propto t^{-0.3*}$ $x_{max} = A_1 \cdot E_v^{-0.35**}$	$x_{max} \propto P_v^{-0.5}$ $x_{max} \propto t^{-0.3*}$ $x_{max} = A_2 \cdot E_v^{-0.4**}$	$x_{max} = A_3 \cdot E_v^{-1}$
High-pressure homogenizer	$x_{max} = A_4 \cdot E_v^{-0.6}$	$x_{max} = A_5 \cdot E_v^{-0.75}$	$x_{max} = A_6 \cdot E_v^{-0.1}$

$$\text{In general:} \quad x_{3,2} = A_i \cdot E_v^{-b}$$

$x_{3,2}$ = Sauter diameter (µm)
$E_v = P/V$ = energy density (J m^{-3})
$A_i = f(\eta_d, \eta_d/\eta_c, \rho_c, \gamma_L, \ldots)$
* Experimentally determined exponent.
** Approximated exponent.

used for droplet disruption. Fundamental work in this field has been done by Walstra and Smulders (1998). Table 8.1 shows the maximum droplet size x_{max} appearing under different types of stress in turbulent and laminar flow under the influence of inertia, shear or expansion forces. The table differentiates rotor-stator systems, ultrasound systems and high-pressure homogenizers, and also shows the different Reynold numbers, Re_{flow} of the continuous phase and $Re_{droplet}$ of the flow around droplets. In $Re_{droplet}$, the droplet diameter is the characteristic length. It becomes obvious that maximum droplet size depends on both power density P_v and residence time t. The concept of energy density (see Karbstein, 1994; Schubert, 1998) is based on these functions. Experiments have shown that a simple relationship exists between maximum droplet size and the Sauter diameter $x_{3,2}$ (mean droplet size of the droplet collective) (Armbruster, 1990). Maximum droplet size shown in Table 8.1 may hence be replaced by the Sauter diameter, if the respective proportionality factor is adequately adjusted. In high-pressure homogenizers, the Sauter diameter is a direct function of energy density resulting from energy input related to the volume flow of the emulsion. In rotor-stator systems and ultrasound homogenizers, Sauter diameter and energy density are only linked by approximation on the basis of experimental data, as noted in Table 8.1.

8.2 Energy density and emulsion formation

In general, the Sauter diameter in continuous emulsification with droplet disruption may be described by the relation:

$$x_{3,2} = a_i \cdot E_V^{-b} \tag{8.1}$$

As mentioned above (see Schubert, 1998), some secondary conditions must be fulfilled to make sure this relation applies. Attacking forces, first of all, must exceed a critical threshold above which droplets are disrupted. The time, furthermore, allowed for droplet deformation must exceed a critical deformation time in order for droplets to become deformed to such extent that they break up. This means that a critical power density P_v must be exceeded to disrupt droplets.

The concept of energy density for emulsions according to Eqn 8.1, developed at the author's institute (Schubert and Karbstein, 1994), has been found to be a useful approximation in practice (Karbstein, 1994). Energy density:

$$E_V = \frac{P}{\dot{V}} \tag{8.2}$$

is easy to measure because power input P into a machine and volume flow $\dot{V}$ of the emulsion are easy to measure. The concept of energy density may also be applied to compare different mechanical emulsifying processes. A comparison of this kind is shown in Fig. 8.2. In membrane emulsification, mean droplet sizes (Sauter diameter) depend not only on energy density, but also on volume proportion φ of the disperse phase (Schröder, 1999). The comparison illustrates that, given equal energy densities, different emulsifying equipment produces very different droplet sizes. In the present comparison, droplet coalescence, which is minimized by an adequate selection of emulsifiers, has been neglected. Droplet coalescence during emulsification will be discussed in the following section.

8.3 Emulsion formation, microstructure and properties

The results presented so far provide a basis for product design and formulation of emulsions. Product design in this context means creating a product with certain desirable properties which are adjusted by engineering methods. As it is very expensive to vary all essential parameters of a technical apparatus in order to achieve the desirable property of a product, an interim attribute is used. In the case of emulsions, the emulsion microstructure is taken as interim attribute which is mainly determined by mean droplet size, droplet size distribution and type of emulsifier. In many instances it may be sufficient simply to characterize the microstructure by the mean droplet size. A physical value is obtained which is easy to measure and to adjust by means of emulsifying equipment.

The link between the emulsion's microstructure and the process or emulsifying apparatus, respectively, is called process function. In the present simplified case, the process function is described by Eqn 8.1. Mean droplet size, accordingly,

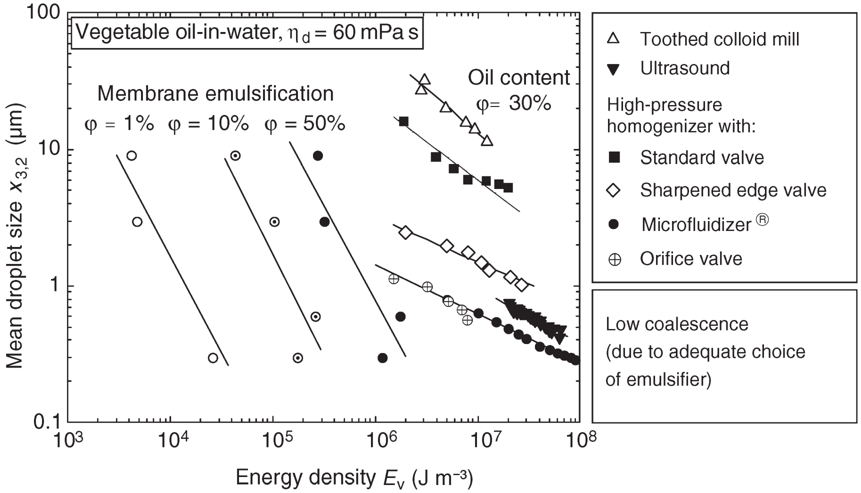

Fig. 8.2 Comparison of various emulsifying processes based on the energy-density concept.

depends on the energy density which, given a certain apparatus, can either be determined or taken from Fig. 8.2. Linking microstructure and emulsion properties has been found to be much more difficult. This link, according to Rumpf (1967), has been called property function. In many cases it is difficult to define the property function, i.e. to link product properties and physical characteristics (microstructure). In the majority of cases, the property function, therefore, is far from being a simple equation; it rather stands for a complex connection between properties and microstructure. Figure 8.3 illustrates the links between emulsion properties, microstructure and process. The concept of product design and formulation is being further advanced at present. In the case of emulsions, the process function provides a useful approximation while property functions, in the majority of cases, remain to be developed (see Schubert, 1998). If in the present case of carotenoid formulations bioavailability were merely a function of droplet size, it would be easy to derive a property function. Process and property function would then provide the possibility of achieving the desirable properties by the process selected, i.e. to produce emulsions as desired. Current investigations will show whether, and to what extent, this is practicable.

8.4 Emulsion stability

The physical stability of emulsions during and immediately after emulsification presents special problems. We have called this state short-term stability. It is indicative of whether the droplets of an emulsion are protected against coalescence during or immediately following emulsification. Short-term stability decides the success or failure of the emulsifying process. Figure 8.4 demonstrates that

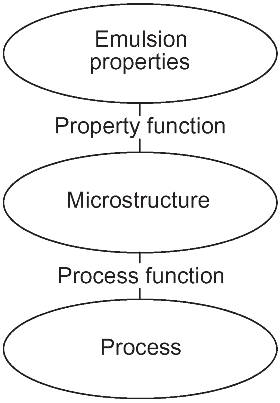

Fig. 8.3 Property and process function exemplified by emulsions.

mechanical emulsifying processes are expected not only to reduce size, but also to stabilize droplets immediately. If droplets of the raw emulsion are deformed and disrupted by a high mechanical energy input, two extreme cases are conceivable. Droplets successfully protected against coalescence remain small, producing the desirable fine-disperse emulsion. If coalescence is not prevented, droplets will flow together and form larger droplets. In a critical case, this could completely negate the size reduction. In unfavourable cases, emulsions may even break. Avoiding droplet coalescence during and immediately following the formation of new droplets, i.e. achieving sufficient short-term stability, is therefore of utmost importance for any emulsifying process.

In many cases, a satisfactory short-term stability is achieved by suitable emulsifiers. Using a novel method (Danner, 2001), droplet coalescence during emulsification can be detected and described in terms of quantity by a stochastic model. The method, furthermore, is also a practice-oriented test to judge the stabilizing efficiency of different emulsifiers and stabilizers (Danner, 2001).

8.5 Emulsion formulation in practice: a case study

By adding carotenoids to the fine-disperse oil droplets of an oil/water emulsion, it is possible to produce a formulation in which carotenoids are not only readily bioavailable, but also present in a water-dispersible form which is easy to handle (Ax *et al.*, 2001). Carotenoid is expected to be dissolved in the disperse oil droplets. For the following formulations, the carotenoids astaxanthin and lycopene were used. Astaxanthin is a polar carotenoid present in salmon, for example; it is employed as a feed additive. Lycopene is a non-polar, acyclic carotenoid mainly present in tomatoes and tomato products. Epidemiological studies give reason to assume that a high consumption of tomatoes is associated with a lower risk of cancer (Giovannucci, 1999). Both astaxanthin and lycopene in particular have

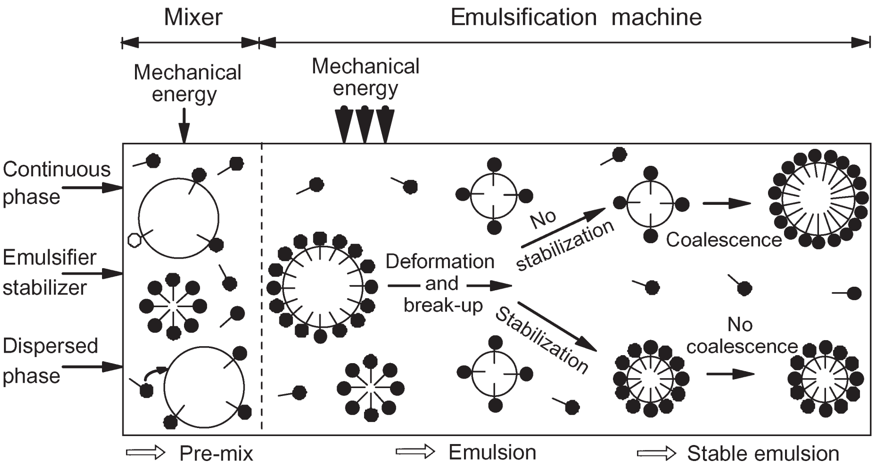

Fig. 8.4 Size reduction of droplets and extreme cases of droplet stabilization in a mechanical emulsifying process.

been found to show greater reactive singlet oxygen quenching ability than β-carotene (Hiramaya *et al.*, 1994; Stahl and Sies, 1996; Baltschun *et al.*, 1997; Di Mascio *et al.*, 1989). The chemical structures of the two compounds are shown in Fig. 8.5.

8.5.1 Solubility of carotenoids

Carotenoids are insoluble in water. Solubility in vegetable oil at room temperature is usually below 1 g l^{-1} and depends on the presence of polar groups in the carotenoid and in the fatty acid pattern of the vegetable oil (Bauernfeind *et al.*, 1958; Borel *et al.*, 1996). Because of their poor solubility at room temperature, carotenoids are dissolved in hot vegetable oil to add them to the oil/water emulsion. As carotenoids, because of their molecular structure, are susceptible to heat, the time of of exposure

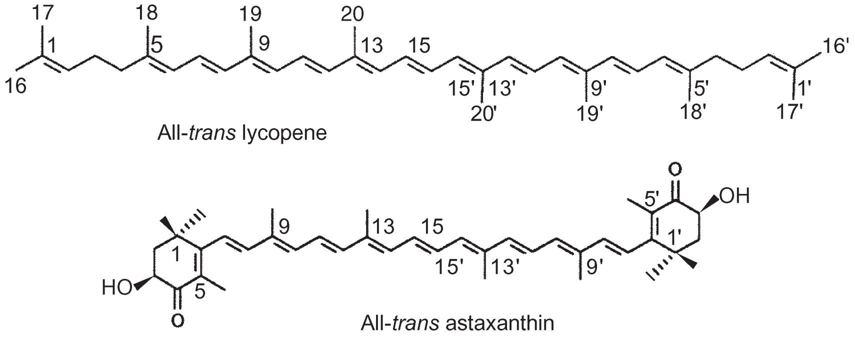

Fig. 8.5 Structure of the all-*trans* isomers of lycopene and astaxanthin.

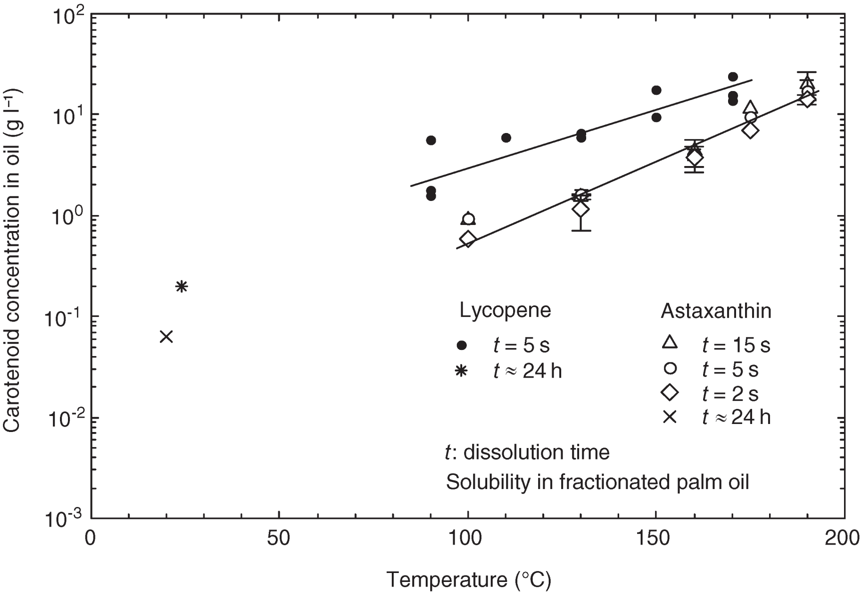

Fig. 8.6 Solubility of lycopene and astaxanthin in oil at different temperatures.

to high temperatures should be limited. Figure 8.6 shows the concentration of the carotenoids astaxanthin and lycopene in vegetable oil at different temperatures. The carotenoid concentrations increase with increasing oil temperature. As carotenoids degrade at high temperatures, no extended dissolution times to determine equilibrium solubility could be applied. In order to achieve approximately maximum carotenoid concentrations after only short periods of time, an excess amount of crystalline carotenoids was added to the hot oil. As different dissolution times led to similar concentrations of dissolved carotenoids in the vegetable oil, the excess of crystalline carotenoids was obviously large enough to achieve equilibrium concentration. The results shown in Fig. 8.6 demonstrate that carotenoid concentrations of 3 g l^{-1} require oil temperatures of at least 100 °C for lycopene and at least 140 °C for astaxanthin. In the experiments presented here, higher temperatures were employed to make sure the carotenoid crystals had dissolved completely.

8.5.2 Preparation of oil/water emulsions containing carotenoids in supersaturated solution

The procedure to produce carotenoid-containing emulsions is schematically shown in Fig. 8.7. The principle selected relies on a method patented by BASF AG (Schweikert and Kolter, 1997). The carotenoids were suspended in fractionated palm oil primarily containing triglycerides with medium-chain fatty acids (Bergabest MCT, Schumann & Sohn, Karlsruhe). The oil content of the emulsion was 20 wt%.

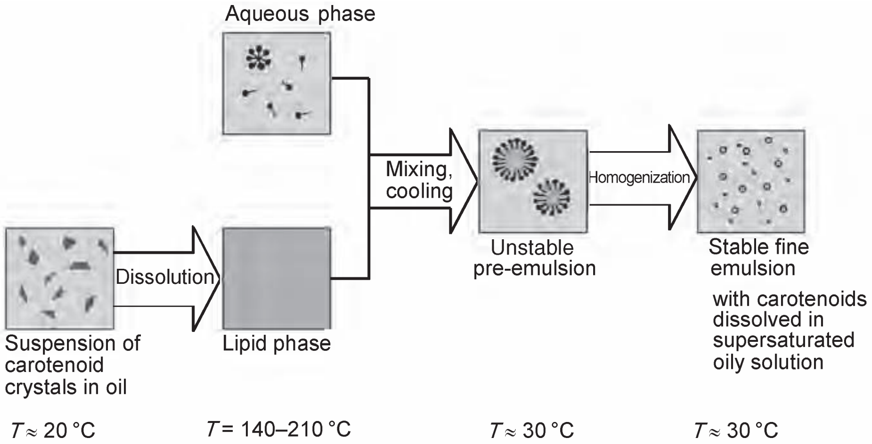

Fig. 8.7 Procedure to produce carotenoid-containing oil/water emulsions.

Crystalline carotenoids of more than 95% purity were mainly present in the form of their all-*trans* isomers (BASF AG, Ludwigshafen). Polyoxyethylene(20) sorbitan monolaurate (Tween 20, Carl Roth GmbH & Co. KG, Karlsruhe), at a concentration of 1 wt% related to the total emulsion, was used as emulsifier.

The crystalline carotenoid was added to the lipid phase first. The suspension was heated in a heat transmitter to dissolve the carotenoid crystals. For this, temperatures of 140–210 °C were required. Because carotenoids are susceptible to heat, the time of exposure to such high temperatures should be limited and not exceed about 5 seconds. To make sure the carotenoid crystals dissolved completely during this short time, they were fine-ground in oily suspension in a ball mill to a particle size of less than 20 μm. The heated lipid solution was subsequently pre-emulsified into the aqueous phase also containing the emulsifier. This was accomplished by means of a rotor-stator system (Ultraturrax®, IKA, Staufen). The Sauter diameter of the resulting pre-emulsion was between 2 μm and 10 μm, depending on the dispersing time. The oil droplets containing the dissolved carotenoid were further size-reduced in a high-pressure homogenizer to yield a fine-disperse stable oil/water emulsion of a Sauter diameter below 1 μm. In order to achieve high pressures in the presence of low volume flows during high-pressure homogenization, an orifice valve made of sapphire, with an aperture diameter of 80 μm, was used as an emulsifying nozzle. The return system shown in Fig. 8.8 allowing the emulsifying process to be repeated several times was not employed in the experiments presented here.

8.5.3 Characterization of emulsions containing carotenoids

Droplet size

Droplet sizes were analysed by static laser light scattering in combination with PIDS (polarization intensity differential scattering; LS 230, Beckmann-Coulter

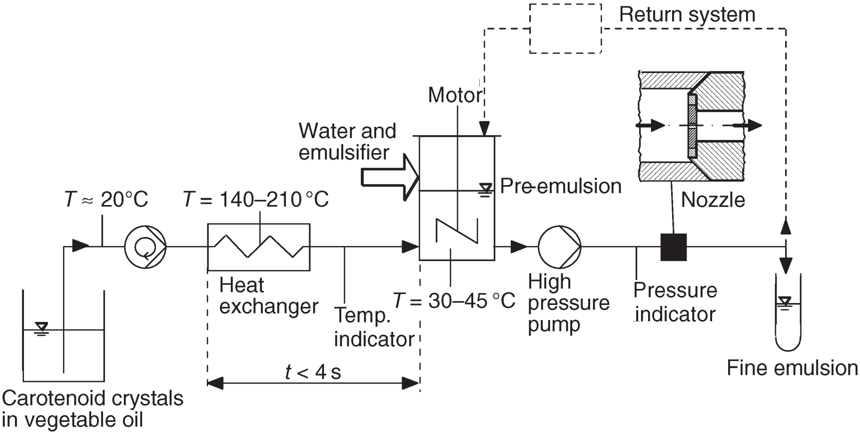

Fig. 8.8 Schematic of the experimental arrangement to produce carotenoid containing oil/water emulsions.

GmbH, Krefeld). Figure 8.9 shows the Sauter diameters of carotene-containing and non-loaded emulsions as a function of the emulsifying pressure. Accordingly, Sauter diameters of less than 1 µm are achieved at a pressure of 250 bar. The Sauter diameters decrease further with increasing pressure. At a homogenizing pressure of 650 bar, Sauter diameters of about 0.6 µm are obtained. No significant differences in droplet sizes have been found for non-loaded and carotenoid-containing emulsions. These experiments have shown that the high-pressure homogenizing system applied allows fine-disperse emulsions to be obtained after only a single emulsifying step.

Carotenoid isomers

At high temperatures, the all-*trans* forms of the carotenoids which prevailed in their crystalline state changes into *cis*-isomers. In high-performance liquid chromatography (HPLC) analyses conducted at the Institute of Botanics II of the University of Karlsruhe (TH) to determine the isomer pattern of the carotenoids (C-30 column; YMC S5 carotenoid, Europe GmbH, Schermbeck), the all-*trans* isomer and *cis*-isomers 9-*cis*, 13-*cis* and 15-*cis* were identified. Other *cis*-isomers were recognized; however, the configuration of these was not explored.

Figure 8.10 presents the concentrations of the different isomers, after the carotenoids were dissolved in heated oil at different temperatures. Accordingly, increasing temperatures led to increasing *cis–trans* isomerization. While astaxanthin has been shown to be relatively resistant to isomerization up to temperatures of about 120 °C, lycopene responded to a temperature of 90 °C by a sharp increase of *cis*-isomers. After a residence time of 5 s in oil at 130 °C, *cis*-isomers accounted for about half the lycopene.

As the carotenoids, according to the method described to produce carotenoid-containing emulsions, are exposed to high temperatures during dissolution, one

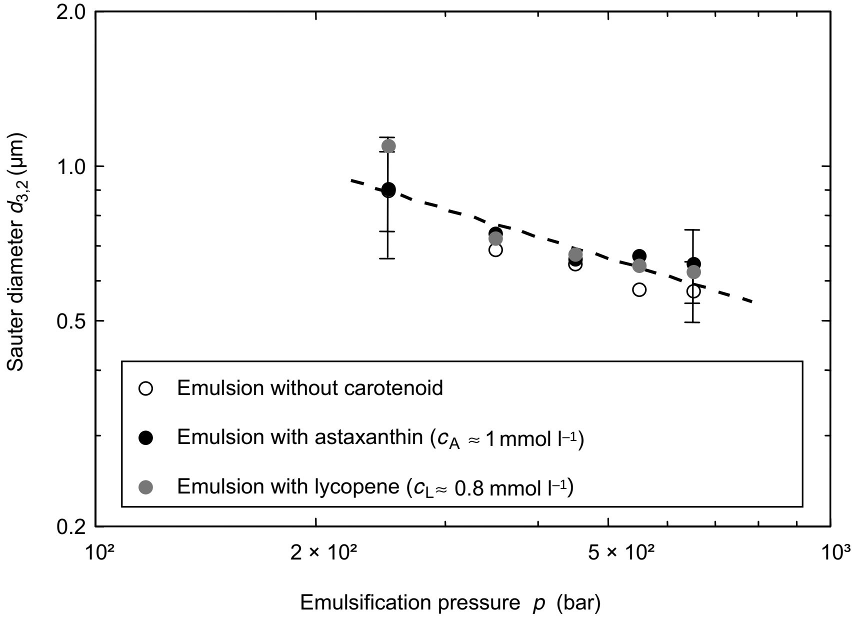

Fig. 8.9 Droplet size of carotenoid-containing emulsions as a function of emulsifying pressure.

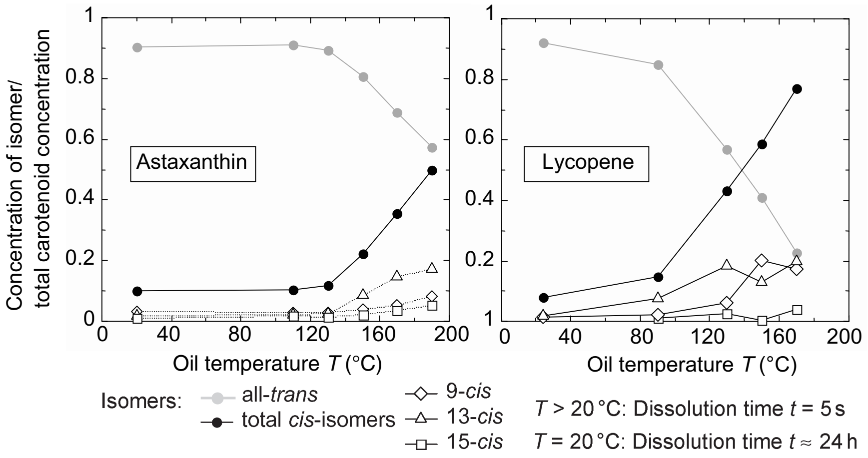

Fig. 8.10 Occurrence of isomers after dissolution of the carotenoids in heated oil.

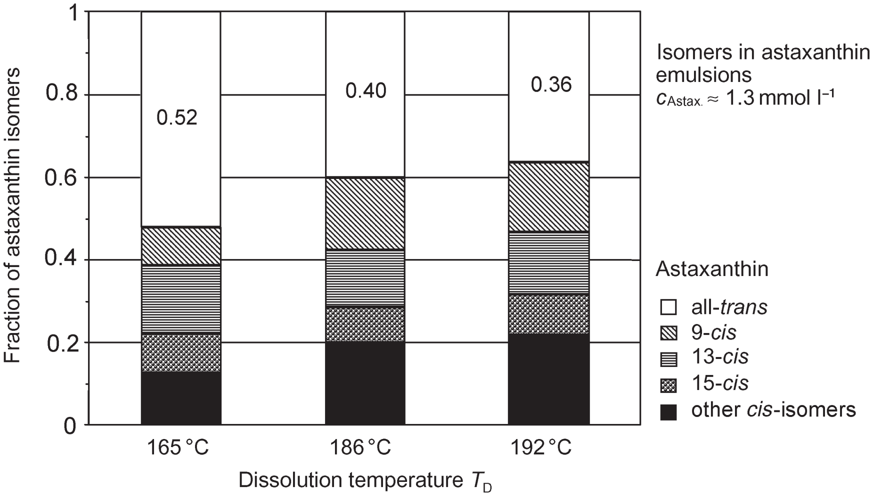

Fig. 8.11 Distribution of isomers in astaxanthin emulsions.

finds a high concentration of *cis*-isomers in the finished emulsion. In Fig. 8.11, the isomeric pattern is exemplified by three astaxanthin emulsions in which the crystalline carotenoid was dissolved in oil at different temperatures. *Cis*-isomers accounted for 50–60% of the total amount of astaxanthin. The share of *cis*-isomers increased with increasing dissolution temperature.

Stability of emulsions containing carotenoids

As far as the stability of carotenoid-containing emulsions is concerned, physical and chemical stability can be distinguished. Emulsions produced by use of the high-pressure homogenizer described above are so finely dispersed that they hardly cream. Repeated emulsifying steps at high pressure are a means of even further narrowing the droplet size distribution and thus enhancing physical stability. In the emulsions discussed here, insusceptibility to creaming is not a critical point.

The physical form in which carotenoids are present is decisive for stability. The carotenoids are dissolved at high temperatures in oil which is cooled during emulsifying into the aqueous phase. Carotenoid concentrations in the oil droplets of the finished emulsion were much higher – about 50 times (astaxanthin) and 10 times (lycopene) the concentrations which could be dissolved in an oily suspension within 24 h at room temperature. Although the oily phase of the carotenoid emulsions was strongly oversaturated, carotenoids in the emulsions have not been found subsequently to crystallize over a period of one month. In oversaturated continuous oily phases, however, carotenoids crystallized within one day. The finding that carotenoids in the oil droplets of emulsions, despite oversaturation, do not crystallize has also been described in the literature (Bunnell *et al.*, 1958; Schweikert and Kolter, 1997).

The emulsions described here were to be used in cell-culture tests to study the physiological functions of carotenoids. Additional antioxidants to improve the

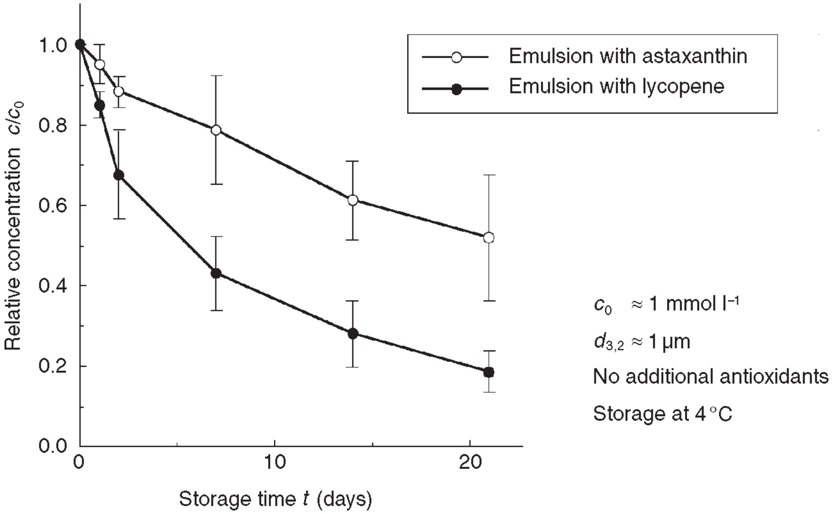

Fig. 8.12 Degradation of carotenoids in emulsions not containing additional antioxidants.

chemical stability of the carotenoids were, therefore, not applied. Carotenoid concentrations in the emulsions have been found to decrease gradually; this is probably due to oxidation. Figure 8.12 illustrates the degradation of astaxanthin and of lycopene in two emulsions. The initial concentration of astaxanthin had decreased to half after about three weeks, and that of lycopene after about one week. In commercial formulations carotenoids are protected against degradation by antioxidants. In cell-culture tests, recently produced emulsions were employed to ensure sufficiently high carotenoid concentrations.

Studies on the physiological functions of carotenoids

Carotenoid emulsions were tested *in vitro* in colon-carcinoma cells (HT 29) as a model for human colonic epithelium cells (Ax *et al.*, 2002) which are not cultivable *in vitro*. In this model system carotenoid absorption by the cells is taken as an indicator of carotenoid bioavailability *in vivo*. The studies showing that the carotenoids are well absorbed by cells suggest good bioavailability of carotenoids in oil/water emulsions. It became obvious that only fine-disperse emulsions with a Sauter diameter below about 1 µm are suitable for cell tests as oil droplets neither cream nor coalesce during incubation.

The results of these *in vitro* tests give reason to assume that carotenoids in emulsions are bioavailable. The formulation, furthermore, provides the possibility of loading cells with carotenoids to study function and mechanism of action of these on the cellular level. The emulsions presented here could be helpful in studies on the physiological action of carotenoids like, for example, the presumed protection against oxidative stress.

A summarizing statement about carotenoid formulations should refer to the following facts:

- It is possible to place carotenoids into the disperse oily phase of oil/water emulsions. Dissolved at an oversaturated concentration they will stay in the oil droplets.
- Carotenoids isomerize into *cis*-forms under the influence of heat. Emulsions therefore contain a mixture of different geometric isomers of the carotenoid.
- *In vitro* experiments suggest a good bioavailability of carotenoids formulated in oil/water emulsions.
- Carotenoid containing emulsions have been found to be a suitable formulation to study the physiological mechanism of action and function of carotenoids in cell cultures. They also allow carotenoids to be supplied as bioavailable, water-dispersible ingredients of functional food.

8.6 Future trends

For food, an adjustment of its microstructure and, thus, its product properties is of growing importance. This applies to the design of functional food in particular, because microstructure could also influence the food's health-promoting properties. An adequate formulation, for example, of carotenoids and phytosterols which are usually available as poorly soluble lipophilic compounds in crystalline form, may enhance their beneficial action.

In many cases, oil/water emulsions are suitable carrier systems for poorly soluble lipophilic compounds. If these compounds are available in the disperse oily phase, oil/water emulsions provide the advantages of water-dispersible systems and still ensure bioavailability. Because the fine-disperse oil droplets may contain the dissolved active ingredients at an oversaturated concentration, it is also possible to place relatively high concentrations of poorly soluble compounds into oil/water emulsions.

8.7 Sources of further information and advice

The information needed to produce emulsions and to formulate poorly soluble compounds in emulsions is presented in a course of lectures, held at two yearly intervals by the Institute of Food Process Engineering, University of Karlsruhe, D-76128 Karlsruhe. For further information contact lvt@lvt.uni-karlsruhe.de.

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9

The creation of new food structures and textures by processing

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9.1 Introduction: processing, microstructure and gel formation

Processing conditions are powerful tools for the creation of structures. Of special importance for structure formation is the stress distribution, determined by the velocity fields in the process equipment and material properties. The stress distribution is often not constant during processing, since transient structures are formed, causing variations in flow behaviour and velocity fields in the processing unit. Furthermore, transient structures, together with the multi-phase nature of a food product, set restrictions on the expansion of structure during processing. Thus, structure formation and process conditions have to be considered as closely connected factors and the relations between them are complex and often unexplored. Today, the choice of process equipment and parameters are not set by the desired microstructure, which determines the behaviour and quality of the product, but rather by tradition, available equipment or simply lack of knowledge. Relations between structure formation and process conditions have been intensely studied.[1–8] Increased knowledge opens up new routes to meet the demands from the market for development of new products with desired properties. Through a careful choice of process equipment and parameters, the possibility of designing structures with tailor-made properties increases.

Mixed gels are widely used as ingredients by the food industry. Compared with pure gels, mixed gels have the ability to show a completely different microstructure and, furthermore, a wider array of textural and functional properties. The behaviour of a mixed gel is largely dependent on the distribution of phases. An emulsion morphology in a phase separated mixed system shows properties similar to the continuous phase, whereas a bi-continuous system shows properties similar to either of the two phases, depending on their individual strength. A third distribution of phases is where one phase becomes depleted of polymers whereas the second phase becomes enriched in polymers. This type of mixed system is denoted as complex coacervation[9] or associative phase separation.[10] The properties of this type of mixed gel are hard to predict and may be completely different from those of the pure gels.

The phase distribution in mixed gels is affected to a large extent by process conditions. Hermansson and co-workers have characterised the structure after every unit operation during production of a spread.[1] The final phase distribution in the spread product was found to be set in the later stages of the production line, in the cooling tubes and the pin mixer. In the early stages of the production line, a coarse emulsion prevailed, which showed proof of being affected by the flow conditions during the production process. The inclusions were far from being spherical. Many were ellipsoidal; some had irregular shapes with 'hooks' that seemed to cause complex interactions between the droplets. The flow-induced structures in the early stages of the production line can have a bearing on the final product structure.

In this chapter, a review is given of the possibilities of creating new structures of whey protein gels, pure or in mixtures with gelatin, by means of different processing techniques. For the pure system, the aggregation behaviour of whey proteins is targeted, whereas for the mixed system, the distribution of phases is of interest. Whey proteins consist of a mixture of globular proteins, of which β-lactoglobulin is dominant, both in amount and in determining the overall behaviour. A major advantage of whey proteins is that they are sensitive to process conditions, such as solution properties, heating conditions and flow patterns.[3,11] Whey proteins form heat-set, irreversible gels, and, depending on pH, two types of network structures can be formed: fine-stranded and particulate. Close to the IP (iso-electric point) of around 5.2, particulate network structures are formed owing to a low repulsion in the system, which show network strands in micrometre dimensions. At high and low pH values, the repulsion in the system is high, leading to the formation of fine-stranded gels with network strands in nanometre dimensions. The shift in microstructure characteristics has been studied in detail by Langton and Hermansson.[12]

Being a by-product of cheese making, whey proteins exist in large quantities and are consequently rather cheap. Whey proteins have proved to be a potential food ingredient in various products. In low-fat products, microparticulated whey proteins are frequently used as surrogate to the dispersed fat phase.[13] Several methods exist for producing protein microparticles and they generally involve steps of heating and shearing, which leads to aggregation of the proteins to hard, spherical particles.

In contrast to whey proteins, gelatin is inert and insensitive to various processes.[3] Even so, gelatin is a potential food ingredient and is widely used in food products as a stabiliser, thickener and a gelling agent. One of the major advantages of gelatin is that the gel is reversible with a melting point around 30–35 °C, i.e. it melts in the mouth. Gelatin forms a cold-set gel with a fine-stranded structure, independent of pH.

In this chapter, various types of processing are applied to create new types of structures. The distribution of phases in whey protein/gelatin mixed gels has been targeted by using different techniques to induce gel formation; conventional heating and cooling and high-pressure processing. The use of the two gel formation techniques allows an alteration in the order of gel formation between whey protein and gelatin. The aggregation behaviour of pure whey proteins has been affected by subjecting whey protein suspensions in various states of aggregation to fluid flow, both on the laboratory scale and on the pilot plant scale. Relations between the created structures and their rheological behaviour are given. The chapter builds on previously published papers by the author.[3–6,14–17]

9.2 Selecting process conditions

9.2.1 High-pressure versus conventional heating and cooling

High-pressure and conventional heating and cooling have been used as gel formation techniques for the mixed whey protein/gelatin system. By using both techniques it is possible to change the order of gel formation between the biopolymers, which has a direct impact on the expansion of the process-induced structure.

Temperature is the conventional technique used for gel formation of biopolymers. To obtain aggregation and gel formation of whey protein, the temperature is increased above the denaturation temperature, which often is around 70 °C. In this chapter, whey proteins are set under conditions of 90 °C for one hour in the presence of dissolved gelatin. Gelatin is then subsequently set upon cooling, in the presence of a whey protein network.

Upon the application of high pressure, whey proteins denaturate and aggregate. In contrast, the gelatin sol does not form a gel by the application of high pressure, indicating that a temperature decrease is necessary to induce the formation of triple helices, the building blocks of the gelatin network. The suspensions have been subjected to a pressure of 6000 bar for 20 minutes. Generally, a pressure above 1.5 kbar is required for protein denaturation.[18,19] Thermodynamically, the effects of pressure on the systems are described by the variation of the molar volume between two states at constant temperature.[18] High-pressure processing has been applied according to two different routes:

- the mixed system has been subjected to high pressure at room temperature;
- the mixed system has been subjected to high pressure during a temperature decrease from 40 to 20 °C.

During the first route, the gelatin network is set prior to the application of high pressure, leaving the whey protein to denaturate and aggregate in the presence of the gelatin network. During the second route, the gelatin and whey protein networks are set at the same time. Details regarding high-pressure processing are given elsewhere.[14,15]

9.2.2 Flow processes

Prior to gel formation, whey protein suspensions in various states of aggregation have been subjected to fluid flow, both in the laboratory and at pilot plant scale. After processing, the suspensions were set at 90 °C for one hour.

On *laboratory* scale, defined shear flow of various strengths was applied to whey protein suspensions by means of a rheometer and a concentric cylinder system. The equipment creates a batch process and the volume required for a run is around 20 ml. In Fig. 9.1, the aggregation states of whey proteins at different temperatures are reflected by changes in the viscosity with temperature increase during a shear flow of 78 s^{-1}. At approximately 40 °C, the viscosity starts to increase, indicating aggregation. In agreement, an increased opaqueness is found for the whey protein suspensions at this temperature. Aggregation prior to denaturation of whey proteins close to IP at pH 5.1 has been reported.[5,20,21] Around 50 °C, the viscosity starts to decrease and around 75 °C, close to denaturation, it increases rapidly and strongly (see Fig. 9.1). The shear flow has been applied for various temperature ranges, all starting at 20 °C and ending at different aggregation states of the whey proteins according to Fig. 9.1, as well as during a short period of time only at the gel point, denoted as shear-pulsing.

On *pilot* plant scale, the experiments were performed with a continuous rotor/stator device, denoted as shear crystalliser,[22] using different rotational speeds and rotor geometries. A minimum volume of 3000 ml was required for a run in the equipment. Results from a concentric cylinder system are focused on in this chapter, since that rotor geometry results in a similar flow patterns to those on the laboratory scale, i.e. a defined shear flow. However, the effects of a complex flow, based on shear and elongation flow as well as vortices, resulting from a rotor geometry with two scraping blades attached, have been investigated as well. A comparison of the effects created by the different rotor geometries is given elsewhere.[6,17] The flow processing has been applied at constant temperatures, 30, 50 and 70 °C, during a mean residence time of 3.5 minutes. The temperatures were chosen in accordance with the various states of whey protein aggregation (see Fig. 9.1).

9.3 Process-induced structures

9.3.1 Phase distributions caused by different gel formation techniques

By using both temperature and high-pressure processing as gel formation techniques, the order of gel formation between whey protein and gelatin can be altered. As a consequence, alterations in the space available for a structure to

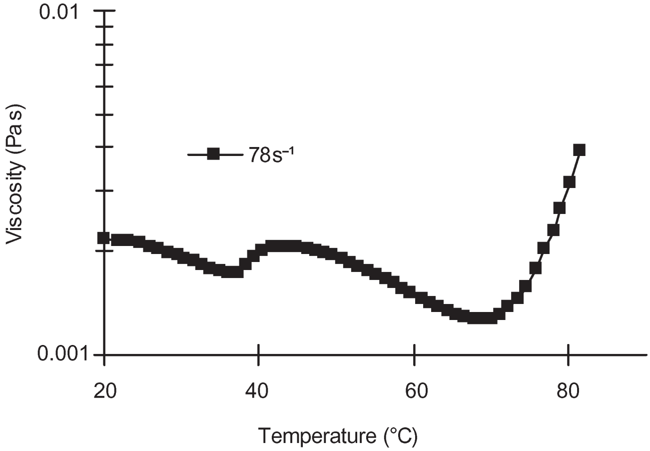

Fig. 9.1 Viscosity versus temperature increase from 20 °C to 82 °C under shear flow of 10% w/w whey protein suspensions at pH 5.4.

evolve and expand in the sample during processing are initiated, which has a major impact on the distribution of phases in a mixed gel. Since food products are often based on multi-phase systems and since transient structures are often formed during processing, restrictions on the expansion of process-induced structures occur generally and are among several factors that come into play to determine the final food structure.

Particulate whey proteins and fine-stranded gelatin

Bi-continuous (Fig. 9.2a) and gelatin-continuous (Fig. 9.2b and c) network structures are formed at pH 5.4 when varying the order of gel formation. At the magnification used, only the whey protein network is revealed since the gelatin network is too fine. The bi-continuous network is formed by conventional heating and cooling, under which the whey protein network sets first, i.e. in an environment where there are minor restrictions on the expansion of the whey protein network. For the gelatin-continuous mixed gels, the microstructure shows an emulsion morphology with spherical whey protein inclusions of various sizes. The size of the whey protein inclusions varies according to the state of the gelatin phase during high-pressure processing, i.e. whether it is a sol or a gel. Whey protein inclusions in the range of 1 μm are formed when the two networks set simultaneously, by means of simultaneous cooling and high-pressure processing (Fig. 9.2b). Under these conditions, the space available for the whey proteins to aggregate is less restricted and, among other things, competition arises between the kinetics of gel formation of gelatin and whey proteins to determine the final phase distribution. Smaller inclusions result from high-pressure processing when the space available for the whey proteins to aggregate is restricted by the pore size of the gelatin network, i.e. when the gelatin network sets first (Fig. 9.2c).

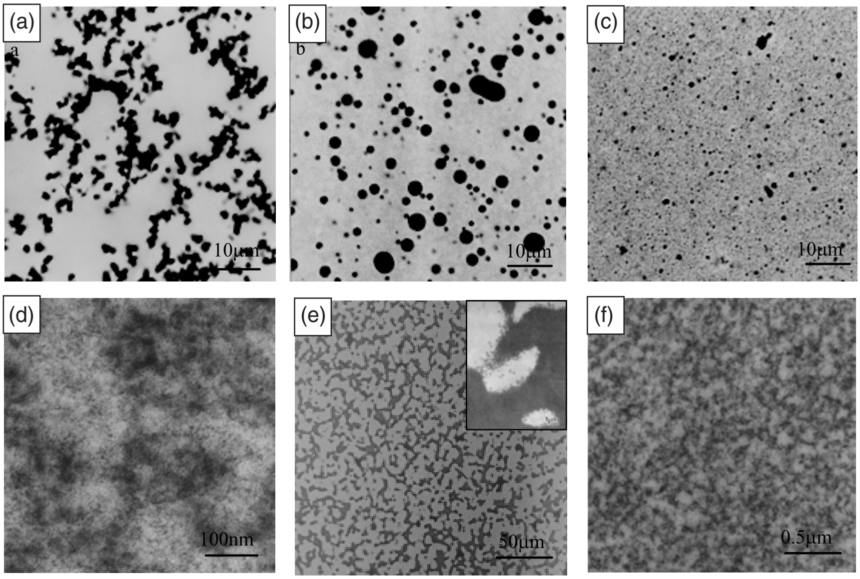

Fig. 9.2 Sections of 12% whey protein + 3% gelatin mixed gels at: (a)–(c) pH 5.4; (d)–(f) 7.5. Gel formation technique: (a), (d) conventional heating and cooling; (b), (e) high-pressure processing during cooling from 40 to 20 °C; (c), (f) high-pressure processing at room temperature.

Fine-stranded whey proteins and gelatin

Bi-continuous (Fig. 9.2d), complex coacervate (Fig. 9.2e) and dense, compact structures are formed at pH 7.5, depending on the order of gel formation. In agreement with the phase distribution found for particulate whey proteins in mixtures with gelatin (Fig. 9.2a), a bi-continuous network is formed by conventional heating and cooling (see Fig. 9.2d). Both networks are fine-stranded and, in Fig. 9.2d, the whey protein network is represented by the darker areas and the gelatin network by the finer, thinner structure.

When the two networks set simultaneously, a complex coacervate is formed (see Fig. 9.2e). The coacervate structure is highly aggregated and shows a network composed of large structural features (notice the difference in scaling). In the inset, the complex coacervate is shown at a higher magnification. The formation of one single network structure from the two biopolymers, which have otherwise proved to be incompatible, is unexpected. The different techniques used for gel formation and the fact that the two networks set simultaneously are important factors, as well as the solvent quality, volume decrease during the high-pressure processing, volume expansion during the pressure release, etc. It has been suggested by Bungenberg de Jong[9] that an opposite charge of individual polymers frames the phase behaviour. However, the condition is not fulfilled for the whey protein/ gelatin mixed system at pH 7.5, since gelatin is close to its IP whereas whey proteins are above theirs. The coacervate structure has been studied in more detail in a separate paper.[15] From temperature treatments of the coacervate structure it was concluded that both the gelatin and whey protein phase were homogeneously distributed within the aggregates.

In the presence of a gelatin network, the aggregation of the whey proteins is restricted to the pore size of the gelatin network. Under these conditions, a dense and compact microstructure is formed (Fig. 9.2f), in which it is difficult to determine the phase distribution and differentiate between the whey protein and the gelatin network. In contrast, in the corresponding gel at pH 5.4, a gelatin continuous network with spherical whey protein inclusions was formed (see Fig. 9.2c).

9.3.2 Influence of fluid flow on the aggregation behaviour of whey proteins

During manufacturing, food products are often exposed to various types of flow conditions at high temperatures. For instance, pumping in pipes, a run through a scraped surface heat exchanger, filtration, etc. are common processes during manufacturing. The process conditions can cause major effects on the aggregation of proteins. In this section, the effects on whey protein aggregation of fluid flow at high temperatures under controlled but different process conditions on laboratory and pilot plant scales are outlined.

Processing conditions

The stress distribution in the processing equipment as well as the time/temperature conditions for the application of flow vary with the scale of the process. Illustrations of the different equipment used are given in Fig. 9.3. Even though the equipment has been chosen to achieve a similar flow pattern, i.e. both are based on concentric cylinder systems, the flow patterns vary owing to the difference in scale of the process and various effects, i.e. difference in gap size and an additional direction of flow on the pilot plant scale. The applied shear rate is higher on the pilot plant scale. For instance at 100 rpm, the flow is laminar and corresponds to a mean shear rate of 215 s^{-1}. The mean residence time on the pilot plant scale is 3.5 minutes, whereas on the laboratory scale, it is around 20 minutes. On the laboratory scale, the fluid flow has been applied during heating from 20 °C up to the gel point at 78 °C, whereas on the pilot plant scale, the fluid flow has been applied at a constant temperature of 70 °C, in relation to the viscosity increase at denaturation (see Fig. 9.1).

Flow-induced structures

The large effects on coarse, aggregating whey proteins of fluid flow at high temperatures are shown in the microstructure of the subsequent gels in Fig. 9.3. On comparison with the microstructure of the unsheared whey protein gel, it is clear that flow processing prior to gel formation causes a coarsening of the network structure (compare Fig. 9.3a with 9.3b–e). The pre-processed gels are inhomogeneous, composed of pores of various sizes as well as loose, sparse network

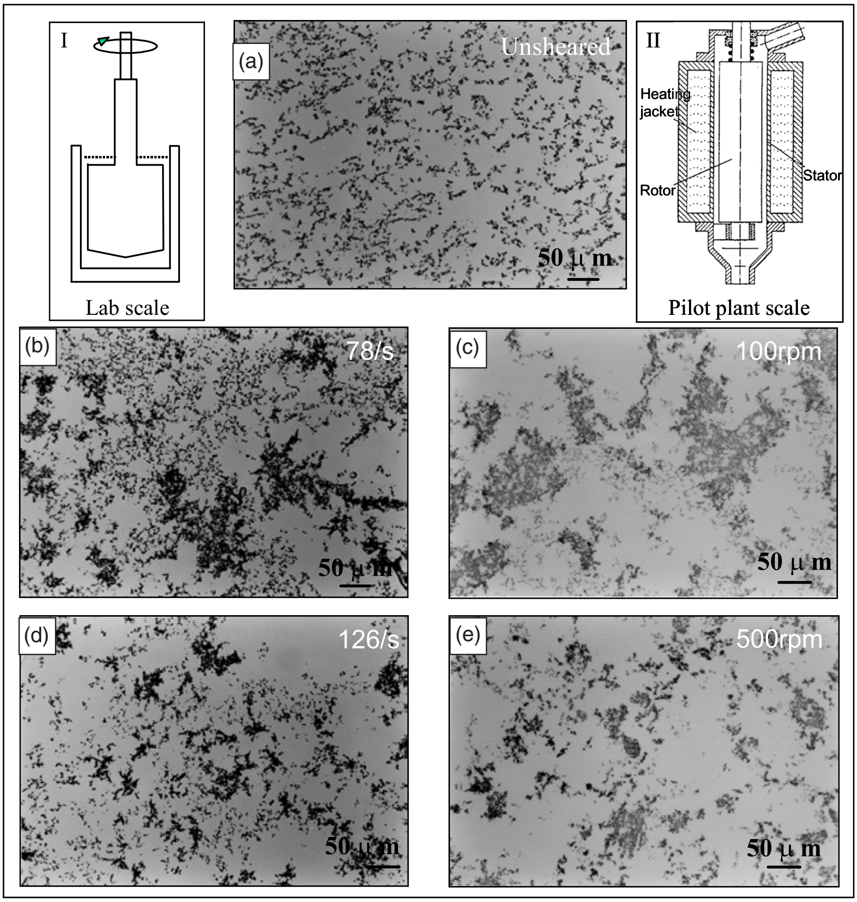

Fig. 9.3 (I) Laboratory scale equipment. (II) Pilot plant scale equipment. Sections of 10% whey protein gels at pH 5.4: (a) unprocessed gel; (b)–(e) pre-processed gels. Suspensions subjected to flow processing in flow equipment on (b), (d) the laboratory scale and on (c), (e) the pilot plant scale.

regions and dense, compact regions. The compact regions appear as aggregates, embedded in the sparse network. In contrast, the unsheared gel is represented by a homogeneous network structure, composed of pores of equal size. The size of the flow-induced, dense aggregates decreases with an increase in flow strength, indicating that they are sensitive towards the stresses from the flow.

It is interesting to note that the flow-induced structures are similar in Fig. 9.3, independent of the difference in scale of the process and in process conditions. This suggests that the more accurate processing that occurs on the pilot plant scale can be mimicked on the laboratory scale. However, the conditions are not directly transferable, which is obvious from the similar results in Fig. 9.3, in spite of the different processing. Care and pre-experiments are necessary precautions when mimicking conditions on the laboratory scale. For instance, a more or less direct transfer of the conditions on the pilot plant scale to the laboratory scale, denoted as shear-pulsing, results in minor effects on the microstructure.[5,16] An explanation of the similarity in structures achieved in Fig. 9.3 may lie in the frequency of particle collision and the energy input, which are direct targets for the parameters that vary between the processes.

9.4 Structure-related rheological properties

9.4.1 Effects of phase distribution

The distribution of the phases in a mixed gel to a large extent determines the behaviour. In agreement, the phase distributions achieved when varying the gel formation technique (see Fig. 9.2) show completely different rheological behaviour; see Fig. 9.4 for the effects on G' (storage modulus) of various gels over time as the temperature increases.

The influence of the continuity of the phases on the overall behaviour of a mixed gel is clearly illustrated in Fig. 9.4a, where a comparison is made between the melting behaviour of the bi-continuous and the gelatin-continuous mixed gel at pH 5.4 (Fig. 9.2a and b). For comparison, the melting behaviour of a pure gelatin gel is included. The gelatin-continuous gel follows the behaviour of the pure gelatin gel more or less exactly and no influence of the whey protein inclusions is detectable in the behaviour. The bi-continuous mixed gel shows a much higher G' at start of melting and, in contrast to the gelatin-continuous gel, does not melt down fully under the temperature increase, which is a clear indication of the continuity of the whey protein network.

The phase distributions created for the mixed gels at pH 7.5 (see Fig. 9.2d–f) show completely different behaviour, both in gel strength and melting characteristics (see Fig. 9.4b). In comparison, the complex coacervate shows the highest gel strength, whereas the bi-continuous system shows the lowest. Furthermore, the complex coacervate, as well as the dense network structure, show an extensive decrease in G' of the temperature increase, while the bi-continuous network shows very small effects. The difference in melting characteristics suggests that the systems have different distributions of the gelatin phase, which is reversible upon the temperature increase. Since neither of the gels melt down fully, a continuous whey protein network is indicated in all gels, even in the dense structure (Fig. 9.2f) for which the whey proteins set in the presence of a gelatin network. In relation to the difficulties in the interpretation of the distribution of phases in the complex coacervate (Fig. 9.2e) and the dense structure (Fig. 9.2f), the information from rheological studies explains further the phase distribution in the gels.

9.4.2 Effects of flow-induced structures

Fluid flow on the laboratory scale versus the pilot plant scale

The aggregate structure of whey protein gels is crucial for their rheological

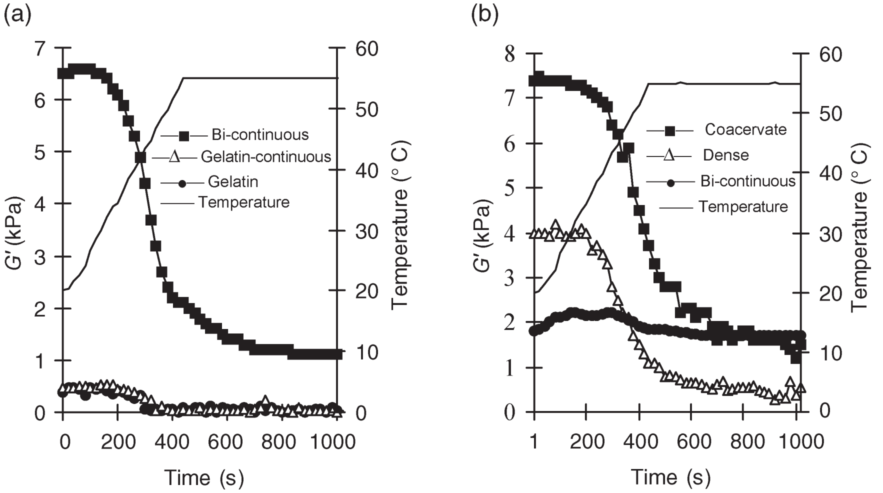

Fig. 9.4 *G′*, during temperature increase, of 12% whey protein + 3% gelatin mixed gels with different phase distributions and of a 3% pure gelatin gel at pH (a) 5.4 and (b) 7.5.

behaviour. In Fig. 9.5, the rheological behaviour of corresponding gels shown in Fig. 9.3 is given. A frequency sweep at 90 °C is given for gels pre-processed on the laboratory scale while a time sweep at 20 °C is given for gels pre-processed on the pilot plant scale. In order to avoid cluttering the graphs, the standard deviation is given for some representative curves.

The rheological behaviour of the gels is similar, independent of the different processing applied. A similarity was also found in the microstructure of the processed gels. On the laboratory scale, the pre-processing performed caused a decrease of the *G′* to a value half of that for the unsheared gel (Fig. 9.5a). As the shear rate increases, there is a small but clear decrease in *G′*. A decrease in *G′* is also found for the gels pre-processed on the pilot plant scale (Fig. 9.5b). *G′* drops from around 11 kPa for the unsheared gel to less than 3 kPa for the pre-processed gels. In contrast, for the pre-processed gels on the pilot plant scale, no effects can be found of the increase in flow rate on *G′*. The similarity in rheological behaviour of the gels further strengthens the suggestion made above that more accurate process conditions that occur on the pilot plant scale can be mimicked on the laboratory scale.

Fluid flow at the various aggregation states of whey proteins

The aggregation state of the whey proteins when subjected to fluid flow is crucial to the effects caused. From shear pulsing on the laboratory scale at the gel point, an increase or decrease in *G′* of the gels results when comparing with an unsheared gel, depending on the flow strength (see Fig. 9.6). The parameters for the shear pulsing are given in the key. As mentioned above, the conditions under shear pulsing on the laboratory scale are comparable to those under the pilot plant

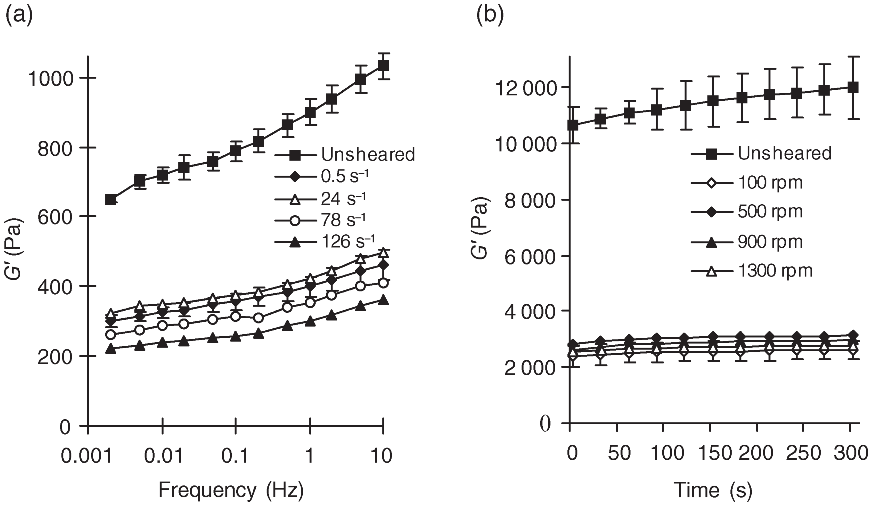

Fig. 9.5 Gels of 10% whey proteins at pH 5.4: (a) frequency dependence of *G′* at 90 °C; (b) time sweeps for gels at 20 °C. Suspensions subjected to flow processing in flow equipment on (a) the laboratory scale and (b) the pilot plant scale.

scale experiments. Even so, the opposite rheological behaviour can result. There is also a discrepancy regarding the caused effects on the microstructure, as already mentioned, since shear pulsing on the laboratory scale has a very small effect on the microstructure of the gels, whereas similar conditions on the pilot plant scale cause large effects (see Fig. 9.3).

Fluid flow in the early stages of a processing cycle can have a dramatic effect on *G′* of the final gel structure. On the laboratory scale, effects of shearing at various states of aggregation for the whey proteins have been investigated. The shearing was applied under heating for various temperature ranges, starting at 20 °C. The temperature range under which shear flow has been applied is given in the key to Fig. 9.7, with the applied stress in the caption. Even at around 40 °C, large effects on the *G′* of the fluid flow were noticed (see Fig. 9.7). *G′* drops from roughly 800 Pa to 400 Pa when subjected to pre-shear during heating from 20 to 40 °C. In relation to the viscosity behaviour of the whey proteins shown in Fig. 9.1, it is clear that the aggregation occurring around 40 °C, prior to denaturation, has a major impact on the strength of the final gel. In contrast to the large effects on the rheological behaviour of the pre-shear around 40 °C, effects on the microstructure are noted first after a pre-shear up to temperatures around 50 °C.[5]

9.5 Conclusions

The microstructure of a food product is influenced by the processing conditions during manufacturing. Unfortunately, how, why and when a structure is formed

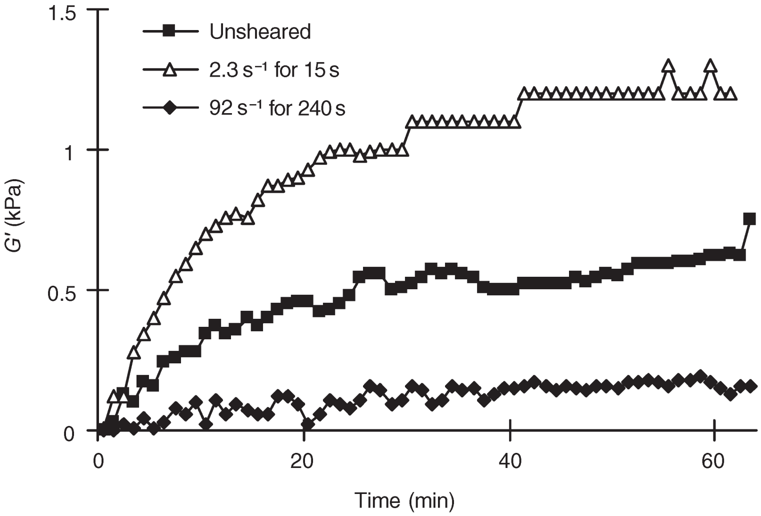

Fig. 9.6 Gel formation of 12% whey proteins at pH 5.4. Suspensions subjected to shear pulsing at the gel point at 80 °C for different rates and times on the laboratory scale.

and affected by the processing is not fully clear. The lack of knowledge restricts the ways of designing manufacturing processes for the purpose of creating structures with controlled texture. In this chapter, the ways of affecting the structure formation of whey proteins, pure or in mixtures with gelatin, by different process conditions in a controlled manner have been explored. The process conditions studied are fluid flow and different gel formation techniques which cause an alteration in the order of gel formation between biopolymers in a mixed system.

The order of gel formation between biopolymer and, thereby, the space available for a structure to expand during processing is crucial to the final structure of a food product. In this chapter, numerous phase distributions, such as bi-continuous, gelatin-continuous and more complex structures of coacervate nature, have been achieved simply by varying the order of gel formation between the biopolymers and the pH. The order of gel formation has been varied by changing the gel formation technique, i.e. conventional heating and cooling and high-pressure processing.

The relations between phase distribution and rheological behaviour are strong. For some phase distributions, the behaviour is predictable, as for the gelatin-continuous system, which shows a similar behaviour to a pure gelatin gel. In contrast, for more complex phase distributions, such as the coacervate structure, the behaviour is impossible to predict and totally different from that of the corresponding pure gels. In applications, the formation of complex structures during processing can be an advantage since desired properties can appear that better meet the demands from consumers. For some phase distributions, a strong connection is found between the formation of the structure during processing and

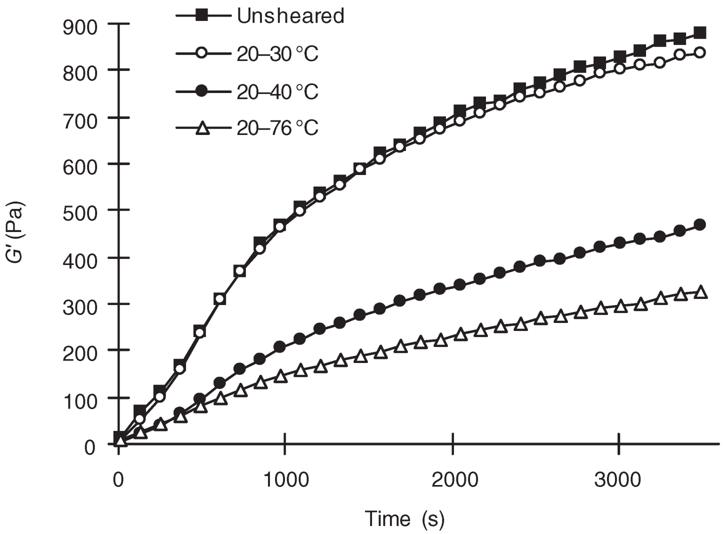

Fig. 9.7 Gel formation of 10% whey proteins at pH 5.4. Suspensions subjected to shear stress of 0.04 Pa under various temperature ranges on the laboratory scale.

the state of the surrounding phase. During manufacturing, restrictions on the expansion of process-induced structures are often set by the multi-phase nature of food products and by the formation of transient structures during processing. Therefore, the knowledge gained is of great relevance for manufacturers and can possibly be used to improve the control of texture by a careful choice of processing conditions.

Flow-induced structuring is a powerful tool to influence the aggregation behaviour of proteins. Often, the microstructural effects created are uncontrolled; however, some food products also exist where conditions such as temperature and shear forces have been tailored to create an aggregated protein structure. For instance, the microparticulated whey protein product *Simplesse*® is formed under more controlled temperature and flow conditions in a concentric cylinder system at high pressure.[13] Thus, it is clear that fluid flow has a good potential in creating desired structures.

In this chapter, the influence on the aggregation behaviour of fluid flow at the various aggregation states of whey protein has been outlined. Flow-induced whey protein aggregates with a dense, compact nature resulted from processing on both the laboratory and pilot plant scale. The flow-induced aggregates caused a decrease in strength of the subsequent gels. Apart from apparent differences, such as temperature conditions and flow pattern, the residence time was longer and the flow stresses weaker on the laboratory scale than on the pilot plant scale. On the laboratory scale, the dense aggregates appeared after a continuous shearing from 20 °C up to the gel point, whereas on the pilot plant scale, the aggregates appeared after fluid flow close to the denaturation point for a short residence time. In spite of the differences in process conditions, similar effects of the fluid flow applied were found, both in microstructure and in rheological behaviour of the subsequent gels. When trying to mimic the process conditions prevailing on the pilot plant scale, denoted by shear pulsing on the laboratory scale, the opposite effects can be created, which indicates that conditions prevailing on the pilot plant scale are not directly transferable to the laboratory scale. Minor effects were found in the microstructure of shear pulsing and a decrease or increase in the gel strength resulted, depending on the flow strength. Further investigations showed that the drop in gel strength had already been initiated by a fluid flow around 40 °C, which suggests that the whey proteins are sensitive to fluid flow far below the denaturation temperature. Thus, even in the early stages of a processing cycle, effects can be initiated that have a bearing on the final product quality.

9.6 Acknowledgements

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10

Using emulsifiers to improve food texture

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10.1 Introduction

Industrially produced food represents an increasing part of our daily diet, requiring emulsifiers to facilitate processing and ensure finished products with a uniform quality and long shelf-life. Food emulsifiers are surface-active lipids that display many functions in relation to food texture and rheological properties. They can either increase colloidal stability or provide controlled destabilisation in aerated food products. Interactions between emulsifiers, proteins and carbohydrates in bakery products or other starch-based foods improve both texture and shelf-life.

The functional properties of emulsifiers have been studied extensively in model systems and foods. Interfacial interactions between proteins and emulsifiers at the oil/water interface are important to emulsion stability or for controlled destabilisation and fat network formation as in aerated emulsions. Interactions between emulsifiers and fat crystals, delaying the fat recrystallisation that causes sensory-perceived defects in fat-based foods, are also highly important.

This chapter describes the chemical structure of food emulsifiers followed by the physical properties and finally interactions of emulsifiers with other food components, emphasising interfacial interactions between emulsifiers and food components such as water, proteins, carbohydrates and oils or fats in relation to the effects of emulsifiers on food texture and rheology.

10.2 The chemistry of emulsifiers

This section gives a brief description of the most common types of food emulsifiers, their chemistry and physical properties with the emphasis on the differences in polarity and crystal properties. The functions of food emulsifiers are controlled by their chemistry and composition with regard to hydrophilic and lipophilic properties. Many food emulsifiers have a relatively low polarity and are soluble in oils and fats, but may not be dispersible in water. Such products adsorb at interfaces affecting emulsion stability positively or negatively, or they influence the crystallisation behaviour of fats and oils. Other emulsifiers with stronger hydrophilic properties, because of a larger polar group or anionic properties, may be directly dispersible in water. Such emulsifiers are strongly adsorbed at oil/water (O/W) interfaces or may react with other food components, such as proteins, at interfaces or in bulk solution, affecting the texture and shelf-life of foods. The chemistry of food emulsifiers is described in detail by Feuge and Bailey,[1] Sonntag,[2] Schuster,[3] Krog,[4] Hasenhuettl and Hartel[5] and Stauffer.[6]

10.2.1 Monoglycerides E. 471; US FDA §184.1505

The term monoglyceride is commonly used for commercial products produced by interesterification of fats or oils (triacylglycerols) with glycerol. This process is referred to as glycerolysis, and the products manufactured by this process without further purification by solvent fractionation or molecular distillation techniques are referred to as mono-diglycerides. Concentrated monoglycerides are usually referred to as distilled monoglycerides.

The content of monoacylglycerols in the equilibrium mixture obtained after glycerolysis may vary from 10 to 60% depending on the glycerol/fat ratio in the reaction mixture. Commercial mono-diglycerides usually contain 45–55% monoacylglycerides, 38–45% diacylglycerides and 8–12% triacylglycerides with traces of glycerol and free fatty acids. An alternative production method is direct esterification of fatty acids with glycerol. Using purified fatty acids, mono-diglycerides with a narrow fatty acid distribution are produced.

Figure 10.1 shows a typical reaction diagram of the glycerolysis process with the chemical structure of the reaction products. Both the monoacylglycerols and the diacylglycerols exist in two isomeric forms. During the glycerolysis process there is a preference for esterification of the terminal OH groups in glycerol. The equilibrium of 1 and 2-monoacylglycerides is highly in favour of 1-monoacylglycerides, while the equilibrium ratio of 1,2 and 1,3-diacylglycerols in mono-diglycerides is approximately 40:60 in the finished product. Mono-diglycerides are oil-soluble, low-polar emulsifiers that are dispersible in water only in the presence of co-emulsifiers (sodium salts of fatty acids).

Distilled monoglycerides

Monoacylglycerols can be separated from the di- and triacylglycerols by a process referred to as molecular distillation, comprising a thin-film, high-vacuum distillation. The commercial products manufactured by this process are referred to as distilled monoglycerides, and contain a minimum of 90% monoacylglycerols, typically 95–97%, with a balance consisting mainly of 2–4% diacylglycerols and traces of glycerol and free fatty acids. Distilled monoglycerides usually contain no

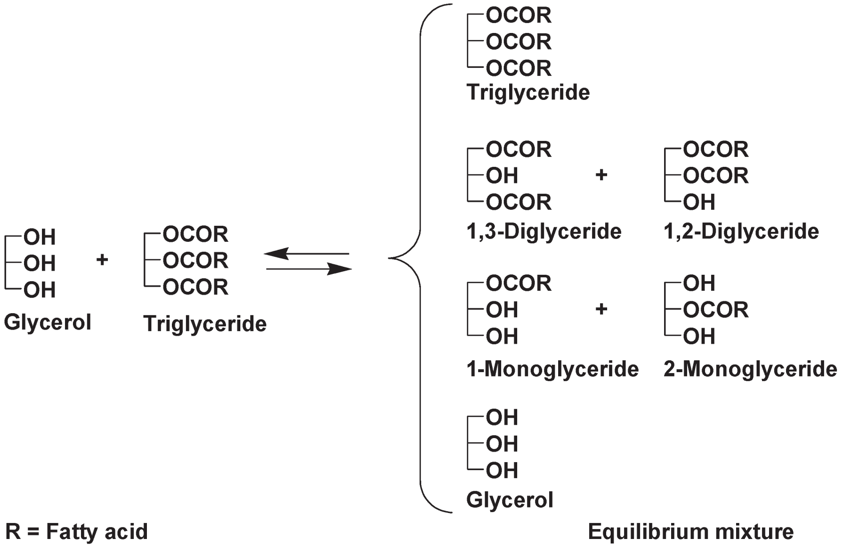


Fig. 10.1 Chemical reaction diagram showing the production of monoglycerides by glycerolysis.

triacylglycerols. The monoacylglycerols are predominantly present in the form of 1-monoacylglycerols, where the fatty acids are esterified to the terminal OH group, and the ratio between 1-monoacylglycerols and 2-monoacylglycerols is 90:10 in the melt after glycerolysis. The equilibrium ratio is dependent on temperature, and, at a low temperature in the crystalline state, the content of the 1-isomer increases to above 95%.

This process can produce distilled monoglycerides with a high purity, single fatty acid composition (e.g. glycerol monopalmitate or glycerol monooleate, etc.). In general, monoglycerides are low-polar, non-ionic lipophilic emulsifiers, which have many different functions in foods. Distilled monoglycerides differ from mono-diglycerides because of their mesomorphic properties in aqueous systems, which are described later in this chapter. Therefore, distilled monoglycerides have better functionality than mono-diglycerides in many foods.

The melting point of mono-diglycerides varies according to the fatty acid composition of the fats or oils on which they are based. However, the melting point of monoglycerides is always higher than the melting point of the corresponding triacylglycerols used in the glycerolysis process.[2] The increase in the melting point of distilled monoglycerides, compared with that of the corresponding fats used as raw materials, may be 10–15 °C.

Like triacylglycerols and other long chain compounds, monoglycerides show polymorphism. Depending on external conditions such as temperature, pressure, solvents and so on, various crystalline states occur. When cooled from the melt, monoglycerides transform from meta-stable to stable higher melting forms via different modes of polymorphism.

Table 10.1 Melting point of distilled monoglycerides

Monoglycerides	Melting points[†] (ºC) Sub-α	 α	 β
Monolaurin, 90% C12	16	45	61
Monomyristin, 90% C14	25	56	67
Monopalmitin, 90% C16	35	66	73
Monoolein, 90% C18:1	–	30	34
Monobehenin, 90% C22	56	82	85
Saturated monoglycerides (hydrogenated soya bean oil)*[a]	37	71	75
Saturated monoglycerides (from hydrogenated lard)*[b]	20	66	72
Saturated monoglycerides (hydrogenated palm oil)*[c]	16	68	72
Unsaturated monoglycerides (palm oil)*[d]	8º	(48)	60

* Danisco products: [a] DIMODAN® HS, [b] DIMODAN® HL, [c] DIMODAN® HP, [d] DIMODAN® P/M.
[†] Differential scanning calorimetry (DSC) peak temperatures.

The molecular organisation in the solid state of polar lipids is controlled by the chemical structure of the polar group and the fatty acid composition. Generally, because of their amphiphilic characteristics, the polar head groups are oriented in sheets, allowing interactions involving hydrogen bonds, dipol–dipol or ionic interactions. Hydrocarbon chains separate the polar groups, forming a bilayer structure similar to the structure of biological membranes.

Distilled monoglycerides crystallise into a meta-stable α-crystal form that transforms into a higher-melting, stable β-crystal form. If the α-crystal form is cooled, a solid-state transition to a third crystal form, termed sub-α, takes place.[7] The melting points of the various crystal forms of distilled monoglycerides are shown in Table 10.1.

The crystallisation behaviour of mono-diglycerides with a high proportion of diacylglycerols differs from that of distilled monoglycerides. This crystallisation pattern is related to the chemical conformation of the diacylglycerols. Synthetically pure 1,2-diacylglycerols are stable in the β'-crystal form, while 1,3-diacylglycerols crystallise in the higher-melting β-crystal form.[8] Commercial products always contain a mixture of both isomeric forms of diacylglycerides.

10.2.2 Organic acid esters of monoglycerides

The free hydroxyl groups in monoglycerides can be esterified with organic acids, forming derivatives of monoglycerides with modified hydrophilic/lipophilic properties. Emulsifiers with functional properties other than those of monoglycerides can, thus, be made by esterification with acetic, lactic, diacetyl tartaric or citric acid. The chemical structure and molecular models of monoglycerides and their organic acid esters are shown in Fig. 10.2.

Esterification of one or both of the free hydroxyl groups of monoglycerides changes the crystallisation pattern to monomorphic. All types of organic acid esters crystallise from their molten state in the α-crystal form, which is the stable

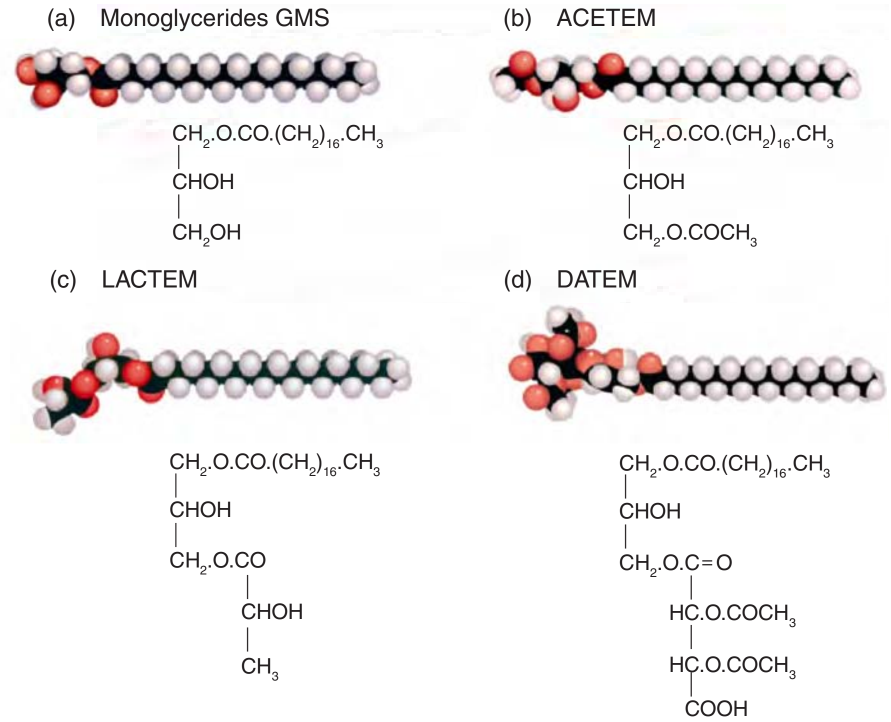


Fig. 10.2 Chemical structure and molecular models of glycerol monostearate (GMS) and the main component of organic acid derivatives of monoglycerides. ACETEM, mono-acetylated monoglycerides; LACTEM, lactylated monoglycerides; DATEM, diacetyl tartaric acid ester of monoglycerides.

solid state of such products. The melting points of organic acid esters of monoglycerides are, thus, considerably lower than those of corresponding monoglycerides.[7]

Acetylated monoglycerides (ACETEM) E. 472a; US FDA §172.828

Acetylated monoglycerides are based on distilled monoglycerides that are reacted with acetic acid anhydride to form acetic acid esters. The degree of acetylation may vary from 50% to 90%, forming either mono-acetylated or di-acetylated monoglycerides. ACETEMs may also be produced by interesterification of a blend of triacetin, triacylglycerols and glycerol. In this case, the final composition includes a minor content of mono-, di- and triacylglycerides. The most common fatty acids in ACETEM are palmitic and stearic acid, forming plastic products. Liquid types of fully acetylated monoglycerides based on unsaturated fatty acids, such as oleic acid, are also available. Partially acetylated monoglycerides are lipophilic, non-ionic, low-polar emulsifiers with α-crystalline properties. Fully acetylated monoglycerides are not surface active, and may be classified as speciality fats.

Lactylated monoglycerides (LACTEM) E. 472b; US FDA §172.852

Lactic acid esters of monoglycerides are made by reacting 15–35% lactic acid with monoglycerides, or by direct esterification of glycerol, fatty acids and lactic acid. LACTEMs contain a number of isomeric compounds, where the lactic acid is esterified to OH groups in the 2- or 3-position on the monoglyceride molecule.

Water-soluble components, such as esters of glycerol and lactic acid, must be removed as they cause unpleasant off-flavours. A further complication is that lactic acid has a strong tendency to polymerise, forming dilactate and trilactate esters of monoglycerides. When considering whether to vary the lactic acid content and fatty acid profile, the overall composition of LACTEMs can be very complex, and a number of commercial products with different analytical specifications exist. The main fatty acids in LACTEM are palmitic and stearic acid. LACTEMs are low-polar emulsifiers with non-ionic, α-crystalline properties.

Diacetyl tartaric acid esters of monoglycerides (DATEM) E. 472e; US FDA §184.1101

Esterification of monoglycerides with diacetyl tartaric acid forms one of the most hydrophilic monoglyceride derivatives. The tartaric acid, which is obtained as a by-product from the wine industry, is first of all reacted with acetic acid anhydride, forming diacetylated tartaric acid anhydride, and then reacted with mono-diglycerides or distilled monoglycerides.[3,4,5] The composition of DATEM is very complex and may vary considerably depending on manufacturing conditions. According to the Food Chemicals Codex in the USA, DATEM may contain 17–20% esterified tartaric acid by weight and 14–17% esterified acetic acid. In Europe the EU regulation prescribes a wider variation in composition, allowing 10–40% esterified tartaric acid and 8–32% esterified acetic acid. Monoglycerides of saturated or unsaturated fatty acids may be used as raw materials and DATEMs are available in powder, bead or soft, plastic liquid form. DATEM is an anionic emulsifier because of the free carboxyl group in the molecule and its net charge is, thus, sensitive to variations in pH in aqueous solutions.

DATEM emulsifiers are considerably more hydrophilic than the corresponding monoglycerides. Their anionic properties make them reactive with proteins, a phenomenon utilised in many foods, particularly emulsions and bakery products.

Citric acid esters of monoglycerides (CITREM) E. 472c; US FDA (GRAS (generally recognized as safe) petition)

The manufacture of citric acid esters of monoglycerides is basically an esterification process, where monoglycerides are reacted with 12–20% citric acid. The final citric acid ester mixture may be partially neutralised by alkali-forming sodium or potassium salts, improving water dispersibility and functionality in emulsions. Owing to acyl-isomerisation during the esterification process, the final composition is very complex. CITREM emulsifiers are anionic, water-dispersible emulsifiers which are mainly used in emulsion-type foods.

The properties and typical applications of organic acid esters in foods are summarised in Table 10.2.

Table 10.2 Typical properties and applications of monoglyceride derivatives

Emulsifier	Properties	Typical applications
ACETEM	α-crystalline Non-ionic Fat soluble	Film forming, coating agent (Fruit, frozen meat) Whippable emulsions Cake shortenings
LACTEM	α-crystalline Non-ionic Fat soluble	Whippable emulsions Powdered topping bases Cake shortenings Cake improvers
DATEM	α-crystalline Anionic Water dispersible	Dough strengthener Bread improver Emulsion stabiliser (coffee whiteners, salad, dressings, beverages)
CITREM	α-crystalline Anionic Water dispersible	Emulsion stabiliser (W/O or O/W emulsions, meat products, beverages)

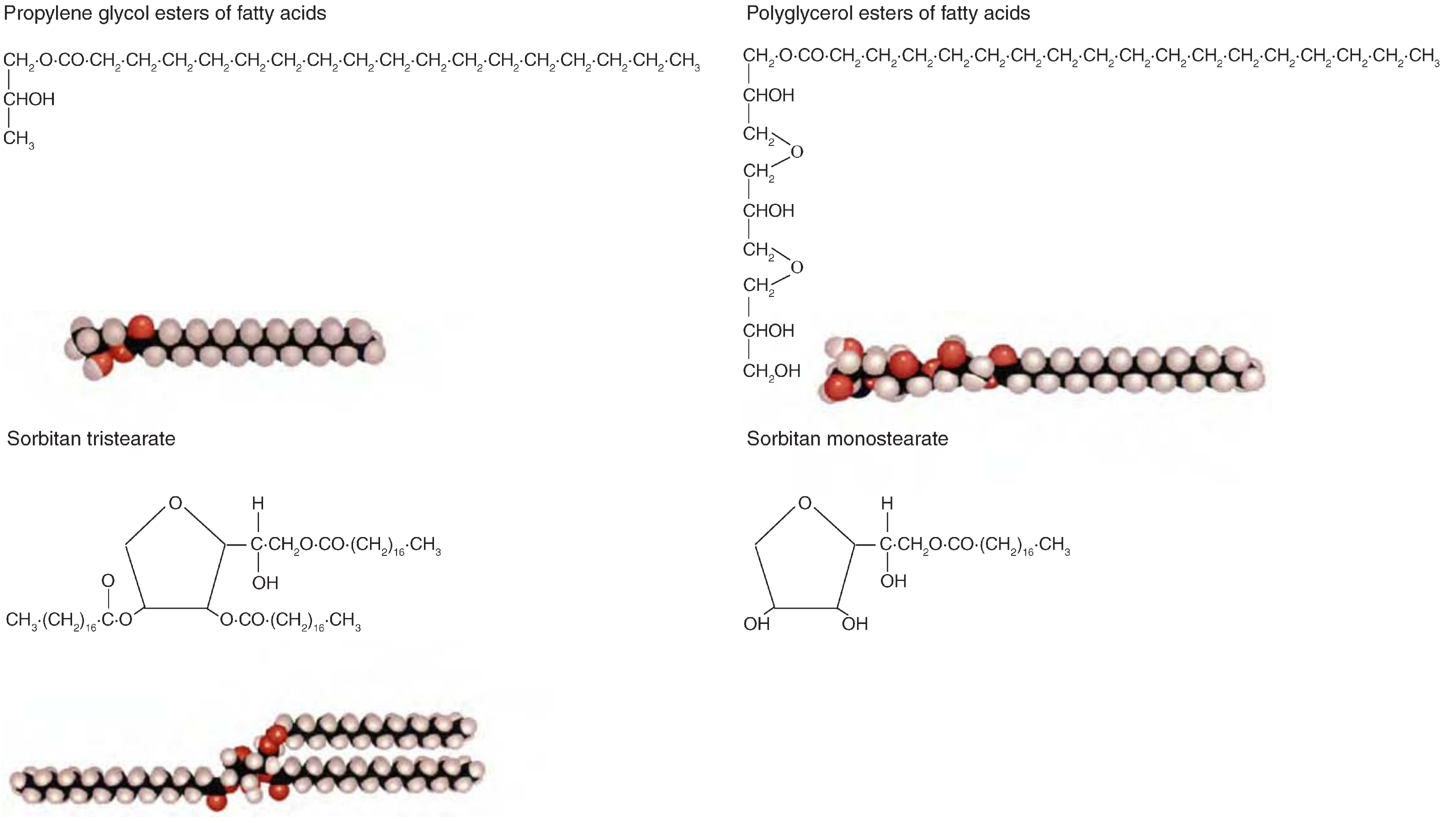
10.2.3 Polyol esters of fatty acids

Esterification of polyols, such as condensed glycerol, propylene glycol, sorbitol/sorbitan or lactic acid with fatty acids, produces a range of emulsifiers with various physicochemical properties, which can be used in food emulsions and fat-based foods. The chemical structure and molecular models of such polyol esters are shown in Fig. 10.3. All fatty acid esters of polyols other than glycerol are generally monomorphic and stable in an amorphous α-like crystal form.

Polyglycerol esters of fatty acids (PGE) E. 475; US FDA §172.854

Glycerol may be polymerised in the presence of an alkaline catalyst at elevated temperatures. The polymerisation is a chain condensation, and a number of different polyglycerols from diglycerol to decaglycerol can be produced. The polymerised glycerol is then esterified with fatty acids, usually palmitic and stearic acids, forming an equilibrium mixture of polyglycerol esters. The aim is usually to optimise the mono-acyl ester content, although di-, tri-, and even tetra-acyl esters may be present because of the high number of free OH groups in polyglycerols. For example, a tetraglycerol has six OH groups as possible esterification sites. Therefore, the composition of polyglycerol esters is very complex with a high number of positional isomers and compounds with a varying degree of polymerisation and esterification.

Esterifying purified diglycerol with fatty acids, followed by a molecular distillation process, can produce concentrated diglycerol monoacyl esters. Such distilled diglycerol esters are hydrophilic, non-ionic emulsifiers with improved properties compared with standard polyglycerol esters.



Propylene glycol esters of fatty acids

Polyglycerol esters of fatty acids

Sorbitan tristearate

Sorbitan monostearate

Fig. 10.3 Chemical structure and molecular models of polyol esters of fatty acids.

Propylene glycol esters of fatty acids (PGMS) E. 477; US FDA §172.856

Esterification of propylene glycol with fatty acids yields an equilibrium mixture of propylene glycol esters, which normally contains approximately 55% monoacyl esters and 45% diacyl esters. The same type of molecular distillation process that is used for monoglycerides is used to produce concentrated propylene glycol monoacyl esters. Distilled propylene glycol esters contain a minimum of 90% monoacyl esters and are normally based on palmitic and stearic acids in a ratio of approximately 1:1.

PGMS is an oil-soluble, non-ionic, low-polar emulsifier, often used in combination with monoglycerides because of its α-crystalline properties.

Sorbitan esters of fatty acids: sorbitan monostearate (SMS) E. 491; US FDA §172.842 and sorbitan tristearate (STS) E. 492; US FDA (GRAS petition)

Sorbitan is derived from sorbitol by dehydration and can be esterified with fatty acids, usually palmitic and stearic acid blends. Depending on the molar ratio between sorbitan and the fatty acids, sorbitan monoacyl esters (SMS) or sorbitan triacyl esters (STS) are the main components of the final product. Several isomeric sorbitan compounds may be formed during the manufacturing process, and all commercial products are complex mixtures containing various sorbitans (1,4-, 1,5- and 2,5-sorbitans) and iso-sorbide in the form of monoacyl, diacyl and triacyl fatty acid esters. SMS is a non-ionic, water-dispersible emulsifier, while STS is an oil-soluble, α-crystalline emulsifier that is predominantly used as a crystal modifier in fat-based products because of its crystalline compatibility with triglycerides.

Polyoxyethylene sorbitan esters (polysorbates): polysorbate 60, E. 435; US FDA §172.836, polysorbate 65, E. 436; US FDA §172.838 and polysorbate 80, E. 433; US FDA §172.840

The hydrophilic properties of sorbitan monoacyl esters can be strongly increased by reacting the free hydroxyl groups with ethylene oxide yielding polysorbates.[9] Such products are highly soluble in water forming micelles, and a range of products with different fatty acid profiles exists. Polysorbates are non-ionic, hydrophilic emulsifiers mainly used in technical O/W emulsions. Their use in foods is somewhat limited because of their low ADI (acceptable daily intake) value of 0–25 mg kg^{-1} body weight per day, assigned by the FAO/WHO Expert Committee on Food Additives. Their legal use in foods varies according to local food laws.

Sucrose esters of fatty acids E. 473; US FDA §172.859

Sucrose esters are made by esterifying sucrose with fatty acids. By varying the ratio between fatty acids and sucrose, a number of esters with different hydrophilic/lipophilic properties can be made. Sucrose esters containing a high content of monoesters (≅70%) are water dispersible, while sucrose esters with a low monoester content (10–30%) are oil soluble.

Sucrose esters can be used in many types of food from emulsions to starch or fat-based products. However, their low ADI value (0–10 mg kg^{-1} body weight per day) limits their use in foods.

10.2.4 Sodium stearoyl lactylate (SSL) E. 481; US FDA §172.846 and calcium stearoyl lactylate (CSL) E. 482; US FDA §172.844

Lactic acid can be esterified with blends of fatty acids, typically palmitic and stearic acids in a ratio of 1:1, in the presence of sodium or calcium hydroxides. This yields a mixture of sodium or calcium salts of stearoyl lactylates, fatty acid salts and free fatty acids. Lactic acid easily polymerises, forming lactoyl-lactic or poly-lactic acids that give rise to a variety of lactylated compounds. Sodium stearoyl lactylate (SSL) is a versatile, anionic, water-dispersible emulsifier, which is used more frequently than the less water-dispersible but oil-soluble calcium stearoyl lactylates (CSL).

10.2.5 Lecithin E. 322; US FDA §184.1400

Vegetable-based lecithin, derived from rapeseed or soya beans, contains a mixture of various phospholipids and a high amount of unsaturated fatty acids such as linoleic acid (C18:2). The main types of phospholipids in soy lecithin are phosphatidylcholin (PC, approx. 34%), phosphotidylethanolamine (PE, approx. 21%), phosphatidylinositol (PI, approx. 18%), the remaining components being phosphatidic acid and other phospholipids.[10,11] Egg lecithin has a high content of phosphatidylcholin (75–80%), the balance being mainly phosphatidylethanolamine (20%). The fatty acid composition of the phospholipids in egg lecithin is mainly palmitic (C16) and oleic (C18:1).

Lecithin is available in many different compositions ranging from purified PC to mixtures containing all the types of phospholipids found in plant seeds.

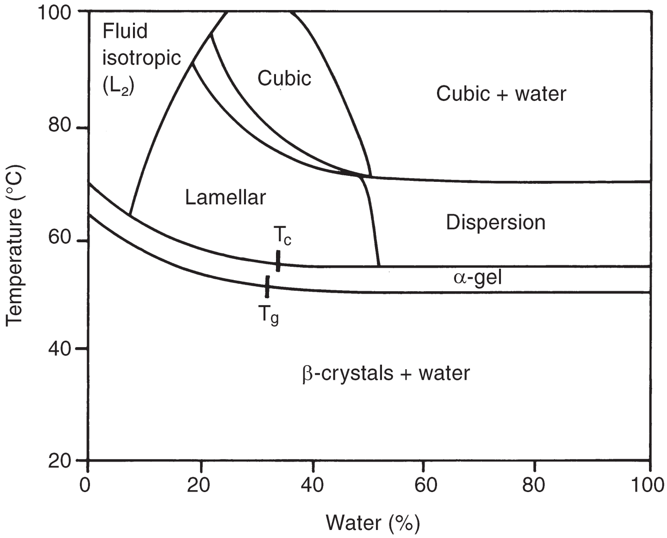
10.2.6 Legal aspects

The local health authorities in each country regulate the use of all food emulsifiers. Evaluation of emulsifiers for foods is done by the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization/World Health Organization (FAO/WHO), which has established the ADI of food additives. In Europe the Commission of the European Communities' Scientific Committee administers food legislation, while in the USA this is done by a department under the Food and Drug Administration (FDA).

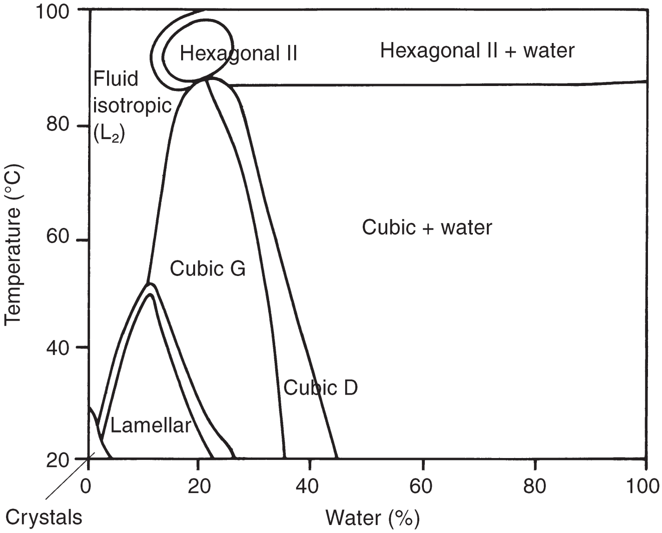
10.3 Association structures with water

10.3.1 Distilled monoglycerides

The ability of distilled monoglycerides to interact with water and form mesomorphic phases and dispersions in aqueous systems is a unique feature, which is utilised in many food preparations where interactions with water-soluble ingredients (e.g. starch components) or the aerating properties of fat-free products are of importance.



(a) Distilled, saturated monoglycerides



(b) Pure 1-monoolein

Fig. 10.4 Binary phase diagrams of monoglyceride–water systems: (a) distilled monoglycerides based on palmitic and stearic acids, (C16/C18 ratio: 35:65); (b) distilled monoglycerides based on oleic acid, (C18:1 content: min. 90 %). (Adapted from Krog.[4])

The phase behaviour of distilled monoglycerides varies according to the fatty acid chain length and the degree of unsaturation,[4,7,12] as shown in Fig. 10.4. The distilled, saturated monoglycerides form lamellar phases or dispersions when heated in water to above their Krafft point, but the lamellar structure is not stable at higher temperatures so a transition to a cubic phase takes place at approximately 70 °C. When preparing diluted dispersions of distilled, saturated monoglycerides in water, it is important not to heat the mixture above this temperature. This is because the cubic phase is not readily dispersible in water, but separates as a viscous, sticky mass adhering to stirring aggregates or container walls.

The distilled, unsaturated monoglycerides form a lamellar phase in water where the water content is very low. At water concentrations above 20%, the cubic phase dominates. The association with water takes place even at low temperatures just above 0 °C. The cubic structure is converted to a hexagonal II structure when heated to higher temperatures. The exact transition temperature is dependent on the fatty acid composition. In the case of distilled monoglycerides based on sunflower oil, hexagonal II phases are formed at approximately 55 °C.

Partially hydrogenated vegetable oils may contain considerable amounts of unsaturated fatty acids with double bonds in *trans* configuration, which has a higher melting point and different phase behaviour in water than corresponding unsaturated monoglycerides with fatty acids in *cis* configuration. The phase behaviour of monoelaidin bears some resemblance to that of distilled, saturated monoglycerides.

The structures of lamellar, cubic and hexagonal II mesophases are illustrated in Fig. 10.5. The swelling behaviour of distilled, saturated monoglycerides is strongly influenced by the degree to which their content of free fatty acids is ionised. The neutralisation of the free fatty acids creates charged molecules in the lipid bilayers, causing electrostatic repulsion effects between the bilayers, which induce higher swelling capacity in dilute systems.[4]

Water purity with regard to ion concentration is highly critical for the swelling process. A minor content of salts shields the electrical repulsion between the ionised groups in the lipid bilayers, reducing the swelling capacity to a minimum where the hydration forces separating the lipid bilayers are in balance with the van der Waals attraction forces which keep the bilayers together. Under carefully controlled conditions with regard to electrostatic repulsion effects and the purity of the aqueous phase, lamellar dispersions of distilled saturated monoglycerides can be made with monoglyceride concentrations of just a few per cent. Such preparations are optically transparent. When cooled to an ambient temperature, they form an opaque, low-viscosity gel with regions of highly ordered lamellar structures.

α-crystalline gel phases and β-coagels

The lamellar phase transforms into a so-called gel phase when cooled below its Krafft point. Owing to the difference in structure, the term 'gel' should not be confused with aqueous gels formed by starch or hydrocolloids. The gel phase has a structure similar to the lamellar state, with lipid bilayers alternating with water layers, as shown in Fig. 10.5a. The difference is that the fatty acid hydrocarbon

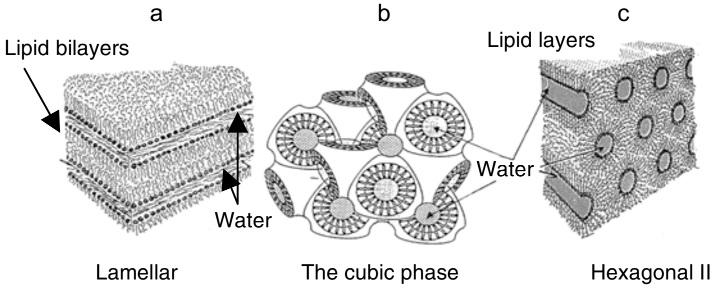


Fig. 10.5 Schematic structure models of liquid crystalline phases. The lamellar phase is two-dimensional with lipid bilayers separated by water layers. The cubic phase consists of a three-dimensional system of curved bilayers, separating two channel systems of water. The reversed hexagonal phase (hexagonal II) contains cylindrical water channels arranged in a hexagonal pattern surrounded by the fatty acid hydrocarbon chains. (From Krog.[4])

chains in the gel are, like α-crystals, in a solid state. By X-ray diffraction analysis, a single short spacing at 4.1 Å can be found, showing hexagonal chain packing.

During storage of the gel phase, the water layer thickness decreases over time unless special conditions are fulfilled with regard to stabilising the α-crystal form of the emulsifier composition. The gel phase of distilled, saturated monoglycerides in water is a very effective aerating agent and commercial emulsifier gel products are often used in the baking industry for the production of cakes and other aerated products. In such emulsifier gels a combination of distilled monoglycerides, α-tending emulsifiers and anionic emulsifiers are used in order to stabilise the α-crystalline structure of the lipid bilayers.

When crystals transform from the α-crystal form to the stable β-crystal form, the monoglycerides lose their ability to hold water between the bilayers. The structure of the β-crystals does not allow interactions with water molecules. The van der Waals attraction forces become dominant, and the hydrogen bonds between OH groups of opposite-oriented bilayers reduce their interaction with water.

These changes result in the formation of a so-called coagel, which is a suspension of monoglyceride β-crystals in water. Commercial monoglyceride–water coagels are often referred to as ‘hydrates’. When the monoglyceride concentration is approximately 20% or higher, the water is bound or entrapped by the monoglyceride crystals. Such a coagel has a viscous, paste-like consistency. Small deformation rheology of monoglyceride–water gels has shown that, with about 2 wt% monoglyceride, a percolating network is formed.[13] Large deformation rheology is typical for a particle gel with a relatively small strain at fracture, both in shear deformation and compression.

The β-crystals in a coagel have a hydrophilic surface of OH groups, because they are formed in an aqueous environment. This is in contrast to crystals formed by cooling a melt (e.g. spray-crystallisation), when the surface of the crystals consists of the non-polar CH_3 end groups of the fatty acid chains. In principle the production of monoglyceride-based ‘hydrates’,[4,14] used in the baking industry as a

Table 10.3 Application of liquid crystalline phases, α-gels and β-hydrates in foods

Function	Lamellar phases	α-gels	β-hydrates
Stabilisation of protein-free O/W emulsions	✓	–	–
Aeration (cakes, creams, fruit purées, etc.)	✓	✓	–
Starch complexing (bakery products, processed potatoes, pasta, cereals)	✓	✓	✓
Texturising of low-fat spreads	–	✓	✓

crumb softener in bread, is based on the hydration of monoglycerides via the lamellar state, followed by cooling and acidification to pH 3 while agitating in order to enhance the formation of the β-crystalline coagel. Commercial 'hydrates' contain 20–50% monoglycerides, and their consistency varies from paste-like to semi-solid.

Monoglyceride coagels are used as texturising agents in low-fat foods (e.g. spreads), owing to their water-binding capacity.[15] The structure of a monoglyceride coagel is very similar to the network of fat crystals formed in fat-based products (e.g. shortening, margarine, spreads). An overview of the application of monoglyceride/water gel phases can be seen in Table 10.3.

Organic acid esters of fatty acids

The esterification of monoglycerides with organic acids considerably alters their polarity and hydration and swelling properties in water. Neither acetylated monoglycerides (ACETEM) nor lactylated monoglycerides (LACTEM) form liquid crystalline mesophases in water owing to their lipophilic characteristics. However, because of their α-crystalline properties, these emulsifiers are capable of absorbing water in the solid state, forming a gel-like structure. When crystals of ACETEM or LACTEM are in contact with water, swelling takes place because of the penetration of water through the polar groups of the emulsifier crystals. The swelling process stops when the hydration force is in balance with the van der Waals attraction between the lipid bilayers. The thickness of the water layers formed is relatively small, usually in the order of 10–20 Å.[4] However, the interaction between water and ACETEM or LACTEM is important for their function in whippable emulsions (non-dairy creams, toppings)[16] and cake shortenings. The formation of α-gel structures in emulsions takes place on the surface of the fat globules, inducing agglomeration under shear and improving aeration properties and foam texture.

Esters of dicarboxylic organic acids and monoglycerides, such as diacetyl tartaric acid esters (DATEM), contain a free carboxyl group. Their swelling properties in water strongly depend on the ionisation of this carboxyl group. When DATEM is dispersed in water, the pH is about 1–2, and very little swelling, if any, takes place. If the free carboxyl group in DATEM is partly or totally neutralised by alkali to a pH above 5, swelling increases and lamellar phases are formed. DATEM based on unsaturated monoglycerides swells readily at room temperature, while DATEM based on saturated C16/C18 fatty acid monoglycerides has a Krafft point of about 45 °C. In contrast to monoglycerides, the lamellar phase is the only crystalline phase formed by DATEM in water at a temperature of 45–100 °C. This lamellar phase, formed at a pH above 5, is stable even when cooled below the Krafft point.

Polyol esters of fatty acids

Fatty acid esters of polyglycerol are highly complex, comprising components with various degrees of polyol polymerisation and esterification. Depending on the content of monoacyl fatty acid esters, they may form liquid crystalline phases in water above their Krafft point, which is the same as the melting point of polyglycerol esters owing to their α-crystalline properties.

Commercial products may contain a small percentage of sodium salts of fatty acids, increasing their swelling properties in water and promoting the formation of lamellar structures. Aqueous preparations of polyglycerol esters in the form of α-crystalline gels are used as aerating agents in the baking industry.

Concentrated diglycerol fatty acid esters with a minimum of 80% monoacyl esters swell in water, forming lamellar phases or multi-lamellar vesicles in diluted dispersions.[17] Figure 10.6 shows the Krafft temperatures (T_c lines) of diglycerol esters of saturated fatty acids with a chain length of C12–C18 and of unsaturated diglycerol monooleate (C18:1*cis*). All diglycerol esters form lamellar phases above their respective T_c line at water concentrations of 10–43%. When the water content is higher, lamellar dispersions or multi-lamellar liposomes or vesicles are formed.

Diglycerol monooleate forms lamellar phases at all temperatures above 0 °C, which is remarkably different from the phase behaviour of the corresponding unsaturated monoglycerides which form cubic mesophases in water. Diglycerol esters can stabilise (O/W) emulsions by forming liquid crystalline interfacial films around the dispersed oil droplets. This may be utilised as a new option in protein-free food emulsions.

Sodium stearoyl lactylate (SSL)

The phase behaviour of SSL in water varies according to the pH of the system as SSL contains a considerable amount of free fatty acids. When SSL is dispersed in water at a pH below 5, it forms hexagonal II phases above its Krafft point (approx. 45 °C) At a higher pH, the fatty acids are ionised and lamellar phases form at a temperature of 45–85 °C.[4] Combinations of SSL and distilled, saturated monoglycerides are used as aqueous dispersions to improve the aeration of bakery products (cakes) or low-fat, non-dairy whipping creams.[15]

Lecithin

The phase behaviour of lecithin in water varies with its chemical composition and origin. When dispersed in water, egg lecithin forms lamellar phases or multi-lamellar vesicles, depending on the concentration and temperature.[12]

The temperature stability of the lamellar phases of lecithin is much higher than that of other lipid/water systems, and lecithin preparations with a high content of

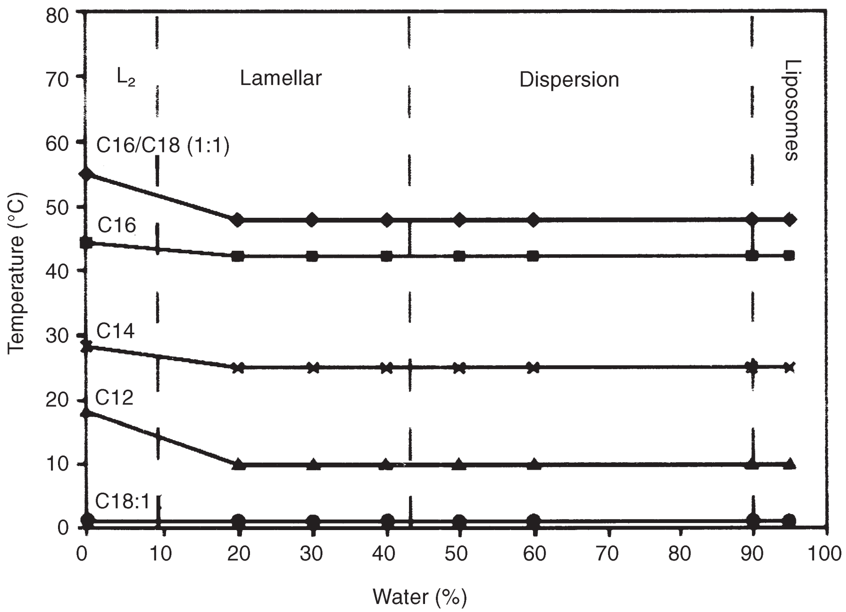


Fig. 10.6 Krafft temperatures (T_c lines) of distilled diglycerol fatty acid esters in water: C18:1, diglycerol monooleate; C12, diglycerol monolaurate; C14, diglycerol monomyristate; C16, diglycerol monopalmitate; C16/18 (1:1)) diglycerol esters of palmitic/stearic acid mixture in a ratio of 1:1. All diglycerol esters form lamellar phases at temperatures above their respective T_c lines at water concentrations between 10 and 43%. At higher water concentrations a dispersion of multi-lamellar vesicles is formed. (From Krog.[7])

phosphatidylcholin (PC) form stable liposomes. The high membrane stability of pure PC is utilised in protein-free O/W emulsions, where pure PC provides good emulsion stability against coalescence owing to the formation of a liquid crystalline layer at the interface.

The phase behaviour of soy lecithin components varies considerably.[18] Phosphatidyl choline and phosphatidylinositol form lamellar phases with water in all combinations, while the more lipophilic phosphatidylethanolamine forms reversed hexagonal phases. Consequently, the phase behaviour of commercial lecithin products is highly dependent on their composition.

10.4 Interfacial properties of emulsifiers

The fundamental functional property of an emulsifier is the ability to reduce the surface or interfacial tension between the air/water or oil/water interface, respectively. The ability to reduce the interfacial tension is caused by the amphiphilic nature of emulsifiers and their interfacial adsorption is energetically favourable.

During the emulsification of oil in water, the oil phase is dispersed in the water

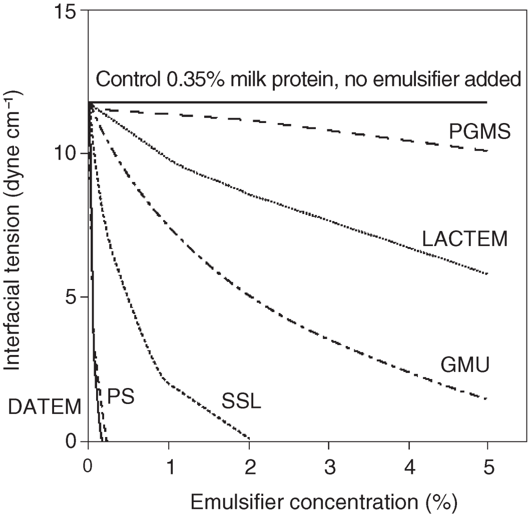


Fig. 10.7 Interfacial tension of different emulsifiers. The graph shows the interfacial tension obtained with different emulsifiers at the sunflower (O/W) interface. The water phase was prepared with 0.35% skim milk protein. Emulsifiers: DATEM, diacetyl tartaric acid ester of monoglycerides; PS, polysorbate 60; SSL, sodium stearoyl lactylate; GMU, unsaturated monoglycerides; LACTEM, lactic acid ester of monoglycerides; PGMS, propylene glycol monostearate.

phase, and the oil droplets are broken into finer droplets by applying homogenisation energy. The oil droplet size obtained is highly important to the properties of the emulsion with respect to stability towards separation caused by flocculation, coalescence and creaming. The disruption of oil particles into finer droplets is affected by both the interfacial tension and the homogenisation energy used to manufacture the emulsion. However, the emulsification of oil-in-water (O/W) emulsions nearly always occurs under turbulent flow conditions,[19] and the relative contribution of interfacial tension to oil droplet disruption is very low compared to the influence of the homogenisation energy applied.

In water-in-oil (W/O) emulsions, such as margarine and spreads which are manufactured with much lower mixing energy input than O/W emulsions, the reduction of interfacial tension is more important to water droplet size, and there is a correlation between the ability of an emulsifier to reduce interfacial tension and the production of fine droplets. Figure 10.7 shows the interfacial tension provided by various emulsifiers as a function of concentration.

In general, then, food emulsifiers are not often used to facilitate emulsification as such, but rather to enhance the stability of different food emulsions and induce partial fat destabilisation in whipped emulsions.

10.4.1 Competitive interfacial adsorption of protein and emulsifiers

Almost all foods are highly complex colloidal systems, where at least two classes of molecule have a strong affinity for the interface – the proteins and the surface-

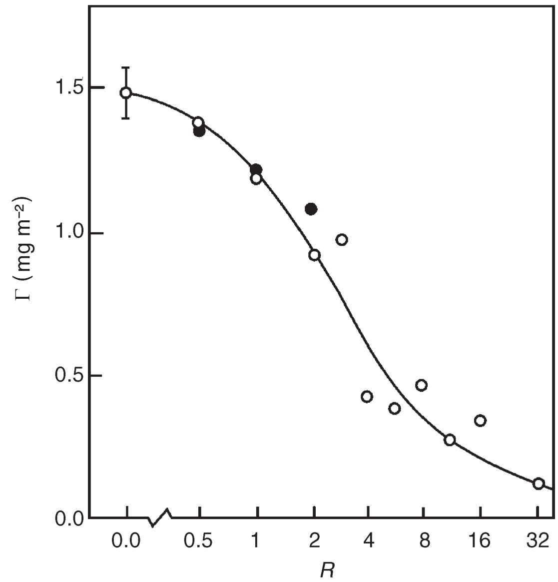


Fig. 10.8 Protein surface coverage of α-lactalbumin stabilised O/W model emulsion (10% (w/w) *n*-tetradecane, 0.45% (w/w) protein) as a function of *R*, which is the molar ratio of added Tween 20:protein. (Adapted from Dickinson.[20])

active lipids, which compete for interfacial adsorption. In dairy products and many other foods where milk proteins are added, the interfacial film is, thus, a mixture of milk proteins and surface-active lipids (native phospholipids or added emulsifiers). The actual composition of the interfacial film depends on the chemical structure and relative concentrations of the proteins and surface-active lipids. A randomly ordered protein, such as β-casein, adsorbs, providing a thick film with long tails protruding from the interface, whereas β-lactoglobulin adsorbs with the whole molecule closer to the interface, resulting in a thinner film.[20] The presence of emulsifiers exerts a great effect on the adsorbed proteins, in many cases by displacing them quantitatively from the interface.

Non-ionic hydrophilic emulsifiers, such as Tween 20, are particularly effective in displacing proteins from the interface at room temperature compared with more hydrophobic non-ionic emulsifiers, such as monoglycerides[20–22]. Figure 10.8 shows the displacement of α-lactalbumin with Tween 20 as a concentration of molar ratio (*R*) of emulsifier to protein. It is seen that, at a low emulsifier concentration ($R < 1$), the protein surface coverage is effectively unaffected by the addition of emulsifier, whereas a high emulsifier concentration ($R > 8$) leads to almost complete displacement of the protein from the interface, resulting in the loss of emulsion stability.

Whereas lipophilic emulsifiers are less efficient at displacing protein from the interface,[22,23] there is a synergistic displacement of protein when hydrophilic and lipophilic emulsifiers are used in combination.[21] Figure 10.9 shows the competitive displacement of β-casein as a function of Tween 20 concentration (*R* = the

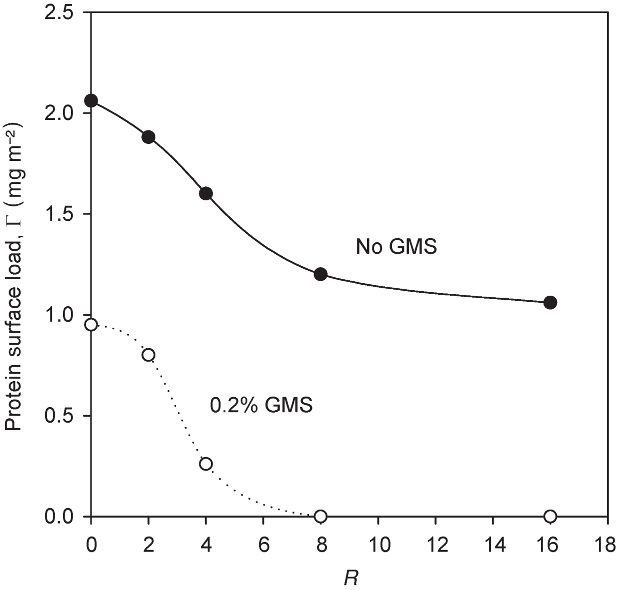


Fig. 10.9 Protein surface coverage of β-casein emulsion droplets (0.4 wt% protein, 20 wt% soy oil, 20 °C) as a function of *R*, which is the molar ratio of Tween 20:protein. The graphs show the effect of adding Tween 20 when no monoglyceride (GMS) is present (filled symbols) or when 0.2 wt% GMS is present (open symbols). (Adapted from Dickinson and Tanai.[57])

ratio of Tween 20 to β-casein), when monoglycerides are added in different concentrations. It can be seen that, with 0.2% monoglycerides present Tween 20 completely displaces β-casein at $R = 8$, whereas without monoglycerides, the protein load is only reduced by 50% at the same concentration of Tween 20.

Proteins are adsorbed to the surface of oil droplets in segments.[24] At a low emulsifier concentration, a mixed interfacial film of adsorbed emulsifiers and proteins is formed. The emulsifiers may be adsorbed in between segments of the hydrophobic parts of the protein molecules, explaining why low emulsifier–protein ratios do not cause protein desorption. It has even been found that low concentrations of monoglycerides can increase the adsorption of proteins to the interface.[23]

The mechanism of protein displacement has been studied by atomic force microscopy (AFM) which shows that, initially, the emulsifiers are adsorbed at the defects in the protein film, and these sites grow with increasing emulsifier adsorption, resulting in compression of the protein film,[25] as shown in Fig. 10.10. When the emulsifier has generated a sufficiently high surface pressure, the interfacial protein film buckles into thicker layers, before it is finally released into the serum phase in the form of protein aggregates. These findings concur with earlier studies, where freeze–fracture transmission electron microscopy showed protein desorption from fat globule surfaces in the form of aggregates.[26]

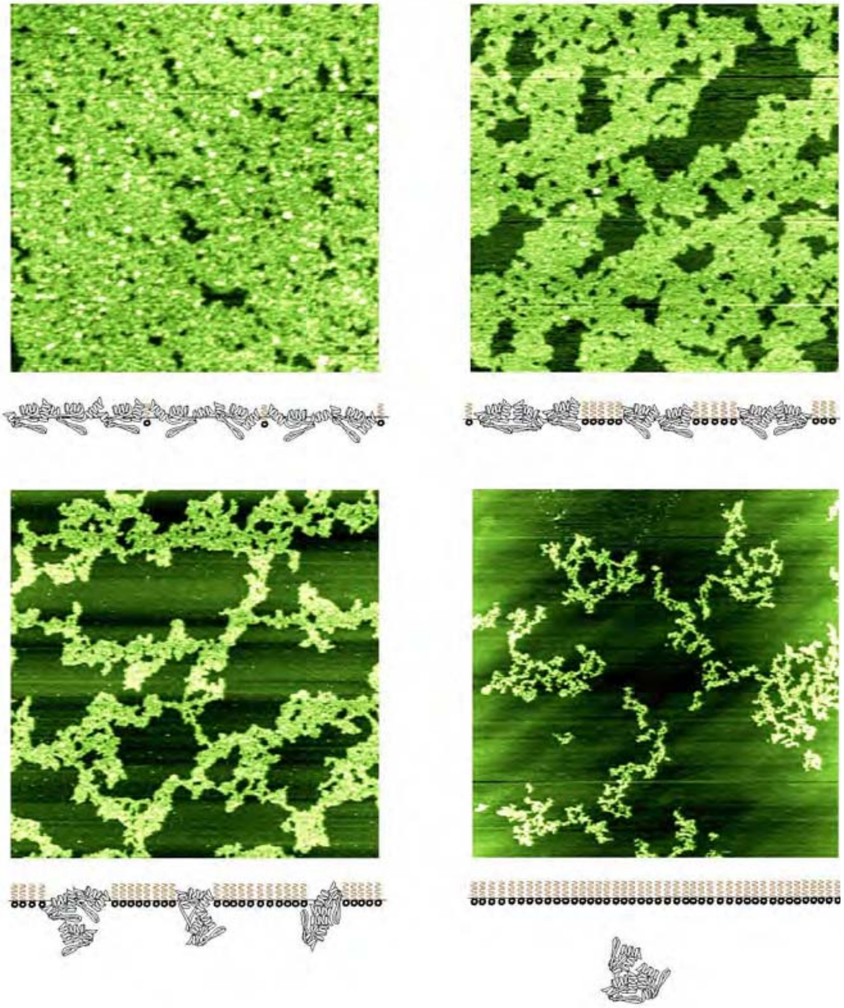


Fig. 10.10 AFM images showing the progressive displacement of an interfacial β-lactoglobulin film induced by addition of Tween 20. Initially, the surfactant areas (black) increase, resulting in folding of the protein molecules and causing the protein film to thicken locally. Finally (bottom right) the protein network breaks up into unconnected aggregates. The protein is then displaced into the bulk solution. Beneath each image, the mechanism is illustrated schematically. (Courtesy of Institute for Food Research, Norwich, UK, www.ifr.bbsrc.ac.uk.)

The effectiveness of a given emulsifier with regard to displacing protein from the interface greatly depends on the affinity of the emulsifier for the interface. For many lipid-based emulsifiers, this depends on the temperature. Low-polar emulsifiers (i.e. monoglycerides) have been found to increase the protein surface load at high temperature (70 °C), when added in low molar concentrations (R = 1–2),

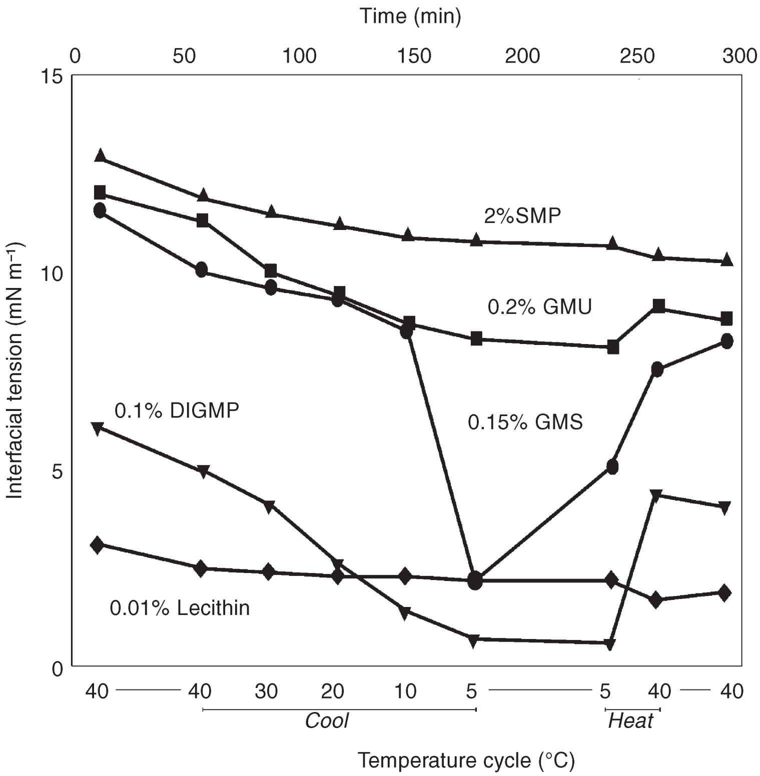


Fig. 10.11 Interfacial tension of oil/water interfaces as a function of temperature. Added emulsifiers are melted in the oil phase prior to the formation of the interface, and 2% milk protein (SMP) is dissolved in the water phase. Temperature programme: start at 40 °C, hold for 1 hour, cool at 0.3 °C/min to 5 °C, hold for 1 hour, heat at 2 °C/min to 40 °C, hold for 30 min. GMU, unsaturated monoglycerides; GMS, saturated monoglycerides; DIGMP, diglycerol monopalmitate.

while a decrease in protein surface load occurs at higher emulsifier concentrations ($R = 8$). Diacylglycerides have no effect on protein adsorption or displacement[23]. Interfacial tension measurements of O/W systems containing proteins and emulsifiers are correlated to the quantitative protein displacement and can be used to predict such phenomena.[27]

Figure 10.11 demonstrates the effect of emulsifiers on the interfacial tension between sunflower oil and water as a function of temperature. The emulsifiers are dissolved in the oil phase and the water phase contains 2% skim milk protein. The interfacial tension between pure sunflower oil and water is 27 mN m^{-1}, and this is reduced to about 12 mN m^{-1} by milk proteins. After the addition of emulsifiers to the oil phase the tension is further reduced, depending on the temperature and the type of emulsifier used. Both saturated monoglycerides and unsaturated monoglycerides reduce the interfacial tension slightly below the values for protein in the temperature interval between 40 °C and 10 °C. This corresponds to the formation of a mixed emulsifier–protein surface film with low emulsifier adsorption (effectively low *R*). Below 10 °C a strong difference between saturated and unsaturated monoglycerides is found: saturated monoglyceride shows a strong reduction in tension, while this is not the case with unsaturated monoglyceride. The difference between the effect of saturated and unsaturated monoglycerides on interfacial tension at a low temperature is related to the crystallisation of the emulsifiers. The saturated monoglycerides have higher Krafft temperatures than the unsaturated ones.

The more hydrophilic emulsifiers, diglycerol monopalmitate, lecithin or polysorbates (not shown), cause a strong reduction in interfacial tension because of their hydrophilicity, and their effect on interfacial tension is less affected by temperature. A strong reduction in surface tension is due to strong adsorption of emulsifiers at the interface, and consequently more interfacial protein is displaced. However, total displacement of protein does not take place in food emulsions where the protein concentration is fairly high.

Just as there are protein–emulsifier interactions in emulsions, protein–protein interactions take place in complex food emulsions. More surface-active proteins may displace less surface-active proteins, e.g. β-casein will displace α-casein to a larger extent than vice versa.[28] It has also been proved that gelatine can be displaced by casein when casein is added to emulsions stabilised by gelatine.[29] Ageing phenomena also play an important role with regard to the displacement of one protein by another, and some proteins are more difficult to displace owing to a specific structure at the interface. For example, it has been shown that β-lactoglobulin is more difficult to displace from the oil/water interface than α-lactalbumin when adding β-casein.[30] The difficulties in displacing β-lactoglobulin from the interface are possibly due to the polymerisation of β-lactoglobulin through disulfide bonds at the interface.[31]

Destabilisation of the fat phase under well-defined conditions is very important in whipped emulsions (creams, ice cream, etc.) and can be achieved by adding emulsifiers of various polarities. The destabilisation process involves several physical changes in the emulsion at low temperature, such as partial desorption of interfacial protein, as previously described, and crystallisation of the fat phase; both result in a decrease in emulsion stability under shear. It is well known that fat crystallisation is important for obtaining partial coalescence,[32] and adding various emulsifiers can influence the degree of fat crystallisation and type of fat crystals. It has been shown that different fat crystals are formed in the presence of water with saturated and unsaturated emulsifiers, respectively. Both in a model system and in ice cream mix, it has been found that the application of unsaturated monoglycerides results in the growth of large, spiky fat crystals, whereas saturated monoglycerides produce smaller, more regularly structured fat crystals.[33,34]

Although it is well documented that some protein desorption takes place in whippable emulsions, it should be noted that extensive protein displacement from the fat globule surface is not necessary in order to trigger flocculation of fat globules under shear, referred to as orthokinetic instability.[35] A comparison of the effect of small amounts of hydrophilic emulsifiers on the destabilisation of protein-

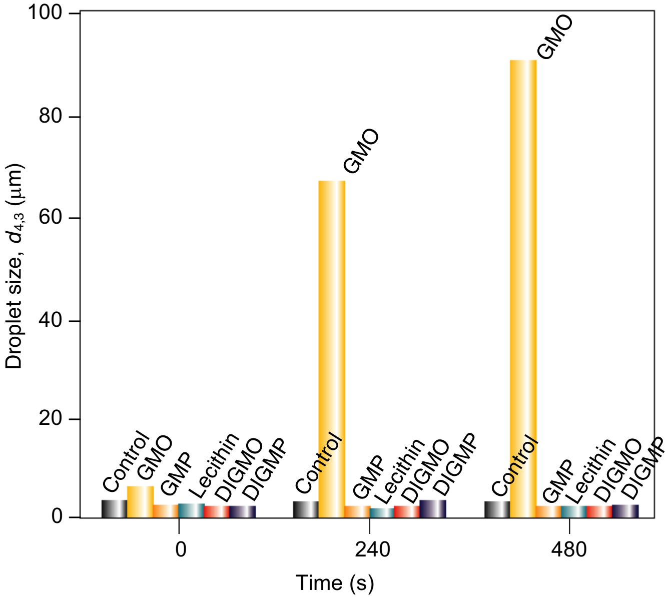


Fig. 10.12 Shear-induced destabilisation of model emulsions (10% HCNO). The graph shows the average droplet size in the emulsion ($d_{4,3}$) as a function of shearing time and emulsifier type. The emulsions were sheared at 5 °C. Control, without emulsifier, 3% skim milk protein; GMO, 0.1% glycerol monooleate; GMP, 0.1% glycerol monopalmitate; lecithin, 0.1% concentrated lecithin; DIGMO, 0.05% diglycerol monooleate; DIGMP, 0.05% diglycerol monopalmitate. (Courtesy of Danisco, Denmark.)

stabilised emulsions under shear has shown that the concentration of emulsifier required to destabilise and aggregate the oil droplets is lower than the concentration needed for protein desorption (R = 1). Furthermore, a decrease in the apparent surface shear viscosity of the interfacial film appears to be correlated to the decrease in emulsion orthokinetic stability and takes place at emulsifier concentrations below those required for actual protein desorption (R = 1)[,35] Thus, the influence of emulsifiers on the rheological properties of the interfacial film may be more important than their ability actually to displace protein with respect to the partial destabilisation needed in aerated emulsions.

Figure 10.12 shows the destabilisation of vegetable cream-type emulsions prepared with different emulsifiers. Among the tested emulsifiers, it is clear that the highest degree of destabilisation is achieved with unsaturated monoglycerides. The difference between the effects of unsaturated and saturated monoglycerides respectively does not correlate with protein displacement, but may instead be related to differences in the structure of the interfacial film. This is supported by

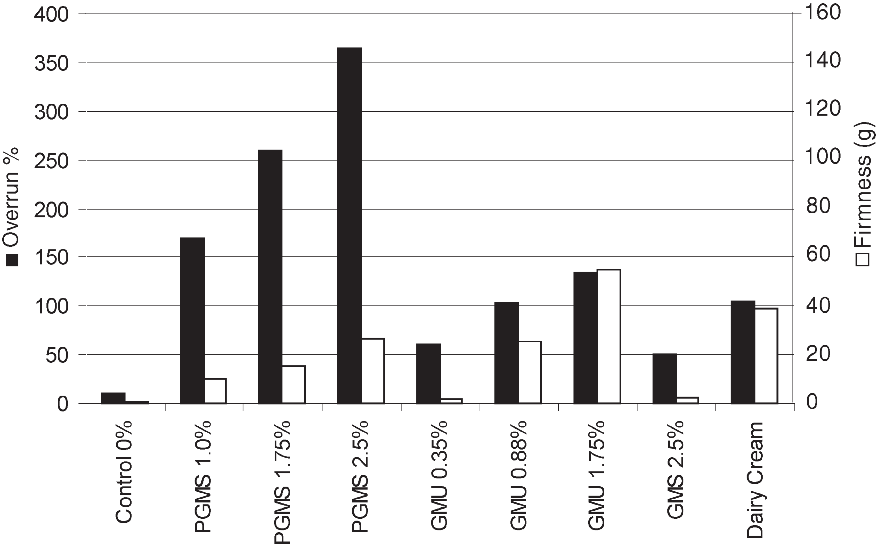


Fig. 10.13 Properties of topping foam made from reconstituted topping powders containing different emulsifiers. Control, no emulsifier added; PGMS, propylene glycol monostearate; GMU, distilled, unsaturated monoglycerides; GMS, distilled, saturated monoglycerides. The black columns show the percentage overrun of the foams, and the white columns show the firmness of the foams.
(Courtesy of Danisco, Denmark.)

results from surface monolayer film studies which have shown that, at the air/monoglyceride interface, the condensed monopalmitin–protein mixed films has a higher surface dilatational modulus (*E*) than the more expanded monoolein/protein films at an identical surface pressure.[36]

Hence, the function of emulsifiers in aerated emulsions is to increase shear-induced destabilisation of the emulsion, resulting in the formation of clusters of agglomerated fat particles that, during aeration, form a structure around and between the air cells, stabilising the foam structure. The functionality of an emulsifier with regard to the destabilisation and aggregation of the fat phase in whipped emulsions is explicitly important to the final volume and texture of the finished product. The agglomerated fat phase contributes to the stability and creaminess of the aerated product. In ice cream, the texture and meltdown resistance are directly related to the amount of destabilised fat.[37] During studies of the function of emulsifiers in ice cream mix and other emulsions[37–41] polysorbates (Tween 60) and unsaturated monoglycerides (GMU) have been found to have a stronger destabilising effect than saturated monoglycerides (GMS).

In general, the fat–emulsifier–protein interactions observed at the air/water interface of many different whipped products provide a unifying mechanism for the stabilisation of air in different food systems. Figure 10.13 shows the properties

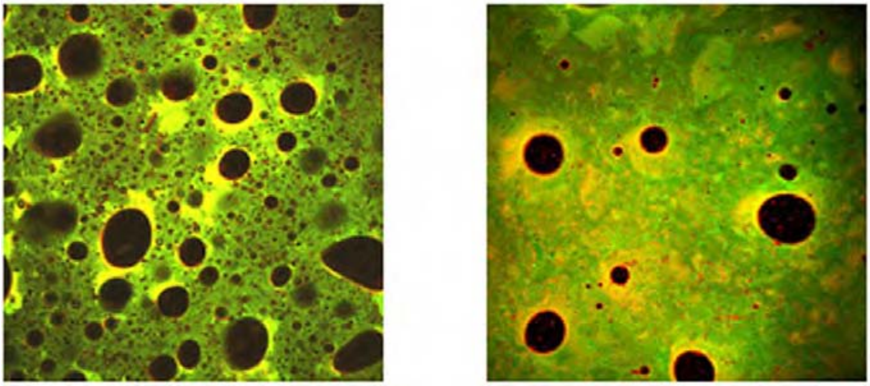


Fig. 10.14 Confocal laser scanning micrographs of mousse. Left**:** mousse prepared with unsaturated monoglycerides (GMU). Right: mousse prepared with saturated monoglycerides (GMS). Image size is 1.5 mm × 1.5 mm. Fat was stained with Nile Red and protein with FITS, both appearing white in the images, while the air bubbles are black. It is clear that GMU provides many smaller air bubbles, owing to its increased destabilisation of the emulsion during whipping. The average air bubble diameter is shown in each image. (Courtesy of Danisco, Denmark.)

of reconstituted toppings prepared with different emulsifiers. It appears that an emulsifier such as PGMS provides a high overrun but a relatively soft foam. Unsaturated monoglycerides, on the other hand, provide a moderate overrun, but a very firm foam, owing to the considerable ability of unsaturated monoglycerides to destabilise the fat phase. Saturated monoglycerides provide low overrun and low foam firmness in spray-dried emulsions (toppings). The difference between saturated and unsaturated monoglycerides when destabilising whipped products can also be seen in mousse products. Figure 10.14 shows the microstructure of mousses prepared with saturated and unsaturated monoglycerides, respectively. Whereas the unsaturated monoglycerides secure mousses with excellent aeration (small air bubbles) and a firm texture, saturated monoglycerides result in a soft mousse (with few, large air bubbles) which partially collapses during storage.

10.4.2 Cooperative interfacial adsorption of protein and emulsifiers

In contrast to the protein displacement caused by non-ionic emulsifiers (polysorbates, monoglycerides), cooperative interactions have been found to take place at O/W interfaces between protein and anionic emulsifiers,[23] such as diacetyl tartaric acid ester of monoglycerides (DATEM). This does not displace interfacial protein significantly, even at a high molar emulsifier to protein ratio (*R*).

The use of DATEM in protein-stabilised emulsions results in the formation of a mixed emulsifier–protein film, where the protein is bound at the oil droplet surface by both hydrophobic and electrostatic forces. Studies of the electrophoretic mobility of emulsion droplets have shown that, by adding DATEM to the emulsion,

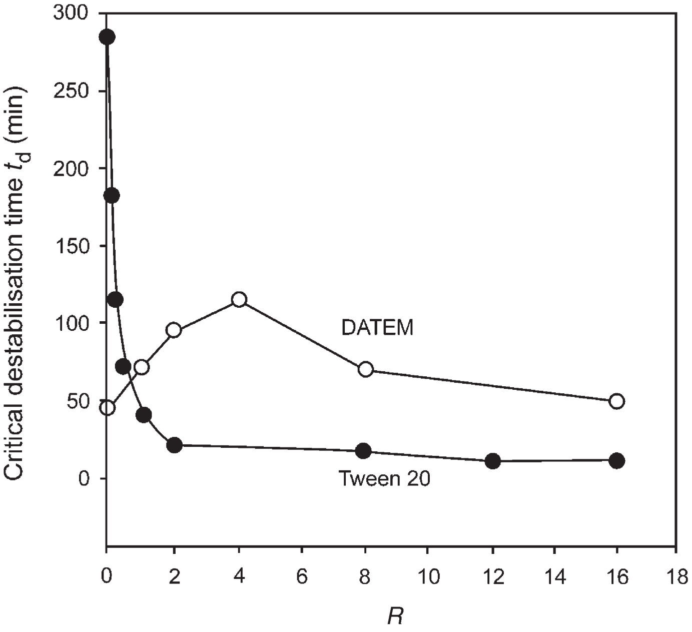


Fig. 10.15 Influence of different emulsifiers on the critical destabilisation time, (t_d), of emulsions. DATEM or Tween 20, respectively, were added to fresh β-lactoglobulin stabilised emulsions, and the emulsions were sheared in order to induce orthokinetic destabilisation. The graph shows t_d when DATEM is added to the emulsion (0.4% protein, 10% oil) (open symbols) and when Tween 20 is added to the emulsion (0.45% protein, 20% oil) (closed symbols). (Adapted from Hong[42] and Dickinson *et al.*,[35] respectively.)

the net negative charge (zeta potential) of the protein-stabilised droplets is increased,[42] indicating that DATEM forms a complex with interfacially adsorbed protein, strengthening the film towards rupture.

The strengthening of the interfacial film by the addition of DATEM can also be measured by interfacial rheology. Measurements have shown that, when adding DATEM, the surface shear viscosity of the interfacial protein film is maintained or even increased.[23] The increase in surface viscosity with time after DATEM addition contrasts with the effect of polysorbate (Tween 20), which causes a strong decrease in the surface shear viscosity.

The cooperative interactions between DATEM and protein at the interface increase the orthokinetic stability of oil-in-water emulsions as an additional energy barrier has to be overcome before droplet coalescence can take place.[43] Figure 10.15 compares the critical destabilisation time for emulsions prepared with DATEM or polysorbate (Tween 20), respectively. The critical destabilisation time is the time it takes for the oil droplet to begin increasing in size owing to droplet coalescence under shear. Whereas the addition of Tween 20 decreases the critical destabilisation time, DATEM increases the critical destabilisation time until added in a molar ratio of 8, which is about four times the relative amount used in typical

Table 10.4 Amylose-complexing effect of emulsifiers

Type of emulsifier	Amylose-complexing index*
Distilled monoglycerides:	
based on hydrogenated palm oil	92
based on hydrogenated soy bean oil	87
based on palm oil	35
based on soy bean oil	28
Mono-diglycerides:	
based on hydrogenated palm oil (50% mono-ester)	42
Organic acid esters of monoglycerides:	
acetylated monoglycerides	0
lactylated monoglycerides	22
diacetyl tartaric acid esters	49
Propylene glycol monostearate (90% mono-ester)	15
Sorbitan monostearate	18
Polysorbate 60	32
Sodium stearoyl-lactylate	72
Lecithin (from soy bean oil)	16

* mg amylose precipitated from a solution containing 100 mg amylose/100 ml by 5 mg emulsifier at 60 °C.

dairy emulsions. DATEM and other anionic emulsifiers are effectively used to increase the emulsion stability of various dairy-based emulsions (such as liquid coffee whiteners).

10.5 Emulsifier–carbohydrate interactions

Starch-based foods, such as bakery products, extruded cereals, processed potatoes and pasta foods, contain emulsifiers in order to facilitate industrial production, prolong shelf-life and improve texture. Starch consists of two types of polysaccharides – amylose and amylopectin. In wheat starch, the ratio between these two components is approximately 1:4. Amylose is the water-soluble part of starch and may cause various undesirable effects, such as stickiness in dough or starch-based foods. Soluble amylose retrogrades 6–8 hours after preparation. This is the reason for the well-known increase in bread firmness after baking and is part of the staling process.[44]

When monoacyl lipids are present, the amylose and lipid will react, forming a water-insoluble complex, thus reducing the stickiness of the dough and reducing the retrogradation of the starch fraction. Wheat starch contains amylose complexes with native lysolecithin, added emulsifiers forming similar helical inclusion compounds with amylose. The amylose-complexing ability of emulsifiers is strongest with monoacyl lipids, such as distilled, saturated monoglycerides with a chain length of C16–C18 or stearoyl lactylates, as shown in Table 10.4.

The complexing effect of various types of emulsifiers varies according to their fatty acid chain length, degree of unsaturation, degree of esterification (content of di- and triacyl esters) and the dispersibility of the emulsifiers in water.[45,46] When heated, the amylose–lipid complexes 'melt' or dissociate at temperatures of around 100 °C. The thermal stability of amylose complexes with monoglycerides increases with an increasing fatty acid chain length and decreases with increasing unsaturation. The resistance of the amylose–monoglyceride complexes to enzymatic hydrolysis varies according to the same pattern.[47] The formation of the amylose complex with monoglycerides is a typical ingredient interaction, improving the texture and extending the shelf-life of starch-based foods.

10.5.1 Crumb softening effects of emulsifiers in wheat bread

When wheat bread is stored, it becomes increasingly firm, partly because of the crystallisation of the free amylose, and partly because of the retrogradation of the amylopectin fraction of wheat starch. When monoglycerides are added, they complex primarily with free amylose and reduce the amount of amylose available for retrogradation. If the concentration of monoglycerides is high enough, complex formation with amylopectin may take place, reducing amylopectin retrogradation.[48] The relationship between the amylose-complexing effect of saturated monoglycerides and breadcrumb firmness is shown in Fig. 10.16.

When monoglycerides are added to wheat bread, the amount of amylose–lipid complexes increases in proportion to the concentration of monoglycerides added and reaches an optimum when the monoglyceride concentration exceeds 1%. At a level of approximately 1.5% monoglyceride, all free amylose is bound. For comparative purposes, the formation of amylose complexes with DATEM emulsifiers is also shown, being considerably lower than that of monoglycerides. Figure 10.16b demonstrates the effect of increased monoglyceride addition on the crumb firmness of wheat bread compared to a control without emulsifiers added. Crumb firmness decreases as a function of added monoglyceride concentration.

The staling of wheat bread is a very complicated process involving many chemical or physical changes in the bread – the retrogradation of the starch fraction being only one of them. Another approach to delaying the staling process in wheat bread is the application of enzymes, which modify the structure of amylopectin and slow the firming rate.[49]

Starch-based foods containing partly gelatinised starch tend to develop a sticky texture, as seen in potato and rice products, cornflakes, pasta, etc. This sticky texture is due to the water-soluble amylose fraction of starch, and can be reduced by adding starch-complexing lipids, such as glycerol monostearate.

10.6 Emulsifier–fat interactions

The crystallisation behaviour of fats (triacylglycerides) is very complicated. In the solid state, several crystalline transformations from one crystal form to another

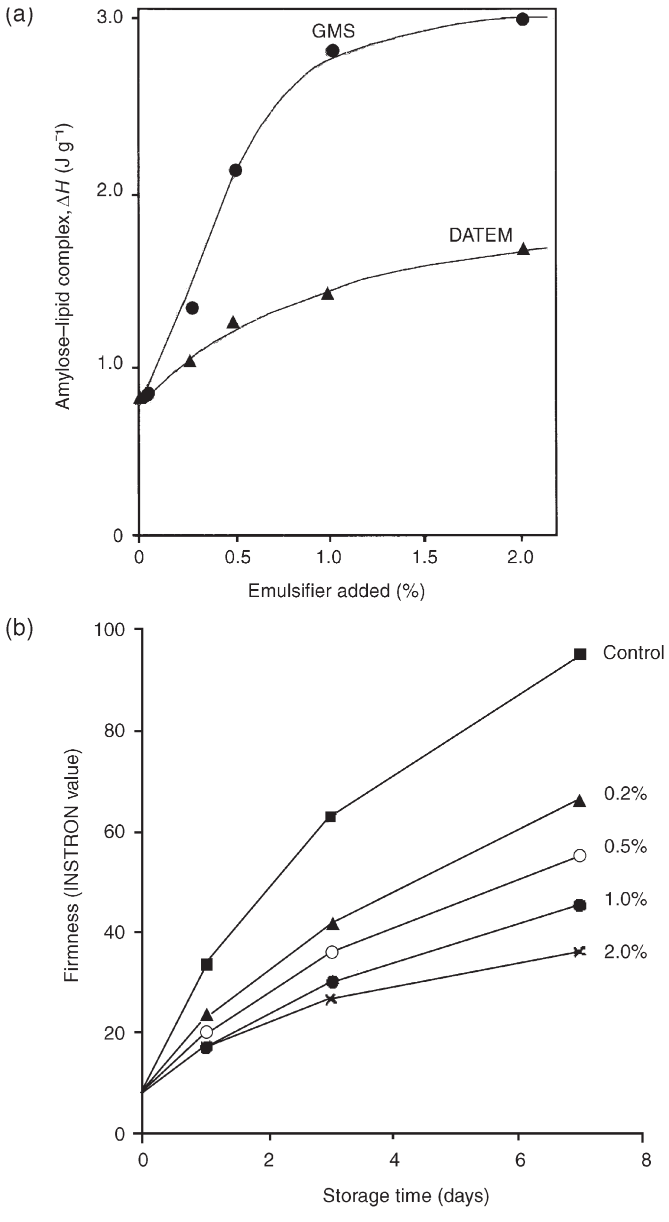


Fig. 10.16 Influence of monoglycerides on amylose-complex formation in wheat bread dough and crumb firmness (anti-staling) of bread. (a) Effect of distilled, saturated monoglycerides (GMS) and diacetyl tartaric acid esters of monoglycerides (DATEM) on the amount of amylose–lipid complexes formed during dough mixing and baking. The concentration of added emulsifiers is based on flour weight. At GMS concentrations above 1%, all free amylose is bound. (b) Crumb firmness of bread containing 0.25–2.0% GMS based on flour weight after storage at 22 ℃, measured by an Instron Tester. (From Krog *et al.*[48])

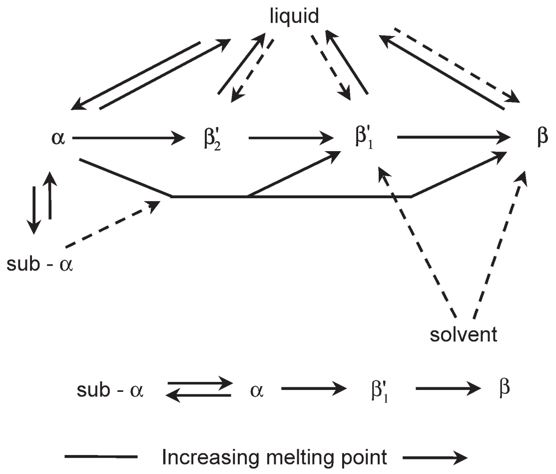


Fig. 10.17 Polymorphic changes occurring in saturated triacylglycerides. All the polymorphic transitions of triacylglycerides, except the α- → sub-α transition, are irreversible. The dotted lines indicate the possibility of direct crystallisation from liquid under tempering conditions or from solvents.

may occur, and this is referred to as polymorphism.[50] The polymorphic changes that typically occur in fats when solidified from melt are shown in Fig. 10.17.

Emulsifiers may affect both the nucleation process, inhibiting fat crystallisation (for instance in salad or cooking oil), and the crystal transitions from one crystal form to another.[51] This prevents the development of large clusters of crystals, which give fat-based products, such as margarine, spreads and chocolate, a 'sandy' texture or poor visual appearance.

10.6.1 Anti-bloom effects in chocolate and chocolate coatings

In chocolate, crystal transitions from one crystal form to another during storage are referred to as 'bloom' and appear as greyish spots on the surface of the product.[52–54] The consumer, who then rejects the product, often mistakes these spots for microbial growth. Bloom develops particularly in chocolate products that have been exposed to fluctuating temperature conditions, causing the fat phase to melt and recrystallise. This causes a change in crystal morphology from crystal platelets, which lie flat along the surface and give a shiny surface, to bundles of bigger crystals protruding from the surface. The new, uneven crystals at the surface reflect the light, giving a dull, grey appearance. The bloom crystals in chocolate coatings are rich in high-melting triacylglycerides, which migrate to the surface during recrystallisation.

Lipophilic emulsifiers in the form of sorbitan tristearate (STS) are used as crystal-modifying agents in fats, where they prevent the formation of the high-melting β-crystal. The function of STS is assumed to be due to its ability to

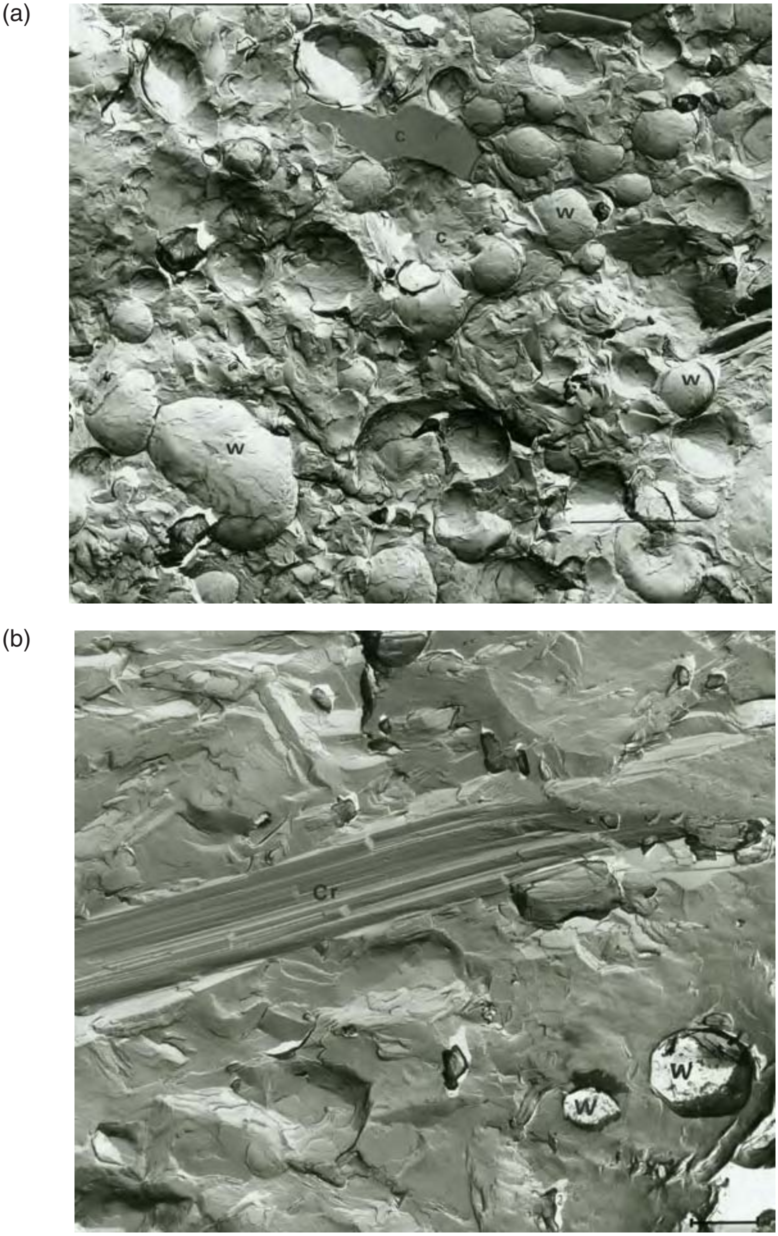


Fig. 10.18 Freeze–fracture transmission electron micrographs of (a) low-fat spread with small β'-crystals. The water droplets (W) are covered with crystals aligned flat along the oil/water interface. (b) Electron-micrograph showing a large fat crystal (Cr) in a spread containing hydrogenated sunflower oil. The large β-crystals cause a 'sandy' texture and may also initiate liquid oil separation from the product. (Courtesy of Dr Buchheim, Kiel.) Bar=2 μm.

Table 10.5 The influence of emulsifiers on fat crystallisation and polymorphism

Product	Function	Emulsifier
Creams, ice cream mix	Promote crystallisation	Monoglycerides (GMS)
Peanut butter	Prevent oil separation	Monoglycerides (GMS)
Low-fat/fat-free spreads	Add texture	Monoglycerides (GMS)
Salad/cooking oil	Inhibit crystallisation	STS, PGE, lecithin
Margarine, spreads	Control polymorphism	STS
Chocolate fats	Provide anti-bloom effect	STS, LACTEM, CITREM

co-crystallise with triacylglycerides in the β'-crystal form, preventing a solid-state crystal transition to the higher-melting β-crystal form during storage.[7] Other emulsifiers, such as LACTEM or CITREM, provide a similar crystal-modifying function in cocoa butter substitutes (CBS) or cocoa butter replacers (CBR), but are less efficient than STS.[55]

10.6.2 Crystal transformation in margarine and low-fat spreads

The normal crystal structure of margarine and spreads is the β'-crystal form.[56] These are small crystals that provide a good plastic consistency. Margarine and low-fat spreads made from fat blends that contain partially hydrogenated sunflower oil or low erucic rapeseed oil (canola oil) have a high tendency to develop large β-crystals. The clusters of β-crystals develop within 2–3 weeks of storage, giving the final products a grainy texture and sandy mouthfeel. Furthermore, owing to the reduced surface area of the crystalline phase, liquid oil separation may occur. Such changes are regarded as defects and often cause the product to be rejected by the consumer.

Figure 10.18 shows electron micrographs of a standard margarine with small fat crystals compared to a margarine containing hydrogenated sunflower oil, which has developed large β-crystals during storage. The change in crystal form from β' to the larger, higher melting β-crystal form causes an increase in the melting enthalpy, but the solid fat content of the margarine is not changed. When using fat blends with a tendency to form β-crystals, such as partly hydrogenated low erucic rapeseed oil or sunflower oil, in the production of margarine or low-fat spreads, the crystal transition from β' to β can be delayed or completely eliminated by adding 0.3–0.5% STS to the fat blend. An overview of the influence of emulsifiers on fat crystallisation and polymorphism is shown in Table 10.5.

10.7 Future trends

The development of new types of surface-active lipids for use in foods must be regarded as unlikely, primarily because of the enormous cost of the toxicological tests needed in order to obtain the final approval of health organisations. However, there is ongoing research to find cost-effective methods to optimise the composition of existing emulsifier types. Enzyme technology represents, perhaps, a new way to produce emulsifiers with a specific composition and application, but has not as yet been used commercially for this purpose. The general trend is to produce well-defined products with a high content of functional compounds in relation to the applications. The fatty acid profile of emulsifiers is an important parameter for their functional properties and is subject to constant development and optimisation.

Synergistic effects between different types of emulsifiers are often found in specific applications and are another target for further development. Surface-active lipids from natural sources, such as phospholipids or galactolipids isolated from wheat and oat cereals or plant seeds, are highly functional in various foods. Their isolation and production are being investigated.

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11

The use of hydrocolloids to improve food texture

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11.1 Introduction

The term ‘hydrocolloid’ is commonly used to describe a group of water-soluble naturally occurring polymers found abundantly in nature. They have evolved to perform many different functions, e.g. act as structural agents and energy reserves in plants and animals, to facilitate cell recognition and adhesion processes, to provide lubrication in bone joints, to act as ion exchangers and blood anticoagulants. Their key function in food products is to control texture and organoleptic properties mainly by enhancing the viscosity and gel characteristics. Even at concentrations of 1 wt% or less some hydrocolloids are capable of producing highly viscous solutions or of forming gels with varying textures. Their thickening ability has led to their use as suspension and emulsion stabilisers where they function by retarding particle sedimentation and droplet creaming caused by bulk viscosity effects. The hydrocolloids may also adsorb onto the surface of particles or droplets and inhibit aggregation by steric or electrosteric forces. Each hydrocolloid has its own unique functional characteristics, which is a consequence of its chemical structure, molecular size and shape.

This chapter first reviews the source, structure and main applications of the major commercial food hydrocolloids and provides details of their market share. It then focuses on the thickening and gelling characteristics imparted by these materials and gives details of the structure, functional properties and applications of individual hydrocolloids. The influence of hydrocolloids on health is discussed and finally the chapter concludes with a perspective on the future prospects.

Table 11.1 Source, function and main applications of hydrocolloids

Hydrocolloid	Source	Function	Application areas
Botanical			
Carboxymethyl cellulose	Trees and cotton	Thickener	Dairy and desserts, ready-to eat meals, bakery products, meat products, sauces and dressings
Methyl- and hydroxypropyl-methylcellulose		Thickener and gelling agent	Reformed vegetables, fish cakes, etc.
Modified starches	Corn, potato, etc.	Thickener and gelling agent	Ready-to-eat meals, dairy and desserts, meat products, soups, bakery products, sugar confectionery
Pectin	Citrus peel and apple pomace	Gelling agent	Jams, fruit preparations, sugar confectionery
Guar gum	Seed endosperm *Cyamopsis tetragonoloba*	Thickener	Dairy and desserts, bakery, petfoods, ready-to-eat meals, sauces and dressings
Locust bean gum	Seed endosperm *Ceratonia siliqua*	Thickener	Dairy and desserts
Tara gum	Seed endosperm *Cesalpinia spinosa*	Thickener	As guar and locust bean gum but limited application at present
Gum arabic	Tree gum exudate *Acacia senegal* and *Acacia seyal*	Produces low-viscosity solutions at high gum concentrations; emulsifier	Sugar confectionery, beverages
Gum karaya	Tree gum exudate *Stercula urens*	Thickener	Dressings but usage limited
Gum tragacanth	Tree gum exudate *Astragalus gummifer*	Thickener but usage limited	Dressings and sauces, icings
Konjac mannan	Tuber *Amorphophallus konjac*	Thickener and gelling agent	Japanese noodles, jelly desserts
Algal			
Red seaweeds			
Agar	*Gelidium, Gelidiella* and *Pterocladia*	Gelling agent	Confectionery, dairy and desserts
Carrageenan			
Kappa type	*Euchema cottonii* and *Chondus crispus*	Gelling agent Gelling agent	Dairy and desserts, meat products, petfoods, sugar confectionery
Iota type	*E. spinosum*		
Lamba type	*Ch. crispus*	Thickener	
Brown seaweeds			
Alginate	*Laminaria hyperborea, Macrocystis pyrifera*	Gelling agent	Dairy and desserts, bakery products, petfoods, sugar confectionery
Propylene glycol alginate		Emulsion and foam stabiliser	Salad dressings, beer

Table 1l.1 cont'd

Hydrocolloid	Source	Function	Application areas
Microbial			
Xanthan gum	*Xanthomonas campestris*	Thickener	Dairy and desserts, ready-to-eat meals, sauces and dressings, petfoods
Gellan gum	*Sphingomonas elodea*	Gelling agent	Sugar confectionery, dessert jellies, fruit preparations
Animal			
Gelatin	Cattle, pigs, fish	Gelling agent	Sugar confectionery, meat products, dairy and desserts
Milk proteins	Cattle	Gelling agent	Bakery products, dairy and desserts, confectionery

11.2 The range and choice of hydrocolloids

Food hydrocolloids are obtained from a variety of sources. Details of some of the more commercially important ones are given in Table 11.1, together with their key functions and main application areas. As a consequence of the increasing consumer demand for convenience foods and the modern trend towards healthier high-fibre and low-fat diets, hydrocolloids are finding ever-increasing application in food products to improve product texture and quality. This is evident from the fact that the market volume increased from 720 000 tonnes with a value of $690 million in 1990 to 1420 000 tonnes at a value of $3.2 billion ($\$3.2 \times 10^9$) in 2001.

The choice of which hydrocolloid to use in a specific application is often a delicate balance between price and performance. Modified starches and gelatin are available in the largest quantities and have the lowest price. They account for 70% and 12% respectively of the total market value. Despite its relatively high price, xanthan gum is finding increasing use in food products because of its unique rheological properties.

Gelatin replacement has been a major issue in recent years due to a number of factors – the advent of BSE (bovine spongiform encephalopathy) in the UK, religious beliefs and also because of the increasing number of vegetarians. A number of other products based on different hydrocolloids such as pectin, carrageenan and xanthan/galactomannan have been introduced; however, gelatin remains the second most utilised hydrocolloid after starch. The possibility of obtaining gelatin from fish may strengthen its position. The situation with gum arabic has been precarious for many years because of shortages of supply and fluctuating prices but because of its unique properties it has proved very difficult to replace and its market share is holding. A summary of the market value and volume of the major hydrocolloids is given in Table 11.2.

The concept of realistic market potential has been developed to allow producers a more accurate estimation of their markets. This consists of two parts: first the

Table 11.2 Market size and value of the main hydrocolloids

Hydrocolloid	Market volume (000 tonnes)	Market value ($million)
Starches	1000	650
Gelatin	162	350
Gum arabic	30	150
Xanthan gum	16	160
Carrageenan	35	270
Pectin	25	250
Alginate	30	90
Guar	30	80
Locust bean gum	10	100
Modified cellulose		34

Source: P.J. Lillford, in *Gums and Stabilisers for the Food Industry 10*, P.A. Williams and G.O. Phillips, (eds), Royal Society of Chemistry Special Publication No. 251 (2000), p 387.

volume of that particular hydrocolloid that is sold and second, a portion of the market for other hydrocolloids, which can realistically be targeted by a given hydrocolloid. For example the potential market for xanthan in 2002 was $219 million, which is the value of xanthan estimated to be sold plus $153 million, which represents the value of xanthan that could realistically be sold in the marketplace when competing with the other hydrocolloids. The estimate for replacement depends on a number of factors, including price, functionality, availability, synergy and, most important, consumer image. The additional replacement potential market for xanthan and the proportion taken from each hydrocolloid are estimated in Table 11.3. The table also shows the value of each hydrocolloid in the market in the year 2000, and illustrates the extent to which the industry is now seeking multi-functional hydrocolloids. This trend is expected to gather pace in the years ahead.

11.3 Thickening characteristics

The viscosity of hydrocolloid solutions shows a marked increase at a critical polymer concentration commonly referred to as C^*. This concentration corresponds to the transition from the so-called 'dilute region', where the polymer molecules are free to move independently in solution without touching, to the 'semi-dilute region' where molecular crowding gives rise to the overlap of polymer coils and interpenetration occurs (Fig. 11.1). Hydrocolloid solutions normally exhibit Newtonian behaviour at concentrations well below C^*, i.e. their viscosity is not dependent on the rate of shear; however, above C^* non-Newtonian behaviour is usually observed. A typical viscosity–shear rate profile for a polymer solution above C^* is given in Fig. 11.2 and shows three distinct regions: (a) a low shear Newtonian plateau, (b) a shear thinning region and (c) a high shear Newtonian plateau. Microstructurally in region (a) it is envisaged that the system is able to rearrange at a rate that is greater than the imposed deformation (i.e. the polymer

Table 11.3 Replacement potential market for xanthan and proportion which could be taken from other gums, in relation to total market for all hydrocolloids (1998)

Hydrocolloid	Replacement ratio	$ million	Market in 2000 ($million)	% Total
Xanthan	100	219.3	219.3	7
Carrageenan	15	39.4	308.2	10
Alginate	4	21.5	90.0	3
Gelatin	22	21.5	693.2	28
Starch	6	15.6	308.2	10
Microcrystalline cellulose	15	13.0	40.4	1
Carboxymethyl cellulose	2	12.9	68.0	2
Methyl and hydroxymethyl cellulose	8	10.4	43.7	1
Gum arabic	9	6.0	127.3	4
Pectin	4	5.9	292.2	9
Guar gum	18	5.8	54.7	2
Gellan gum	6	4.6		
Agar	8	3.7		
Locust bean gum	22	2.4	136.7	4
Tragacanth gum	6	0.3		
Karaya gum	5	0.3		

Source: D. Seisun, in *Gums and Stabilisers for the Food Industry 11*, P.A. Williams and G.O. Phillips (eds), Royal Society of Chemistry Special Publication No. 278 (2002), p 3.

molecules entangle at a greater rate than they disentangle). Above a critical shear rate, however, in region (b) the rate of rearrangement is less than the imposed deformation and shear thinning results. The viscosity drops to a minimum plateau value at infinite shear rate (region (c)). A number of empirical mathematical models have been developed to describe the flow characteristics. The simplest is the power law relationship, which describes the shear thinning portion of the profile:

$$\eta = K\gamma^{n-1} \tag{11.1}$$

where η is the apparent shear viscosity, K is the consistency index, γ is the shear rate and n is the power law index which has a value of 1 for a Newtonian solution and decreases to 0 with increasing shear thinning characteristics.

The most widely used model to describe the whole shear rate range is probably the Cross equation:

$$\eta = \eta_\infty + \frac{\eta_0 - \eta}{1 + (\tau\gamma)^m} \tag{11.2}$$

where η is the apparent shear viscosity, η_∞ is the infinite shear viscosity, η_0 is the zero shear viscosity, τ is a shear dependent constant denoting the onset of shear thinning, γ is shear rate and m is an exponent quantifying the degree of shear thinning with a value of 0 for a Newtonian solution, increasing to 1 with increased shear thinning.

The viscosity of hydrocolloid solutions is influenced significantly by the

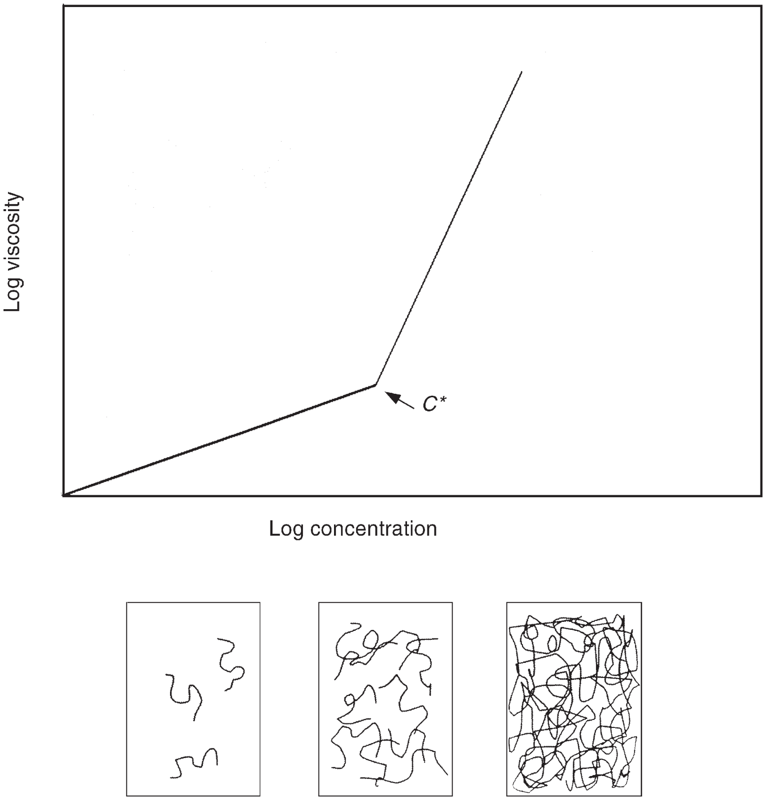


Fig. 11.1 Schematic illustration of the variation of viscosity with hydrocolloid concentration. *C** corresponds to the concentration where polymer chains start to overlap.

polymer hydrodynamic volume, which increases with radius of gyration, R_g. R_g increases with molecular mass, chain rigidity and electrostatic charge density and is greater for linear compared to branched hydrocolloids. The main hydrocolloid thickeners used are listed in Table 11.4. The viscosity shear rate profiles for 1% solutions of some of these hydrocolloids are presented in Fig. 11.3. The most striking feature is the profile for xanthan gum. This hydrocolloid has a very large low-shear viscosity, and hence it is good at suspending particles and oil droplets. In addition, however, it is also extremely shear thinning and, therefore, it readily flows on simple shaking. These characteristics have led to its widespread use in many food applications, notably, mayonnaise, dressings and sauces.

11.4 Gelling characteristics

A number of hydrocolloids are able to form gels by physical association of their polymer chains through, for example, hydrogen bonding, hydrophobic association

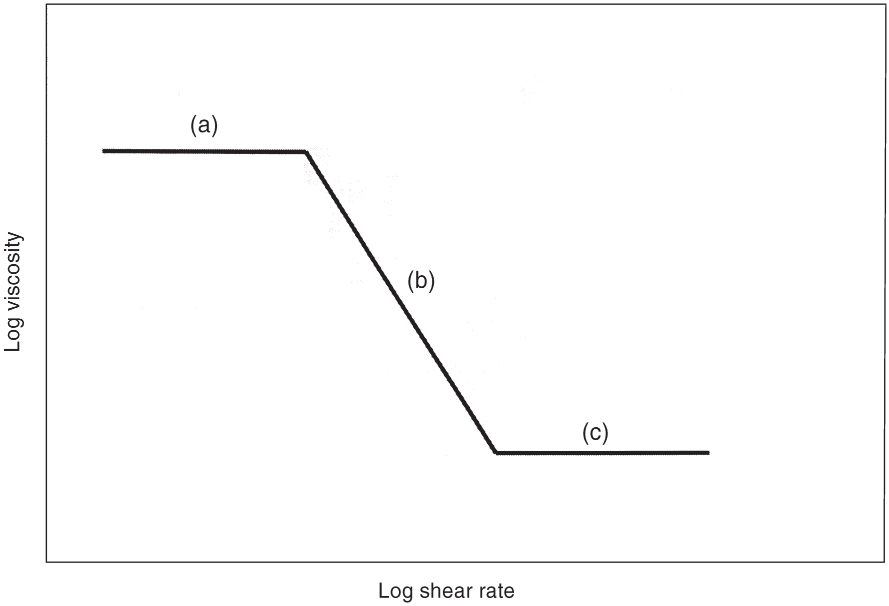


Fig. 11.2 Schematic illustration of the variation of the viscosity of a hydrocolloid solution above *C** with shear rate.

Table 11.4 Main hydrocolloid thickeners

Hydrocolloid	Characteristics
Modified starches	Viscous solutions are formed depending on the type of modification.
Xanthan gum	Has an apparent yield stress and hence can prevent sedimentation and creaming, but solutions are highly shear thinning. Viscosity is not influenced by temperature, addition of salts or to pH changes.
Carboxymethyl cellulose	Viscous solutions are formed but the viscosity decreases on addition of salts and at low pH.
Methylcellulose and hydroxymethyl cellulose	Form viscous solutions which gel on heating.
Galactomannans (guar and locust bean gum)	Form highly viscous solutions which are not influenced by addition of salts or changes in pH.

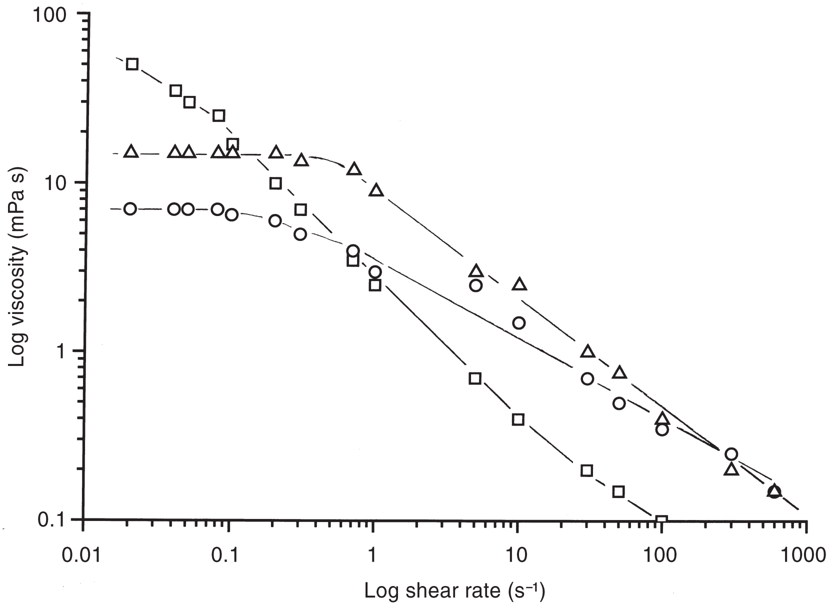


Fig. 11.3 Viscosity shear rate profiles for 1% solutions of □, xanthan gum; ○, CMC (carboxymethyl cellulose); and △, guar gum.

and cation-mediated cross-linking, and differ from synthetic polymer gels which normally consist of covalently cross-linked polymer chains. Certain helix-forming hydrocolloids, for example, agarose, carrageenan, gellan gum and gelatin, form gels on cooling. These hydrocolloids adopt a disordered conformation at high temperatures but on cooling they undergo a conformational change and ordered helices are formed. The helices then aggregate to form a gel. The process is thermally reversible and hence the gels melt on heating. The melting temperature is often higher than the gelation temperature since melting only occurs after disaggregation of the helices. It is interesting to note here that mixing solutions of xanthan gum and locust bean gum (two non-gelling polysaccharides) results in the formation of very strong gels on cooling. This has been attributed to molecular association between ordered xanthan helices and 'bare' mannan regions along the locust bean gum chain. Non-thermoreversible gels can be formed by cross-linking chains with divalent cations. Alginate and low methoxy (LM) pectin are typical examples. Some hydrocolloids, notably methyl- and hydroxypropylmethyl-cellulose form thermoreversible gels on heating. Chain association is believed to be due to hydrophobic bonding.

Gel formation only occurs above a critical minimum concentration, C_0, which is specific for each hydrocolloid (Fig. 11.4). Agarose, for example, will form gels at concentrations as low as 0.2% while for acid-thinned starch a concentration of ~15% is required before gels are formed. Below C_0 precipitation often results. C_0

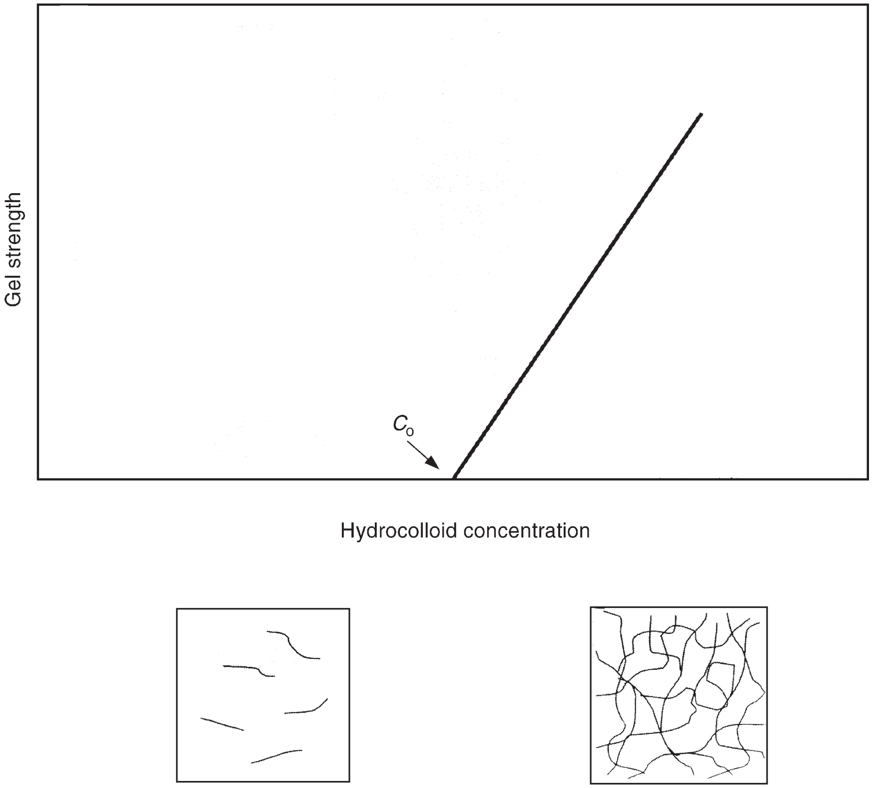


Fig. 11.4 Schematic illustration of the variation of gel strength with hydrocolloid concentration. C_0 corresponds to the concentration above which gelation occurs.

is not the same as the critical overlap concentration, C^*, noted above. The properties of individual hydrocolloid gels vary considerably in strength and elasticity owing to differences in the number and nature of the junction zones and the degree of chain aggregation. The main hydrocolloid gelling agents and their characteristics are summarised in Table 11.5.

11.5 Structure and properties of individual hydrocolloids

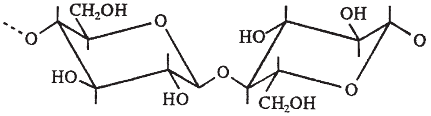
11.5.1 Cellulose derivatives

Cellulose is the most abundant of all the polysaccharides, being the structural component of land plants. The main commercial sources are trees and cotton although bacterial cellulose is now gaining some importance. Cellulose is composed of linear chains of β-(1,4)-linked glucopyranose units, which associate to form a number of crystalline structures (Fig. 11.5a). Although it is insoluble in water, it

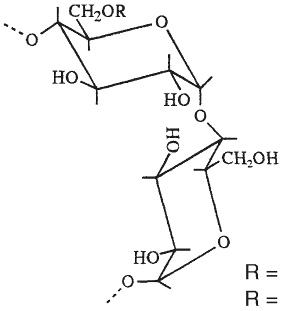
Table 11.5 Main hydrocolloid gelling agents

Hydrocolloid	Characteristics
Modified starch	Amylose containing starches will form thermally irreversible opaque gels.
Gelatin	Forms thermoreversible gels on cooling. Gels are elastic and melt at body temperature.
Agar	Forms thermoreversible turbid, brittle gels on cooling (~40 ºC). Gels melt only at high temperatures (~85 ºC).
Kappa carrageenan	Forms thermoreversible slightly turbid gels on cooling to 40–60 ºC, which is promoted by the presence of potassium ions. Melting occurs at 5–20 ºC above gelation temperature. Gels tend to be brittle and hence it is often used in combination with locust bean gum, which gives increased elasticity, improves clarity and reduces syneresis.
Iota carrageenan	Forms thermoreversible elastic gels on cooling to 40–60 ºC. Melting occurs 5–20 ºC above the gelation temperature.
Low methoxy pectin	Forms thermoreversible gels on cooling in the presence of calcium ions and sequesterant (e.g. citrate) at pH 3–4.5.
High methoxyl pectin	Gels are formed at high-soluble solids content at pH < 3.5. Gels are not thermoreversible.
Gellan gum	Forms highly transparent gels on cooling in the presence of electrolyte. Low-acyl gels are brittle and are often not thermally reversible. High-acyl gels are elastic and thermoreversible. They set and melt at ~70–80 ºC.
Methyl and hydroxypropylmethyl cellulose	Form thermoreversible gels on heating.
Alginate	Gels are formed on addition of polyvalent ions (usually calcium). Homogeneous gels are formed by generating the calcium ions *in situ*. Gels do not melt on heating.
Xanthan gum	Forms highly elastic thermoreversible gels with locust bean gum and konjac mannan.
Konjac mannan	Forms non-thermoreversible elastic gels in the presence of alkali.

(a) Cellulose

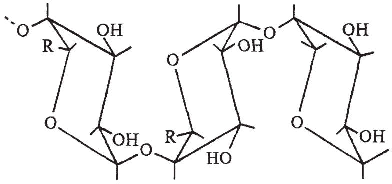


(b) Amylose and amylopectin



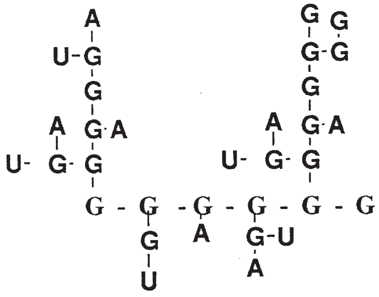
R = H for amylose
R = glucopyranose for amylopectin

(c) Pectin



R = COO$^-$ or COOCH$_3$

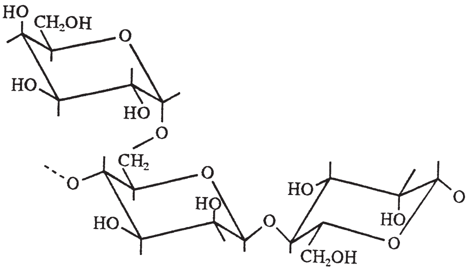
(d) Gum arabic



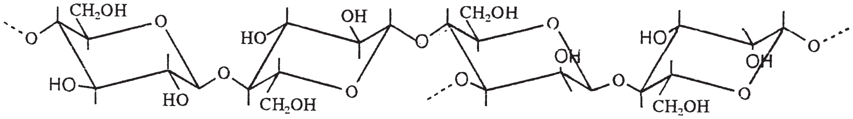
G = Gal*p*
A = L-Ara*f* or L-Ara*p*-terminated short chains of (1,3)-linked L-Ara*f*, or D-Gal*p*-(1,3)-L-Ara*f*
U = Rha*p*-(1,4)-GlcA, or Glc*p*A(OMe)

Fig. 11.5 Primary structures of the various hydrocolloids.

(e) Galactomannans



(f) Konjac mannan



(g) Xanthan gum

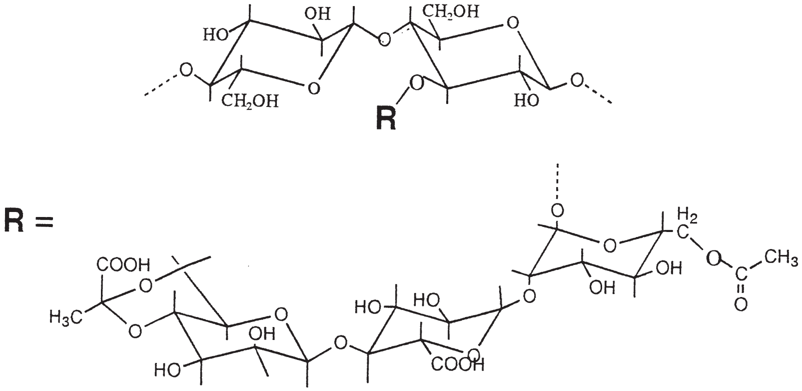
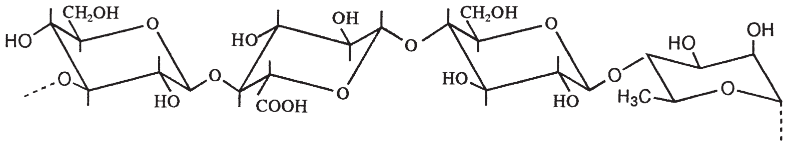
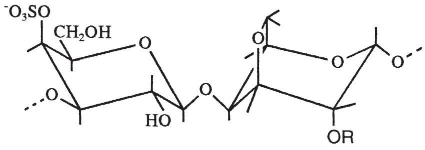


Fig. 11.5 cont'd

(h) Gellan gum

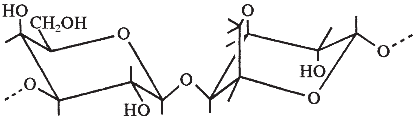


(i) Carrageenan



R = H for Kappa
R = SO_3H for Iota

(j) Agarose



(k) Alginate

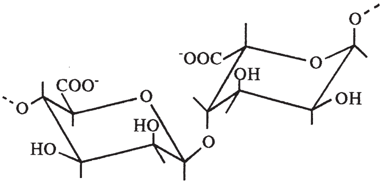


Fig. 11.5 cont'd

can be chemically modified to form a number of water-soluble derivatives with valuable functional properties. This usually involves etherification of the reactive hydroxyl groups on the glucose residues. The most commonly used water-soluble derivatives in foods are carboxymethyl- (CMC), methyl- (MC) and hydroxypropylmethyl- (HMPC) cellulose. Derivatisation is carried out following conversion of the cellulose to the sodium form by treatment with alkali in order to destroy the crystalline structure. The alkali cellulose is then reacted with the appropriate reagent, namely sodium monochloroacetate for CMC, methyl chloride for MC, methyl chloride and propylene oxide for HPMC. Since the reactions are heterogeneous, substitution can be very irregular.

CMC is the most widely used of the cellulose ethers and can be obtained in grades, which vary in molecular mass and degree of substitution (DS). The minimum DS is ~0.4 (i.e. 4 hydroxyls substituted per 10 glucose residues) since below this the CMC is insoluble. Above this CMC dissolves readily in water to form viscous solutions but, since it is anionic, the viscosity of dilute solutions is reduced on addition of electrolyte and at low pH owing to compaction of the polymer chains. It is used as a thickener in a broad range of products. These include low-pH milk products where it forms complexes with the milk proteins, bakery products such as cake mixes and doughs and frozen ready-to-eat meals.

The non-ionic cellulose ethers MC and HPMC are also supplied in a range of molecular sizes and DS. Since the substituted groups may also participate in the reaction, a molar substitution (MS) is also quoted to characterise the polymers. MC and HPMC form thermoreversible gels on heating. The gelation temperature is dependent on the degree of substitution. For MC containing 30% methoxyl groups, gelation occurs at ~50–55 ºC whereas for HPMC containing 20% methoxyl and 8% hydroxypropyl, gelation occurs at ~85 ºC. Gelation is believed to be a consequence of molecular association through the hydrophobic methyl and hydroxypropylmethyl substituents.

The main application of cellulose derivatives is as binders and to aid shape retention on heating in products such as reformed vegetables (potato croquettes, onion rings, etc.) and fish cakes.

11.5.2 Starch

Starch is also a very abundant material and is derived commercially principally from corn and potato and to a lesser extent from waxy corn, wheat, tapioca, rice, cassava, sorghum, pea and sago. It is obtained in the form of granules, which have varying degrees of structural order, and consists of two polysaccharides, namely amylose and amylopectin. The proportions of each depend on the source, for example, corn and potato starch, contain ~27% and 21% amylose, respectively, while waxy corn contains <2% amylose.

Amylose consists of linear α-(1,4)-linked glucopyranose chains with very little branching (10 branch points per molecule) and has a molecular mass of typically $2 \times 10^5 - 2 \times 10^6$. Amylopectin also contains sequences of α-(1,4)-linked glucopyranose units; however, it has extensive branching via (1,6) linkages and has a molecular mass of ~$5 \times 10^7 - 4 \times 10^8$ (Fig. 11.5b). The starch granules are insoluble in water but on heating they swell and then burst, releasing amylose and forming a viscous paste. The actual temperature at which the granules burst is very characteristic of the source of the starch and is (perhaps inappropriately) referred to as the gelatinisation temperature. Typical values for corn and potato starch are 67 °C and 60 °C, respectively. On cooling, the amylose molecules readily self-associate, a process known as retrogradation, and it is then that a gel is formed. The use of native starch is limited because of its tendency to self-associate readily and hence most of the commercial starches used in food applications are derivatives, which have a lesser tendency for retrogradation. Derivatives include hydroxypropyl-starches, starch phosphates, oxidised starch and acid or enzyme degraded starches.

Modified starches are used as thickeners in a broad range of product areas such as batters and breadings, dairy and dessert products, soups and sauces, confectionery, dressings and confectionery. They are also used in meat products as water binders.

11.5.3 Pectin

Pectin is the general term for a group of polyuronans that occur as the structural components of plants. Commercial pectins are obtained from the pomace of apple or the peel of citrus fruits following hydrolysis, which renders the pectin water soluble. Pectin molecules consist of linear chains of (1,4)-α galacturonic acid residues (Fig. 11.5c) up to 80% of which occur as the methyl ester together with up to 4% (1,2)-α-rhamnopyranose units which are distributed along the chain giving rise to kinks. L-Arabinose, D-galactose and D-xylose (10–15%) are linked to the rhamnose units forming ramified side-chains which are referred to as 'hairy regions' along the otherwise smooth galacturonan backbone. If the degree of esterification (DE) is more than 50% it is referred to as high methoxyl (HM) pectin. De-esterified pectin with DE of less than 50% is produced by mild acid or alkali treatment and is referred to as low methoxy (LM) pectin.

Pectin is soluble in water and is most stable at a pH of ~3–4. Above and below this value hydrolysis may occur. Both HM and LM pectins form gels. For HM pectin (DE 60–75%) gelation occurs at high soluble solids content (typically 50–75% sugar) and at pH <3.5 over a period of time. The gels are not thermoreversible. Junction zone formation is believed to be as a consequence of hydrophobic association between ester groups coupled with intermolecular hydrogen bonding between hydroxyl groups on the galacturonan backbone. For LM pectin (DE typically 20–40%) gelation is brought about by the addition of divalent cations. A high soluble solids content and low pH are not prerequisites. Gelation is rapid and is thermoreversible.

Pectins are used as gelling agents in the production of jams and preserves, fruit preparations, low pH milk products and confectionery.

11.5.4 Gum arabic

The main tree gum exudates are gum arabic, gum karaya and gum tragacanth, with gum arabic being, by far, the most important commercially. Gum arabic occurs as a sticky liquid that oozes from the stems and branches of acacia trees (notably *Acacia senegal* and *Acacia seyal*) which grow across the Sahelian belt of Africa, principally in Sudan. The gummosis process occurs when the tree is subjected to stress conditions such as heat, drought or wounding. The liquid dries in the sun to form glassy nodules, which are collected by hand. The gum is a complex polysaccharide consisting of galactopyranose (~44%), arabino-pyranose and furanose (~25%), rhamnopyranose (14%), glucuropyranosyl uronic acid (15.5%) and 4-*O*-methylglucuropyranosyl uronic acid (1.5%). It also contains a small amount (~2%) of protein as an integral part of the structure. Analysis of the carbohydrate structure has shown that it consists of a core of β-(1,3)-linked galactose units with extensive branching at the C6 position. The branches consist of galactose and arabinose and terminate with rhamnose and glucuronic acid (Fig. 11.5d).

It has been shown that the gum consists of three broad molecular fractions, which differ principally in their size and protein contents. Most of the gum (~90%) contains very little protein and has a molecular mass of $\sim 2.5 \times 10^5$. A second fraction, (~10% of the total) contains ~10% protein and has a molecular mass of $1 - 2 \times 10^6$ and has been shown to have a 'wattle-blossom'-type structure where blocks of carbohydrate of molecular mass $\sim 2.5 \times 10^5$ are connected to a common polypeptide chain. The third fraction (~1% of the total) contains up to 50% protein and has a molecular mass of $\sim 2 \times 10^5$. The high degree of branching gives rise to a very compact molecular structure for all of the fractions and results in solutions of very low viscosity. Fraction 2 has been shown to be responsible for the gum's excellent ability to stabilise oil-in-water emulsions.

The main use of gum arabic is in the confectionery industry. It is also used as an emulsifier for flavour oils for incorporation in soft drinks. Encapsulation of the flavour oil can be achieved by spray drying the emulsion to form a solid powder which can be added to dried soup and cake mixes.

11.5.5 Galactomannan seed gums

Locust bean (or carob), tara and guar gums are storage polysaccharides obtained from the endosperms of leguminous seeds of *Ceratonia siliqua*, *Caesalpinia spinosa* and *Cyamopsis tetragonoloba*, respectively. They consist of a linear main chain of β-(1,4)-linked mannopyranosyl units with galactopyranosyl units linked α-(1,6) to varying degrees (Fig. 11.5e) and have a molecular mass of the order of 10^6. The mannose to galactose ratio, (M/G), is approximately 4.5:1, 3:1 and 2:1 for locust bean, tara and guar gums, respectively. The galactose residues have been shown to be non-uniformily distributed along the mannan chain. The presence of galactose tends to inhibit intermolecular association; hence, whereas guar gum is readily soluble in cold water, tara and locust bean gums have to be heated to high temperatures to achieve complete dissolution. Once dissolved, all three yield highly viscous solutions.

Locust bean gum will self-associate in solution and can form thermally irreversible gels on freezing. It is commonly used in combination with other polysaccharides, particularly κ-carrageenan, since it leads to the formation of stronger, more elastic gels, which have improved transparency and are less prone to undergo syneresis. Locust bean gum also forms strong thermoreversible gels with xanthan gum. For both mixtures it has been argued that the synergistic behaviour is due to association of the ordered carrageenan and xanthan chains with mannose sequences along the backbone which are devoid of galactose residues. The model, therefore, explains why such behaviour is not observed in mixtures containing guar gum.

Guar gum and locust bean gum are used as thickeners in a broad range of food products including dairy products, desserts, bakery, petfoods, ready-to-eat meals, sauces and dressings.

11.5.6 Konjac mannan

Konjac mannan is a glucomannan obtained from the tuber of the konjac plant, notably the *Amorphophallus konjac* species which grows in Southeast Asia, particularly Japan, China and Indonesia. It is a high molecular mass ($>10^6$) polysaccharide consisting of linear chains of glucose and mannose units linked β-(1,4). Possible structures for the repeating unit are:

(G-G-M-M)-M-M-G-M and G-G-M-G-M-M-M-M

The main chain has branches, approximately every 10 residues, of up to 16 sugar units linked to the C3 position of the glucose and mannose. The mannose to glucose ratio is 1.6:1 and it is believed that there are no block sequences of glucose or mannose along the chain (Fig. 11.5f). Konjac mannan dissolves in water to form highly viscous solutions. It is acetylated (~1 acetyl group for every 19 sugar residues) and in the presence of alkali, deacetylation occurs and thermally irreversible gels are produced. Konjac mannan interacts with kappa carrageenan and xanthan gum in much the same way as locust bean gum, although the gels formed are considerably stronger.

Konjac gel has been a popular traditional Japanese food (konnyaku) for over a thousand years. It is also used to produce noodles and jelly desserts.

11.5.7 Xanthan gum

Xanthan gum, discovered in the 1950s, was the second microbial polysaccharide after dextran to be commercially exploited and is now finding extensive application in the food industry. The gum is obtained from the genus *Xanthomonas*, notably *X. campestris*, by aerobic fermentation. The xanthan molecules have a β-(1,4)-linked glucopyranose backbone as in cellulose and in addition have a trisaccharide side-chain on every other glucose residue linked through the C3 position. The side-chain consists of two mannopyranosyl residues linked on either side to a glucuropyranosyl uronic acid group. The inner mannose residue connected to the backbone may be acetylated while the terminal mannose residue may be pyruvated (Fig. 11.5g).

The molecular mass of the xanthan molecules is very high (>3 × 10^6) and the gum dissolves in water to yield highly viscous solutions. The xanthan molecules undergo a thermoreversible coil–helix transition in solution, which is shifted to higher temperatures by the addition of electrolyte. In the disordered coil form the side-chains are envisaged as protruding away from the backbone into solution, while in the ordered form the molecules form a stiff five-fold helical structure with the side-chains folded in and associated with the backbone. It is now generally recognised that the helix is double stranded. The stiffness of the xanthan chains gives rise to highly shear thinning rheological properties and unlike other polyelectrolytes, the viscosity of xanthan solutions can actually increase rather than decrease on addition of electrolyte since the electrolyte will promote helix formation and association.

Xanthan gum is finding increasing use in a variety of applications including batter coatings, cake batters, frozen and chilled dairy products, sauces and dressings.

11.5.8 Gellan gum

Gellan gum has only recently been commercialised. It is produced from *Sphingomonas elodia* by aerobic fermentation and consists of a linear tetrasaccharide repeat unit of β-(1,3)-glucopyranose, β-(1,4)-glucuronopyranose, β-(1,4)-glucopyranose and α-(1,4)-rhamnopyranose. In the native form the β-(1,3)-glucose residues contain glycerate and acetate moieties (Fig. 11.5h). X-ray fibre diffraction studies indicate that the gellan molecules form a three-fold double helical structure and in solution undergo a thermoreversible coil–helix transition. The transition shifts to higher temperatures in the presence of electrolyte. Once formed, the helices tend to self-associate, leading to the formation of a transparent gel. Gels formed in the presence of monovalent ions are usually thermoreversible although the melting temperature is normally much greater than the gelation temperature, a consequence of the extensive aggregation. If gels are formed by the addition of divalent ions then they can be thermally irreversible. The native form produces soft elastic gels, whereas the deacetylated material sold commercially forms hard brittle gels.

Gellan gum finds limited application at present in dessert jellies, sugar confectionery and fruit preparations.

11.5.9 Carrageenan

The carrageenans are a family of sulphated galactans obtained from red seaweeds (*Rhodophyceae*) where they have a key structural function. The traditional method of extraction is by treatment of the seaweed with hot alkali for 10–30 hours followed by precipitation with alcohol and then drying. The three major types are kappa, iota and lambda carrageenan. Kappa is obtained from a species of seaweed called *Euchema cottonii* and occurs together with lambda carrageenan in *Chondrus crispus*. Iota carrageenan is obtained from *Euchema spinosum*. They differ essentially in their degree of sulphation. The idealised repeat unit for kappa carrageenan consists of (1,3)-linked galactopyranose-4-sulphate and (1,4)-linked 3,6-anhydrogalactopyranose residues (Fig. 11.5i). Iota carrageenan differs only in that the latter residue is sulphated at the C2 position. Lambda carrageenan is further sulphated and consists of (1,4)-linked galactopyranose-2,6-disulphate and (1,3)-linked galactopyranose which are 70% substituted at the C2 position. The carrageenans are all soluble in water but whereas lambda forms viscous solutions, kappa and iota form thermoreversible gels. The gelation mechanism is similar to that described for gellan gum above, i.e. on cooling the molecules undergo a conformational coil to helix transition and the helices self-associate, giving rise to a three-dimensional gel structure. It is still not conclusively proven whether the helices are double or singly stranded.

The temperature of gelation increases with increasing electrolyte concentration. It has been shown that potassium, rubidium and caesium ions specifically bind to the helical structure of kappa carrageenan and hence promote helix formation and gelation at much lower concentrations than other electrolytes. As a consequence kappa carrageenan gels are much stronger in the presence of potassium chloride compared with, say, sodium chloride. This ion specificity is not observed for iota, which forms weaker, more elastic gels than kappa. This is probably due to the fact that the increased charge on the iota carrageenan chains reduces the extent of helix self-association.

Carrageenan is used in dairy and dessert products such as puddings, milk shakes, ice cream and water dessert jellies. It is also used in meat products where it acts as a water binder.

11.5.10 Agarose

Agarose (the major component of agar) is also obtained from red seaweeds, notably species of *Gelidium* and *Gracilaria*. It is a linear neutral polysaccharide and has a similar structure to the carrageenans, consisting of alternating (1,3)-linked β-galactopyranose and (1,4)-linked 3,6-anhydro-α-galactopyranose units (Fig. 11.5j). It dissolves in near-boiling water and gels on cooling to ~ <40 °C. The gelation mechanism is as described for the carrageenans but, since the agarose chains are non-ionic, extensive helix aggregation occurs resulting in the formation of very strong gels. The gels melt only on heating to 80–90 °C.

Agarose applications include confectionery and baked goods such as icings and pie fillings.

11.5.11 Alginate

Alginate is obtained from brown seaweeds (*Phyophyceae*) and is a linear (1,4)-linked polyuronan consisting of β-mannuronic and guluronic acids (Fig. 11.5k). The acids are present as blocks of separate or mixed sequences along the chain depending on the seaweed source. *Macrocystis pyriferia* and *Ascophyllum nodosum* have a high mannuronic acid content (61% and 65%, respectively), whereas *Laminaria hyperborea* has a high guluronic acid content (69%). Sodium alginate dissolves readily in water to form viscous solutions and forms thermally irreversible gels in the presence of divalent cations (notably calcium). It is the guluronic acid residues that are responsible for gel formation and their proportion and distribution along the polyuronan chain have a major influence on the properties of the gels produced. Adjacent diaxially linked guluronic acid residues form a cavity which acts as a binding site for cations which interact with the carboxyl and hydroxyl groups. Intermolecular cross-linking of sequences results in the formation of junction zones and a three-dimensional gel network. This mechanism has been described as the egg-box model. If the divalent cations are added rapidly to sodium alginate in solution, inhomogeneous gels are produced. In order to produce homogeneous gels a common practice is to generate the cross-linking ions slowly *in situ*. Sparingly soluble salts such as calcium sulphate are used and the release of ions is controlled by the presence of sequesterants and adjustment of pH.

Alginate is used in many food products including dairy products, desserts, fruit pie fillings, structured fruit and sugar confectionery. Propylene glycol alginate is used to stabilise foam on beer and is particularly effective at stabilising salad dressings.

11.5.12 Gelatin

Gelatin is denatured collagen, which is a protein and the major constituent of the white fibrous connective tissue occurring in the hides, skins and bones of animals. It is obtained mainly from cattle and pigs, although since the UK outbreak of BSE in the 1990s alternative sources, notably fish, have been investigated. The collagen is extracted from the raw material by subjecting it to either acid or alkaline treatment. Acid treatment involves immersing in cold dilute mineral acid (pH 1.5–3.0) for up to 30 hours while for alkaline treatment the raw material is steeped in saturated lime water (pH 12.0). The material is then washed with water, leading to the Type A (acid treatment) and Type B (alkaline treatment) gelatins. The main amino acids are glycine, proline, alanine and 4-hydroxyproline for both types. Type A gelatin contains lower amounts of glutamic and aspartic acids and hence the isoelectric point for Type A is in the range 7–9.4 while for Type B it is in the range 4.8–5.5. In solution above ~40 °C the gelatin molecules are in the form of random coils but on cooling the chains tend to order and form collagen-like triple helices which aggregate to form optically clear elastic gels

Gelatin is widely used in confectionery, meats, dairy and dessert products.

11.6 The health benefits of hydrocolloids

The texture-producing and fibre characteristics of hydrocolloids have recently gained great prominence in maintaining a healthy colon. There is a growing belief throughout the world that natural fibre foods are an integral part of a healthy lifestyle. Therefore, food producers source an increasing proportion of their raw materials from nature itself. An increasingly health-conscious consumer demands reduced fat and enhanced fibre foods of all types. If this can be achieved using materials that have low calorific value, further health benefits will result. Foods containing such ingredients will need to match the quality of the original product and without adverse dietary effects. This target cannot be achieved without the scientific use of thickeners, stabilisers and emulsifiers, particularly of the 'natural type'. This calls for fibres that can interact with water to form new textures and perform specific functions, and calls for this new emphasis in the use of hydrocolloids. This market is set to grow significantly to meet the health aspirations of the consumer during this millennium. It is the task of the food scientist to provide the hydrocolloids in the most appropriate form for inclusion in the food product. This requires an understanding of their structure and the way in which they act to produce the desired texture and function in the food.

Dietary fibre was first described as the skeletal remains of plant cell walls, which are resistant to hydrolysis by human digestive enzymes. Since this excluded polysaccharide fibres in the diet, the definition was subsequently expanded to include all polysaccharides and lignin, which are not digested by the endogenous secretions of the human digestive tract. Dietary fibre thus mainly comprises non-starch polysaccharides, and indeed has been defined as the polysaccharides which are resistant to human endogenous enzymes. Industrialised countries now generally recognise the health-giving properties of increased consumption of fibre and reduced intakes of total and saturated fat. In this respect 'fibre' is used in a non-specific way, but is generally taken to mean structural components of cereals and vegetables. More recently the concept of 'soluble fibre' has emerged which assists plasma cholesterol reduction and large-bowel fermentation. The hydrocolloids discussed meet this definition.

The physical and fibre properties of such soluble and insoluble fibre allow them to perform both in a physical role and also to ferment through colonic microflora to give short-chain fatty acids (SCFA), mainly acetate, propionate and butyrate. These have a very beneficial effect on colon health through stimulating blood flow, enhancing electrolyte and fluid absorption, enhancing muscular activity and reducing cholesterol levels.

11.6.1 The physiological effect of texture

For dietary fibre to be effective it must be resistant to the enzymes of the human and animal gastrointestinal tract. If physically suitable, it can work effectively as a result of its bulking action. This requires a knowledge of the interactions that enable different textural formulations to be produced. In the stomach and small intestine the fibre can increase digesta mass, leading to faecal bulking, which readily explains the relief of constipation, which is one of fibre's best-documented effects. It can increase stool mass and ease laxation very efficiently. This behaviour has considerable human and agricultural importance. The growth of the ruminant animal depends on the fermentable fibre content of the stockfeed.

Soluble as well as non-soluble fibres exert their actions in the upper gut through their physical textural properties. Those that form gels or viscous solutions can slow down the transit in the upper gut and delay glucose absorption, best explained in terms of 'viscous drag'. Thus the reduction in glycemic response by soluble fibres can be explained.

11.6.2 Fermentation product effects

Large bowel microorganisms attack the soluble fibres, in fermentation resembling that in the rumen of obligate herbivores such as sheep and cattle. The products too are similar: SCFAs, gases (hydrogen, carbon dioxide and methane) and an increased bacterial mass. The principal SCFAs are the same in humans as in ruminants, and the concentrations are similar too, particularly for omnivorous animals with a similar digestive physiology (for example, the pig). The increased textural bacterial cell mass also has a positive effect on laxation. Faeces are approximately 25% water and 75% dry matter. The major components are undigested residuals plus bacteria and bacterial cell wall debris. These form a sponge-like, water-holding matrix which conditions faecal bulk and cell debris. The ability of different fibres to increase faecal bulk depends on a complex relationship between chemical and physical properties of the fibre and the bacterial population of the colon.

The production of SCFAs and their beneficial effects in humans and ruminant species has been well established for a considerable time, but the effect was not thought to be relevant to the carnivorous dog and cat. Now this too has been demonstrated.

11.6.3 Health benefits

Whether by physical bulking action or through the production of SCFAs, several health advantages are now established for the use of hydrocolloids in this way: increasing fibre (20–30 grams per day in humans) can eliminate constipation through increased faecal bulking and water-holding. The fermentation to produce SCFAs can also assist, since propionate stimulates colonic muscular activity and encourage stool expulsion.

It was at one time thought that fibre lodged in the colon, leading to inflammation and herniation. This has now been disproved, and fibre can now relieve diverticular disease conditions, probably in the same way as it relieves constipation. Applying a solution of SCFAs into the colon of ulcerative colitis patients or into the defunctioned portion of surgical patients has given rise to substantial remission in colitis. It could be that the condition arises because of a defect in the fermentation process in these patients or in the products (SFCAs).

SCFAs stimulate water and electrolyte absorption by the mucosa and enhance their transport through improving colonic blood flow. Fibre fermentation also reduces the population of pathogenic bacteria such as *Clostridia* and can prevent diarrhoea caused by bacterial toxins. Epidemiological studies have shown repeatedly that populations with high levels of fibre in their diet have reduced risk of colon cancer. Protection may be through the SCFA butyrate, which inhibits the growth of tumour cells *in vitro.*

When applied to the companion animal, the increased production of SCFAs increases gut acidity marginally, which reduces the activity of putrefaction and pathogenic bacteria and so lowers toxin and thus reduces bad odours and bad-smelling faeces. The low level of toxin production reduces the load on the liver and results in better coat and skin quality. Therefore, the ageing animal can look better and produce less offensive faeces.

The behaviour in the intestine can influence the immune system. Thus protection is possible against colonisation by opportunistic bacteria, and the improved colonisation of beneficial indigenous bacteria in the gut gives greater resistance to infectious bacteria.

11.7 Future trends

The pace of modern-day life, the demand for fast food and ready-to eat-meals, coupled with the consumer awareness of the need to eat a more healthy diet, will ensure that the use of hydrocolloids in foods will continue to increase considerably in the future. The added health benefits that some hydrocolloids impart will add further momentum to this trend. The introduction of new hydrocolloids to impart novel thickening and gelling characteristics is unlikely because of the high costs involved in gaining food approval. More likely, however, is the introduction of hydrocolloid materials already used in various parts of the world as food ingredients. An example of this is konjac mannan, which has been used in Japan for thousands of years and has just gained approval in the USA and Europe for use as a food additive.

In recent years the food industry has concentrated on developing new textures in foods by using combinations of hydrocolloids which interact synergistically. Synergy between certain helix-forming polymers such as carrageenan, agarose and xanthan and relatively stiff linear polymers such as galactomannans has been known and utilised since the 1970s. For such systems the synergy is attributed to association of the polymer chains leading to gel formation. Presently there is renewed interest in pectin–alginate combinations, which form mixed gels by calcium ion cross-linking. There has been much interest in hydrocolloid mixtures in which the hydrocolloids do not associate but rather undergo phase separation. In such systems the hydrocolloids tend to separate into two liquid layers much like oil and water. Indeed they are sometimes referred to as ‘water-in-water’ emulsions. If one or both of the hydrocolloids are gel forming, the processes of phase separation and gelation will compete and novel gel structures can be formed. The effect of shear on these systems is the focus of considerable current research work.

Textural modification and control are an integral part of food processing. Functional issues are also important but perhaps even more important will be regulatory and social issues. A food additive that does not have regulatory food approval is not a food additive. Moreover, changes in regulatory approvals can impact on the levels and application within the food industry. Social issues such as vegetarianism, halal and kosher continue to grow in importance. Organic, genetic modification and irradiation are three other social concerns that must be addressed. Overall, however, the food hydrocolloids are in an excellent situation to embrace the changes which may be necessary.

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12

Developing new polysaccharides

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12.1 Introduction

12.1.1 Microbial polysaccharides

Many bacteria, yeasts and fungi can produce polysaccharides. Polysaccharides are present as cellular constituents (e.g. cell walls) or are secreted into the extracellular environment. Although much interest in these polymers is because of their role in infection (e.g. *O*-antigens of the lipopolysaccharides of Gram-negative bacteria as virulence factors) or adhesion, some of them have proved to be useful industrial products that compete with plant and algal polysaccharides as well as synthetic products (Sutherland, 1990, 1998).

In general, microbial polysaccharides are extracellular polysaccharides that are associated with, and often covalently bound to, the cell surface in the form of capsules, or secreted into the environment in the form of slime. They are referred to as capsular exopolysaccharides (CPS) or slime exopolysaccharides (EPS), respectively (Sutherland, 1972). Cell wall polysaccharides (WPS) are another type that, in contrast to EPS, are not released into the medium and are associated with the cell envelope, and may again be either covalently bound to the peptidoglycan layer or loosely associated with it (Delcour *et al.*, 1999). Finally, mucoidness gives a slimy appearance to bacterial colonies but does not produce viscous ropes, while ropiness is phenotypically defined by viscous strings originating from the colonies when these are touched (Knoshaug *et al.*, 2000). A capsule can be visualised microscopically.

12.1.2 Food structure and texture

Polysaccharides can be added as additives to food, or are produced *in situ* and hence contribute as an ingredient to the food structure. In general terms, polysaccharides alter the texture of a food product and influence its rheological behaviour. However, many different technological functions can be distinguished (Sutherland, 1996). Most polysaccharides are employed in foods as thickeners or gel-forming agents. Thickeners are, for instance, used in jams, sauces, syrups, pie fillings, yoghurts, milk drinks, dairy desserts and snack foods. Gelling agents are requested in confectionery, milk-based desserts, jellies, pie and pastry fillings, pet foods and synthetic meat gels. Stabilisers are used in bakery fillings, canned foods, dry mixes, ice cream, salad dressings, yoghurts, dairy desserts, snack foods, sauces, gravies, processed cheeses and juice drinks. Polysaccharides applied in frozen foods and sugar syrups control the stability of the product and prevent or reduce the formation of crystals. Polysaccharides further inhibit syneresis in yoghurt, cheeses and frozen foods. Other applications of polysaccharides are as adhesive (icings and glazes), binding agent (pet foods), swelling agent (processed meat products), coating agent (confectionery), film formation agent (protective coatings and sausage casings), fining agent (wine and beer) and foam stabiliser (beer). Appearance, colour and flavour of a food product may be affected by a polysaccharide as well; in addition, powdered flavours may be encapsulated with polysaccharide. Polysaccharides of a lipophilic nature (less polar groups) may serve as emulsifying agents (salad dressings). Polysaccharides are also incorporated in baking improvers as conditioners, crumb softeners and anti-staling agents. However, it has to be realised that in most cases the consistency and texture of the final product is the result of a complex set of interactions between different food components. For instance, if a polysaccharide-based thickener is desired for usage in a milk-based product, complete compatibility with milk proteins is a prerequisite.

12.1.3 Industrially important microbial polysaccharides

A variety of polysaccharides produced by plants (starch, cellulose, pectin, locust bean gum, guar gum) and algae (alginate, carrageenan, agar) are commonly used as food additives for their thickening, viscosifying, stabilising, suspending, emulsifying, gelling or water-binding properties (Sutherland, 1990, 1998; Tombs and Harding, 1998). While dextran was the first microbial EPS to be commercialised and to receive approval for food use, very few bacterial EPS are currently employed in the food industry. Xanthan is a high-molecular-mass, anionic polysaccharide from the plant pathogenic bacterium *Xanthomonas campestris*. It has a cellulosic backbone on every second glucose residue of which a trisaccharide side-chain is attached; the repeating unit is a pentasaccharide. This unique structure confers special physical properties to the polymer, which is utilised in food and other industries. Xanthan is stable at both acid and alkaline pH and forms a pseudoplastic dispersion in water. Relatively low polysaccharide concentrations produce highly viscous solutions and the viscosity does not change greatly on raising the temperature. The solutions are compatible with many other food ingredients and with salts and other chemicals and give good flavour release. Xanthan is also a good suspending and stabilising agent for oil/water emulsions such as salad dressings. Finally, xanthan can form gels with, for instance, locust bean gum in very low concentrations of total polysaccharide. Such mixtures are widely used in the preparation of manufactured foods.

The use of xanthan as a food additive was accepted by the US Food and Drug Administration (FDA) in 1969. In the EU it has been added to the positive list of additives under number E415. Gellan is a high-molecular-mass anionic polysaccharide from another Gram-negative plant pathogenic bacterium, *Sphingomonas paucimobilis.* It is composed of a tetrasaccharide repeating unit partially esterified with glycerate and/or acetate. Low acyl gellan is an excellent gelling agent providing firm, brittle gels at low concentrations; high acyl gellan gum forms soft, elastic, thermoreversible gels. Further, it yields highly viscous aqueous solutions enabling its use as a stabilising and suspending agent. Gellan has authorisation for food use in both the USA and the EU (E418) but finds other applications as a gelling agent for plant biotechnology as well as for bacterial cultures.

Dextran, although no longer used as a food ingredient, is the base for chromatographic media. Dextran solutions can also be used as a blood plasma substitute, being very poorly antigenic and having the correct physical properties. Curdlan is used as a formulation and processing aid, stabiliser and thickener, or texturiser for use in food and was approved for these uses by the FDA in 1996. Also, bacterial alginates secreted by *Pseudomonas* and *Azotobacter* species, bacterial cellulose from *Acetobacter xylinum*, hyaluronic acid from *Streptococcus equi* and succinoglycan from *Rhizobium* species have found applications, albeit in the non-food sector (Sutherland, 1990).

The functional properties of plant carbohydrates and xanthan in foods are determined by quite subtle structural characteristics. For instance, cellulose, amylose and dextran, which are all essentially linear D-glucans, have completely different physical properties. Cellulose (1,4-linked β-D-glucan) is water-insoluble and highly crystalline, whereas dextran (1,6-linked α-D-glucan) is extremely water-soluble. The degree of branching (non-α-$1 \rightarrow 6$ bonds) and molecular mass affect the water solubility and other physical properties of dextran. Amylose (1,4-linked α-D-glucan) is sparingly soluble in water, but forms strong thermoreversible gels at low concentrations. Xanthan is a natural water-soluble cellulose owing to its side-chains. On the side-chains are both *O*-acetyl and pyruvate ketals. The acyl and other non-sugar substituents may greatly alter the conformation and physical properties of polysaccharides, as does the charge conferred by uronic acids (Sutherland, 1994, 2001). For instance, removal of *O*-acetyl moieties and absence of pyruvate, situated in the side-chains of xanthan polymers, positively influence the strength of the gel network formed with the modified polymers.

An important property of alginates (composed of guluronate and mannuronate) is their ability to form gels in the presence of divalent cations, e.g. Ca^{2+}, through inter-chain cross-linking of guluronate sequences. In galactomannan gels non-substituted mannose regions are supposed to associate to form a three-dimensional network. Regions with a relatively high number of galactosyl side-chains are important for its solubility. Synergistic gelling represents a specific interaction between different polysaccharides, thus reducing the total amount of polysaccharide required to yield firm gels. For example, xanthan can form stable gels when mixed with plant gluco- and galactomannans.

For industrial practice, most of the plant carbohydrates are chemically modified (Tombs and Harding, 1998). For instance, starch esters and starch ethers influence molecular mass (distribution), crystallinity and trace impurities, in turn changing key properties such as gelatinisation temperature, solubility, viscosity, gel formation and ionic charges. The use of modified and native plant polysaccharides in foods is restricted and regulated by law. Unfortunately, the chemical modifications provide these polysaccharides with a non-natural image, at least from the consumer point of view. Nowadays the consumer is more aware but also more concerned about the use of food additives; 100% natural food products that are free of additives and that undergo minimal processing are increasingly popular. Enzymatic treatments of polysaccharides contribute to the natural development of modified structures with enhanced functional properties.

An illustrative example of the use of enzymes in tailoring polysaccharides is the upgrading of alginate as a gel former through conversion of mannuronate into guluronate at the polymer level. Guar gum, another interesting galactomannan, can be successfully upgraded into a polymer with much better gelling properties by decreasing the amount of galactose side-chains with a α-D-galactosidase. Finally, genetic engineering may deliver novel polysaccharide structures with improved properties. However, the use of genetically modified products is hindered by the hostility of the consumer.

Widespread screening of several environments for novel polysaccharides from microbial strains remains interesting. The search for new, naturally synthesised EPS has intensified since the early 1990s. As an example, interesting polymers from numerous isolates from deep-sea thermal vents have been examined (Rougeaux *et al.*, 1996). It is, however, unlikely that many more new products from Gram-negative bacteria will be developed (Sutherland, 2001). In reality few match the robustness of xanthan and few can maintain their physical properties in the presence of salts, at higher temperatures or extremes of pH. The texturising properties of EPS in fermented dairy products in combination with the GRAS (Generally Recognised As Safe) status of EPS-producing lactic acid bacteria (LAB) make these Gram-positive bacteria of interest for the food industry (De Vuyst and Degeest, 1999; Kleerebezem *et al.*, 1999; De Vuyst *et al.*, 2001). Also, these EPS represent natural, safe, food-grade additives for novel food formulations and may have applications in non-food products. Other food-grade bacteria such as dairy propionibacteria and bifidobacteria are known to produce EPS as well (Abbad-Andaloussi *et al.*, 1995; Cerning, 1995; Roberts *et al.*, 1995; De Vuyst *et al.*, 2001). In this chapter, only EPS and more specifically heteropolysaccharides from LAB will be dealt with. For reviews the reader is referred to Cerning (1990, 1995), Cerning and Marshall (1999), De Vuyst and Degeest (1999), Ricciardi and Clementi (2000), De Vuyst and Marshall (2001) and Ruas-Madiedo *et al.* (2002a).

12.2 Developing new polysaccharides: key issues

EPS from LAB can be subdivided into two major groups: (i) the homopolysaccharides (HoPS) that are composed of one type of monosaccharide and (ii) the heteropolysaccharides (HePS) composed of a repeating unit that contains two or more different monosaccharides (De Vuyst *et al.*, 2001).

12.2.1 Production of homopolysaccharides by lactic acid bacteria

Based on their structure, four groups of HoPS can be distinguished (Table 12.1): α-D-glucans (dextrans, mutans and alternan produced by *Leuconostoc* spp., *Streptococcus* spp. and *Lactobacillus* spp.), β-D-glucans (produced by *Lactobacillus* spp., *Streptococcus* spp. and *Pediococcus* spp.), β-D-fructans (levans and inulin-type fructans produced by *Streptococcus* spp., *Leuconostoc* spp. and *Lactobacillus* spp.) and others such as polygalactan (produced by *Lactococcus lactis*). Among all LAB HoPS, strain-specific differences occur that depend on the degree of branching and the different linking sides (Monsan *et al.*, 2001). HoPS are of high molecular mass (up to 10^7 Da). The production of HoPS requires the presence of a specific substrate such as sucrose or related oligosaccharides, which serve as the glycosyl donor.

The polymerisation of glucose or fructose takes place outside the bacterial cell and is thus independent of the intracellular central carbohydrate metabolism. The necessary transglycosylases (glycansucrases) are secreted extracellularly. This enables cell-free synthesis of HoPS, which is of industrial importance. The transglycosylases, for instance glucansucrase or fructansucrase, use the energy of the osidic bond of sucrose to catalyse the transfer of a corresponding glycosyl moiety, glucose or fructose, respectively. Dextran is synthesised by a two-site insertion mechanism. During the primary reaction, the substrate reaction, the D-glucosyl group from sucrose is added to the reducing end of a covalently linked, growing dextran chain. The chain, or the covalently bound glucosyl residue, is released from the active site by a secondary reaction, the acceptor reaction.

At the molecular level, the catalytic action of dextransucrase is still not understood in detail. However, the amino acid sequences of some dextransucrases have been reported and the probable location of the catalytic sites have been identified by sequence comparison and site-directed mutagenesis. In addition to the synthesis of high-molecular-mass polymers, glycansucrases generally catalyse the production of low-molecular-mass oligosaccharides when efficient acceptor molecules, such as maltose, are present in the reaction medium in addition to sucrose. HoPS are produced in large quantities upto 10 g l^{-1} (van Geel-Schutten *et al.*, 1999; Ul-Qader *et al.*, 2001).

12.2.2 Production of heteropolysaccharides by lactic acid bacteria

Biodiversity of heteropolysaccharide production

HePS are produced by LAB in a greater variety with respect to chemical composition,

Table 12.1 Homopolysaccharides produced by lactic acid bacteria. For this classification only the structure of the HoPS has been taken into account. For appropriate references, see Cerning (1990) and Monsan *et al.* (2001)

Homopolysaccharide	Structure	Lactic acid bacteria species
α-D-glucans		
– dextrans	[α-D-Glc*p*(1→6)][a]	*Leuc. mesenteroides* *S. mutans* *S. sanguis* *S. downei* *Lb. reuteri* LB 121[f] *Lb. brevis* G-77[g]
– mutans	[α-D-Glc*p*(1→3)][b]	*S. mutans* *S. sobrinus* *Leuc. mesenteroides*
– alternan	[α-D-Glc*p*(1→3)/(1→6)][c]	*Leuc. mesenteroides*
β-D-glucans	[β-D-Glc*p*(1→3)]	*Pediococcus damnosus* *Streptococcus* spp. *Lb. brevis* G-77[g]
β-D-fructans		
– levans	[β-D-Fru*p*(2→6)]	*S. salivarius* *S. mutans* *Lb. reuteri* LB 121 *Leuc. mesenteroides* NRRL-B512F *Lb. sanfranciscensis*[h]
– inulin-type fructans	[β-D-Fru*p*(2→1)]	*Streptococcus mutans* Ingbritt A *S. mutans* JC-2 *Lb. reuteri* LB 121[i]
Other		
– polygalactan	[α-D-Gal*p*/β-D-Gal*p*][d]	*L. lactis* subsp. *lactis* H414[j]
– glycogen-like	[α-D-Glc*p*(1→4)][e]	*L. lactis* subsp. *lactis* ATCC 19435[k]

[a] Besides >50% α(1→6) linkages, also α(1→3), and less frequently α(1→2) and α(1→4) linkages may occur.

[b] Besides min. 50% α(1→3) linkages, also α(1→6) linkages.

[c] Some degree of α(1→3) branches.

[d] Synthesised as a heteropolysaccharide with a pentameric repeating unit.

[e] Intracellular polysaccharide synthesised when cells are grown on maltose.

[f] With terminal 4-substituted, 6-substituted or 4,6-disubstituted α-D-glucose.

[g] With a 2-substituted single glucose.

[h] For a reference, see Korakli *et al.* (2000).

[i] For a reference, see Van Hijum *et al.* (2002): only in recombinant *E. coli* containing the fructosyltransferase (*ftf*) gene from *Lb. reuteri* LB 121.

[j] For a reference, see Gruter *et al.* (1992).

[k] For a reference, see Levander *et al.* (2001a).

monomer ratio and molecular structure (monosaccharide components, ring forms, anomeric configurations, stereo- and regio-specific linkages) of the repeating unit as well as the conformation and molecular mass of the polymer (De Vuyst *et al.*, 2001). This high diversity is reminiscent of the capsule variation present in pathogenic streptococci such as *Streptococcus pneumoniae* (Henrichsen, 1995). This biodiversity initiated interest in analysis of the chemistry, biochemistry and genetic basis of HePS biosynthesis by LAB.

Monomer composition. HePS are composed of repeating units of a limited number of diverse sugar residues (Table 12.2). The repeating units most often contain a combination of the neutral sugars D-glucose (Glc), D-galactose (Gal) and L-rhamnose (Rha), albeit in different ratios (De Vuyst *et al.*, 2001). In a few cases, acetylated amino sugars, namely *N*-acetyl-D-glucosamine (GlcNAc) and *N*-acetyl-D-galactosamine (GalNAc), are present (Doco *et al.*, 1990; Yamamoto *et al.*, 1994; Robijn *et al.*, 1996a,b; Stingele *et al.*, 1996; Degeest *et al.*, 2001b; Marshall *et al.*, 2001b; Navarini *et al.*, 2001; Vincent *et al.*, 2001; Faber *et al.*, 2002b; Landersjö *et al.*, 2002). L-Fucose (Fuc; Low *et al.*, 1998), D-ribose (Rib; Faber *et al.*, 2002b), D-glucuronic acid (GluA; Robijn *et al.*, 1996a) and D-nononic acid (Sug; Faber *et al.*, 2002b) have been reported only once. Further, non-carbohydrate constituents such as glycerol, and acetyl, pyruvyl and phosphate groups may occur (Nakajima *et al.*, 1992a; Robijn *et al.*, 1995b, 1996b; van Casteren *et al.*, 1998, 2000a; Yang *et al.*, 1999; Faber *et al.*, 2001b; van Calsteren *et al.*, 2002).

Other interesting observations are:

(1) the monosaccharide present with the highest frequency is galactose followed closely by glucose and then, at a much lower frequency, rhamnose;
(2) the rare occurrence of the combination of glucose and rhamnose in the absence of galactose;
(3) the higher number of galactose residues compared with glucose in the HePS from *S. thermophilus*;
(4) the high number of galactose residues in the HePS from some *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* strains;
(5) the high number of rhamnose residues in the HePS from *Lb. rhamnosus* RW-9595M; and
(6) the presence of GalNAc residues only in HePS from *S. thermophilus*, except for that from *Lb. paracasei* 34-1.

Other uronic acids, fructose, mannose, arabinose and xylose have never been detected in purified HePS from LAB whose primary structure is known. Uronic acids, mannose, rhamnose, ribose, etc. are typical for HePS isolated and purified from Gram-negative bacteria (Sutherland, 1985). Uronic acids, aminosugars and rhamnose often occur as contaminants in HePS preparations, for instance elaborated or extracted from the cell envelopes by cell wall degrading enzymes or trichloroacetic acid treatment, respectively (Cibik and Chapot-Chartier, 2000; Quiberoni *et al.*, 2000). Because of the absence of uronic acids, HePS from LAB are neutral to weakly negatively (presence of phosphate) charged polysaccharides.

Table 12.2 Classification of heteropolysaccharides produced by lactic acid bacteria based on the linkages in the backbone of the HePS structure and their monosaccharide composition

Linkage class	Monosaccharide composition								Lactic acid bacteria species	Reference
	Glc	Glc NAc	Gal	Gal NAc	Rha	Fuc	Rib	Sug		
β										
only β (1→4)	2		2		1				*Lactococcus lactis* subsp. *cremoris* SBT 0495	Nakajima *et al.* (1992a)
	2		2		1				*Lactococcus lactis* subsp. *cremoris* NIZO B40	van Casteren *et al.* (1998)
	2		2		1				*Lactococcus lactis* subsp. *cremoris* AHR53	Yang *et al.* (1999)
1 β (1→4) + 2 β (1→6) + 1 β (1→3) +			3	1					*Lactobacillus paracasei* 34-1	Robijn *et al.* (1996b)
β + α										
2 β (1→4) + 1 α										
– no (1→6)	3		2						*Lactococcus lactis* subsp. *cremoris* NIZO B891	van Casteren *et al.* (2000a)
	3		2						*Lactobacillus delbrueckii* subsp. *bulgaricus* 291	Faber *et al.* (2001a)
	3				2				*Lactobacillus sakei* 0-1	Robijn *et al.* (1995b)
	4		2						*Lactobacillus helveticus* K16	Yang *et al.* (2000)
	3	1	2						*Streptococcus macedonicus* Sc 136	Vincent *et al.* (2001)
– with (1→6)	3	1	1						*Lactobacillus acidophilus* LMG 9433	Robijn *et al.* (1996a)
	3		3						*Lactobacillus* sp.	Micheli *et al.* (1999)
	2		4						*Lactobacillus helveticus* 2091	Staaf *et al.* (1996)
3 β (1→4) + 2 α	1		2	1			1	1	*Streptococcus thermophilus* 8S	Faber *et al.* (2002b)
2 β (1→4) + 3 α	3				3				*Lactobacillus delbrueckii* subsp. *bulgaricus* EU23	Harding *et al.* (2003)

1 β (1→4) + 2 α							
– no (1→6)	1		5		1	*Lactobacillus delbrueckii* subsp. *bulgaricus* rr	Gruter *et al*. (1993)
	1		5		1	*Lactobacillus delbrueckii* subsp. *bulgaricus* LY03, 24 and 25	Marshall *et al*. (2001b)
	1		5		1	*Lactobacillus delbrueckii* subsp. *bulgaricus* Lfi5	Lamothe *et al*. (2002)
– with (1→6)	2		3		2	*Streptococcus thermophilus* EU20	Marshall *et al.* (2001a)
1 β (1→4) + 3 α							
– no (1→6)		1	4		1	*Lactobacillus rhamnosus* GG	Landersjö *et al*. (2002)
	5		2			*Lactobacillus helveticus* Lb161	Staaf *et al*. (2000)
– with (1→6)	4		2			*Lactobacillus helveticus* NCDO 766	Robijn *et al*. (1995a)
no β (1→4)							
– no (1→6)	2		2			*Streptococcus thermophilus* SFi39	Lemoine *et al*. (1997)
	2		2			*Streptococcus thermophilus* SY89 and SY102	Marshall *et al*. (2001b)
	1		2	1		*Streptococcus thermophilus* CNCMI 733	Doco *et al*. (1990)
	1		2	1		*Streptococcus thermophilus* Sfi6	Stingele *et al*. (1996)
	1		2	1		*Streptococcus thermophilus* IMDO 01, IMDO 02, IMDO 03, NCFB 859 and 21	Marshall *et al*. (2001b)
	1		2	1		*Streptococcus thermophilus* SFi20	Navarini *et al*. (2001) Degeest *et al*. (2001b)

Table 12.2 cont'd

Linkage class	Monosaccharide composition								Lactic acid bacteria species	Reference
	Glc	Glc NAc	Gal	Gal NAc	Rha	Fuc	Rib	Sug		
	3		3						*Lactobacillus helveticus* TN-4	Yamamoto *et al.* (1995)
	3		3						*Lactobacillus helveticus* Lh59	Stingele *et al.* (1997)
			4		2				*Streptococcus thermophilus* S3	Faber *et al.* (2001b)
	2		1		4				*Lb. rhamnosus* RW-9595M and R	Van Calsteren *et al.* (2002)
– with (1→6)	3		3						*Lactobacillus helveticus* NCC 2745	Jolly *et al.* (2002a)
	3	1	3						*Lactobacillus helveticus* TY1-2	Yamamoto *et al.* (1994)
	2		3						*Lactobacillus rhamnosus* C83	Vanhaverbeke *et al.* (1998)
α										
	1		3		2				*Streptococcus thermophilus* SFi12	Lemoine *et al.* (1997)
			5		2				*Streptococcus thermophilus* OR901	Bubb *et al.* (1997)
			5		2				*Streptococcus thermophilus* Rs and Sts	Faber *et al.* (1998)
			5		2	1			*Streptococcus thermophilus* MR-1C	Low *et al.* (1998)
	2		3		2				*Lactococcus lactis* subsp. *cremoris* NIZO B39	van Casteren *et al.* (2000b)

Structures. Detailed structural analysis of the HePS produced by LAB over the last decade has revealed an immense variety of structures of their repeating units. Since the last review of De Vuyst *et al.* (2001) five new structures have been elucidated, namely the structure of the HePS repeating unit from *Lb. delbrueckii* subsp. *bulgaricus* EU23 (Harding *et al.*, 2003), *Lb. helveticus* NCC2745 (Jolly *et al.*, 2002a), *Lb. rhamnosus* GG (Landersjö *et al.*, 2002), *Lb. rhamnosus* RW-9595M and R (van Calsteren *et al.*, 2002), and *S. thermophilus* 8S (Faber *et al.*, 2002b). The structure for the HePS repeating unit from *Lb. delbrueckii* subsp. *bulgaricus* Lfi5 was identical to one reported before (Lamothe *et al.*, 2002). Of all structures published to date, an octasaccharide is the largest repeating unit reported, indicating that LAB may not be capable of producing larger building blocks. This is also the case for HePS from Gram-negative bacteria (Sutherland, 2001). In general, side-chains of one, two or three monosaccharides are attached to the HePS repeating unit from LAB; only the HePS repeating units from *Lb. rhamnosus* C83 and *S. thermophilus* 8S represent a linear structure (Vanhaverbeke *et al.*, 1998; Faber *et al.*, 2002b). The non-carbohydrate constituents occur, either as an ester linkage, e.g. acetate and glycerate bound to a monosaccharide or phosphate group, or as a ketal linkage, e.g. pyruvate (Robijn *et al.*, 1995b, 1996b; van Casteren *et al.*, 2000a; Faber *et al.*, 2001b; van Calsteren *et al.*, 2002). Phosphate groups are present in a diester linkage between two monosaccharides or between a monosaccharide and a glycerol group, and may provide the HePS with a negative charge (Nakajima *et al.*, 1992a; Robijn *et al.*, 1995b, 1996b; van Casteren *et al.*, 1998; Yang *et al.*, 1999).

To date there are over 25 unique structures of the HePS repeating units. These structures can be grouped according to different linkage patterns in their backbone (Table 12.2). Based on these structures, a number of observations can be made:

(1) for glucose and galactose there is a small preference for the β-anomer, while for rhamnose, there is a clear preference for the α-anomer;
(2) the only hexose to adopt a furanose conformation is galactose;
(3) the most frequent backbone is a pentasaccharide, followed by tri-, tetra-, hexa- and heptasaccharides;
(4) the longest backbone contains seven sugar units and is found in the HePS repeating unit of *S. thermophilus* 8S;
(5) when only α- or β-bonds occur in the backbone, α-bonds are more prevalent than β-bonds;
(6) α (1→3) bonds are most abundant in the backbone, followed by β (1→4), and then α (1→2), β (1→3) and β (1→6) bonds;
(7) (1→6) links are less frequent and are found more widely in HePS repeating units from lactobacilli, once in *S. thermophilus*, and not at all in lactococci;
(8) β (1→7) and β (1→5) bonds are very rare;
(9) in *Lb. paracasei* 34-1 only β-bonds occur, and some repeating units of HePS from *L. lactis* subsp. *cremoris* contain only β (1→4) bonds;
(10) most of the branched structures have only one side-chain composed of one, two or three monosaccharides, of which the latter occurs very rarely;

(11) as a branch terminus, galactose most frequently occurs followed by glucose and then by rhamnose;

(12) in the side-chains β (1→6) bonds occur most frequently, followed by β (1→4) and β (1→3) bonds, while β (1→2) bonds and α-links are less frequent;

(13) if present β (1→3) bonds and α-links only occur at the branching points of the side-chain; and

(14) a heptasaccharide repeating unit has been reported for several strains of *Lb. delbrueckii* subsp. *bulgaricus*.

Identical HePS repeating units do not appear to occur among different genera of LAB. Moreover, the structure of a HePS repeating unit seems to be strain-specific. However, some species may produce an almost identical polysaccharide. For instance, the backbone for the HePS isolated from *Lb. helveticus* K16 (Yang *et al.*, 2000) is the same as that synthesised by *L. lactis* subsp. *cremoris* NIZO B891 (van Casteren *et al.*, 2000a), *Lb. delbrueckii* subsp. *bulgaricus* 291 (Faber *et al.*, 2001a) and *S. macedonicus* Sc136 (Vincent *et al.*, 2001). These structures differ in their side-chains (composition and/or branch point). Similarly, the same -Gal-Glc-Glc-sequence is found in the repeating units of the HePS from some *L. lactis* subsp. *cremoris* (Nakajima *et al.*, 1992a; van Casteren *et al.*, 1998; Yang *et al.*, 1999) and *S. thermophilus* (Lemoine *et al.*, 1997; Marshall *et al.*, 2001b) strains. Yet, a number of the HePS from LAB are structurally related. They include the repeating units of HePS produced by some *S. thermophilus* strains (Bubb *et al.*, 1997; Lemoine *et al.*, 1997; Faber *et al.*, 1998, Low *et al.*, 1998). These HePS are characterised by the presence of a repeating pentameric backbone containing only α-links of Rha-α-(1→2), Glc-α-(1→3) and Gal-α-(1→3). The OR 901, Rs and Sts HePS have identical repeating units. The repeating units differ in the attachment site of the side-chain, as well as in its composition. For instance, the OR 901, Rs and Sts HePS repeating units differ from the structure of the MR-1C HePS repeating unit in a fucose side-chain present in the latter. Finally, a -Gal-Glc-sequence of the side-chain is distributed among the HePS repeating units of some *L. lactis* subsp. *cremoris* (van Casteren *et al.*, 2000a,b), *Lb. delbrueckii* subsp. *bulgaricus* (Faber *et al.*, 2001a) and *Lb. helveticus* (Yamamoto *et al.*, 1994, 1995; Stingele *et al.*, 1997; Jolly *et al.*, 2002a) strains. The nature of the links within this motif as well as the branch linkage and site may differ.

Producing strains. HePS are produced by mesophilic (e.g. *L. lactis, Lb. casei, Lb. paracasei, Lb. sakei*) and thermophilic (e.g. *Lb. acidophilus, Lb. delbrueckii* subsp. *bulgaricus*, *Lb. helveticus*, and *S. thermophilus*) LAB (Table 12.2). HePS from mesophilic LAB have been classified according to their monomer composition (van Kranenburg *et al.*, 1999b). Three major groups have been distinguished: (i) HePS containing only galactose; (ii) HePS containing galactose and glucose, and (iii) HePS containing galactose, glucose and rhamnose. A preliminary grouping has been performed for the HePS from a limited number of *S. thermophilus* strains as well (Marshall *et al.*, 2001b). Each group corresponded with a HePS with a particular subunit structure. Other relationships between species and HePS have not been established.

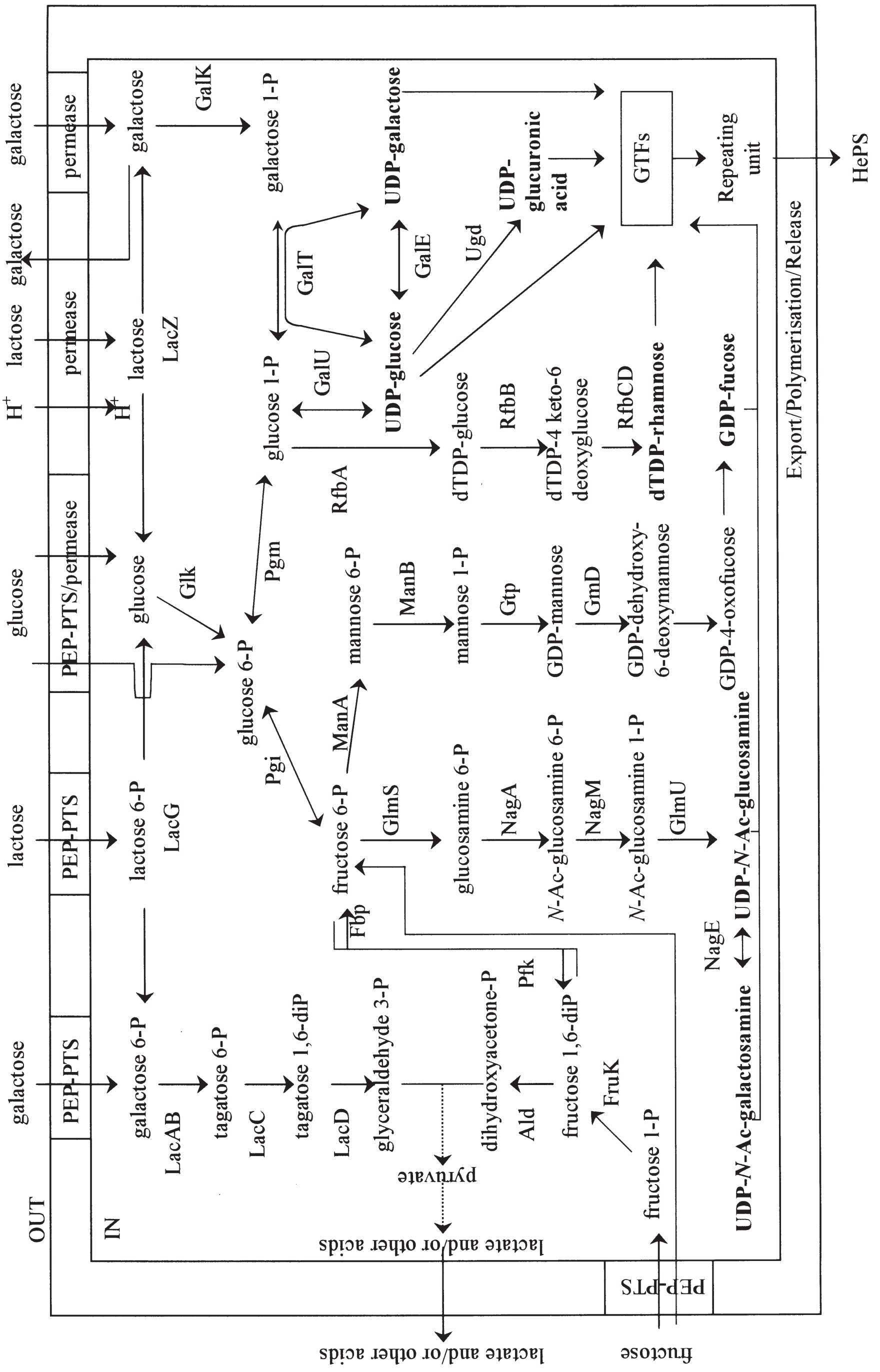
Molecular mass. HePS vary in molecular mass from 1.0 × 10^4 to 9.0 × 10^6 Da (Cerning, 1995; De Vuyst and Degeest, 1999), which are high values compared with many other microbial polysaccharides (Sutherland, 1990). Some LAB strains produce high-molecular-mass HePS, while others produce low-molecular-mass HePS. The simultaneous production of two HePS with different molecular mass has been reported for *L. lactis* subsp. *cremoris* LC 330 (Marshall *et al.*, 1995), *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2772 (Grobben *et al.*, 1997), *S. thermophilus* OR901 (Ariga *et al.*, 1992), *S. thermophilus* LY03 (Degeest and De Vuyst, 1999) and *S. thermophilus* SY (Ricciardi *et al.*, 2002).

Genetics and biosynthesis of heteropolysaccharides

Genes involved in HePS biosynthesis in all the Gram-positive bacteria producing polysaccharides at the surface of the cell identified to date are organised in gene clusters. They appear to be highly conserved. They code for regulation, chain length determination, biosynthesis of the repeating unit, polymerisation and export (Jolly and Stingele, 2001). Genes (*eps*) or gene clusters involved in the biosynthesis of HePS from LAB have been reported for *S. thermophilus* (Stingele *et al.*, 1996; Low *et al.*, 1998; Bourgoin *et al.*, 1999; Almirón-Roig *et al.*, 2000; Germond *et al.*, 2001), *S. macedonicus* (Jolly and Stingele, 2001), *Lb. helveticus* (Jolly *et al.*, 2002a), *Lb. delbrueckii* subsp. *bulgaricus* (Lamothe *et al.*, 2002) and *L. lactis* (van Kranenburg *et al.*, 1997, 1999*a*). The genes are transcribed as a single mRNA (Stingele *et al.*, 1996; van Kranenburg *et al.*, 1997). HePS biosynthesis differs from HoPS biosynthesis in that the precursor repeating units are formed intracellularly. The sugar repeating units are thought to be assembled on a membrane-bound lipophilic carrier (undecaprenyl-phosphate) by sequential addition of sugar residues from activated donors (sugar nucleotides) by glycosyltransferases (GTFs) (van Kranenburg *et al.*, 1999a; Jolly *et al.*, 2002a). Hence, a possible control factor in HePS biosynthesis is the availability of the required sugar nucleotides (Fig. 12.1).

Sugar nucleotides are derived from Glc 1-P, Fru 6-P or Gal 1-P as precursor molecules (De Vuyst and Degeest, 1999; Looijesteijn *et al.*, 1999; Degeest *et al.*, 2001b; De Vuyst *et al.*, 2001; Levander and Rådström, 2001). Glc 1-P can be converted with UDP-glucose pyrophosphorylase (GalU) into UDP-Glc, which subsequently is converted into UDP-Gal by UDP-galactose 4 epimerase (GalE). Glc 1-P can also be converted into dTDP-Rha by the sequential activities of the rhamnose synthetic enzymes, and UDP-Glc can also be converted into UDP-GluA by UDP-glucose dehydrogenase (Ugd). UDP-GlcNAc and UDP-GalNAc are formed from Fru 6-P via the aminosugar metabolism (Delcour *et al.*, 1999; Boels *et al.*, 2001b).

Correlations exist between the enzymes involved in these syntheses and HePS monomer composition and often HePS yields (Grobben *et al.*, 1996; Escalante *et al.*, 1998, 2002; Degeest and De Vuyst, 2000; Mozzi *et al.*, 2001, 2003). GTFs form the actual glycosidic bonds of the HePS repeating unit. Individual enzymes are characterised by a donor- and acceptor-specific action, which appears not to be exclusive (Stingele *et al.*, 1999b). However, it is difficult to characterise the


OUT
IN
galactose
PEP-PTS
lactose
PEP-PTS
glucose
PEP-PTS/permease
H+
lactose
permease
galactose
galactose
permease
galactose 6-P
LacAB
tagatose 6-P
LacC
tagatose 1,6-diP
LacD
glyceraldehyde 3-P
pyruvate
lactate and/or other acids
dihydroxyacetone-P
Ald
fructose 1,6-diP
Pfk
FruK
fructose 1-P
PEP-PTS
fructose
lactate and/or other acids
lactose 6-P
LacG
glucose
Glk
lactose
LacZ
galactose
GalK
galactose 1-P
glucose 6-P
Pgi
Pgm
glucose 1-P
GalU
GalT
GalE
fructose 6-P
Fbp
ManA
mannose 6-P
ManB
mannose 1-P
Gtp
GDP-mannose
GmD
GDP-dehydroxy-6-deoxymannose
GDP-4-oxofucose
GDP-fucose
GlmS
glucosamine 6-P
NagA
N-Ac-glucosamine 6-P
NagM
N-Ac-glucosamine 1-P
GlmU
UDP-N-Ac-glucosamine
NagE
UDP-N-Ac-galactosamine
RfbA
dTDP-glucose
RfbB
dTDP-4 keto-6 deoxyglucose
RfbCD
dTDP-rhamnose
UDP-glucose
UDP-galactose
Ugd
UDP-glucuronic acid
GTFs
Repeating unit
Export/Polymerisation/Release
HePS

specificity of putative GTFs towards its two reactants, the activated sugar nucleotide and the acceptor, owing to these specific individual properties of each GTF, which do not permit the use of one general biochemical assay (Jolly *et al.*, 2002a). Not much is known about translocases and polymerases involved in the secretion and polymerisation of the HePS repeating units. However, the mechanism of biosynthesis of HePS resembles the mechanism of biosynthesis of *O*-antigens and several types of CPS (Roberts, 1996). It is also closely related to the mechanism of biosynthesis of cell envelope components such as peptidoglycan and teichoic acids (Delcour *et al.*, 1999).

Glc 1-P is formed by a phosphoglucomutase (Pgm) from Glc 6-P, an intermediate of glycolysis, and hence the responsible enzyme to link glycolysis with HePS biosynthesis (De Vuyst and Degeest, 1999). Two forms of Pgm are known. In general, α-Pgm is responsible for generating Glc 1-P from Glc 6-P and hence fuels the pathways leading to the sugar nucleotides that are necessary not only for HePS biosynthesis but also for the biosynthesis of peptidoglycan, LPS, WPS, etc. In that sense competition can be expected between cell synthesis and HePS production (De Vuyst and Degeest, 1999). In particular cases β-Pgm is involved in sugar degradation. For instance, *L. lactis* cells grown on maltose form glucose and β-Glc 1-P through maltose phosphorylase activity (Levander *et al.*, 2001a). The glucose formed enters the glycolysis. β-Pgm converts β-Glc 1-P to Glc 6-P before entering the glycolysis. Similarly, trehalose phosphorylase or trehalose 6-P phosphorylase are used by the cell to form Glc 6-P and β-Glc 1-P during trehalose metabolism of *L. lactis* (Andersson *et al.*, 2001; Andersson and Rådström, 2002). Interestingly, β-Pgm mutants of *L. lactis* are grown on maltose accumulated polysaccharides which are probably a combination of intracellular polysaccharides and WPS rather than HePS (Levander *et al.*, 2001a).

←

Fig. 12.1 Schematic representation of pathways involved in lactose, galactose, glucose and fructose catabolism leading to the formation of sugar nucleotides that are involved in heteropolysaccharide (HePS) biosynthesis in lactic acid bacteria. Lactose, glucose, galactose and fructose transport can occur *via* a specific phosphotransferase system (PEP-PTS). In addition, galactose transport can take place *via* a highly specific galactose permease while lactose transport can occur via lactose/galactose antiport. The different enzymes involved are: LacG, phospho-2-galactosidase; LacAB, galactose 6-phosphate isomerase; LacC, tagatose 6-phosphate kinase; LacD, tagatose 1,6-diphosphate aldolase, LacZ, 2-galactosidase; Glk, glucokinase; Pgm, phosphoglucomutase; GalU, UDP-glucose pyrophosphorylase; GalE, UDP-galactose 4 epimerase; GalT, galactose 1-phosphate uridylyltransferase; RfbA, dTDP-glucose pyrophosphorylase; RfbB, dTDP-glucose 4,6-dehydratase; RfbCD, dTDP-4-keto-6-deoxy-D-glucose 3,5-epimerase and dTDP-4-keto-L-rhamnose reductase; GalK, galactokinase; Ugd, UDP-glucose dehydrogenase; Pgi, phosphoglucose isomerase; ManA, mannose 6-phosphate isomerase; ManB, phosphomannomutase; Gtp, mannose 1-phosphate guanylyltransferase; Gmd, GDP-mannose 4,6-dehydratase; GlmS, glutamine-fructose 6-phosphate transaminase; NagA, *N*-acetylglucosamine 6-phosphate deacetylase; NagM, acetylglucosamine phosphomutase; GlmU, UDP-*N*-acetylglucosamine pyrophosphorylase; NagE, UDP-*N*-acetylglucosamine 4-epimerase; Fbp, fructose 1,6-bisphosphatase; Pfk, 6-phosphofructokinase; FruK, 1-phosphofructokinase; Ald, fructose 1,6-diphosphate aldolase. GTF, glycosyltransferase. The sugar nucleotides for incorporation in the HePS repeating unit are represented in bold.

When galactose is used as energy source or made available via the Leloir pathway during lactose breakdown, Gal 1-P serves as a precursor, which can be converted to UDP-Gal that in turn can be interconverted to UDP-Glc by GalE. Evaluation of HePS production in a *L. lactis galE* mutant reveals undetectable levels of UDP-Gal and abolishes HePS biosynthesis when cultured on glucose, while HePS production is recovered by the addition of galactose to the medium. Moreover, this mutant is also affected in cell division when grown on glucose alone, which indicates a critical role for UDP-Gal as a precursor in cell wall biosynthesis (Boels *et al.*, 2001a). Similarly, HePS biosynthesis by *Lb. casei* CRL 87 is correlated with the activity of GalE for growth on glucose and galactose, whereby the enzyme is not essential for growth (Mozzi *et al.*, 2003). It is interesting to see that *S. thermophilus* strains can also use galactose as a precursor molecule for sugar nucleotide biosynthesis, because α-Pgm-negative mutants of *S. thermophilus* LY03 synthesise HePS when grown on lactose but not when grown on glucose (Levander and Rådström, 2001). Similarly, some strains of *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* produce capsules when grown on galactose only (Hassan *et al.*, 2001a). This also suggests that there is no need for a functional α-Pgm in these strains grown on lactose. This is of particular interest knowing that *S. thermophilus* is usually galactose-negative, and the galactose moiety of lactose is secreted when the cells are grown on lactose. However, the genes encoding the enzymes necessary for galactose breakdown are present in the genome, but are not fully expressed (de Vos and Vaughan, 1994). They can be up-regulated by mutations in the promoter region since they are transcribed as a single unit (Vaughan *et al.*, 2001). Indeed, a galactose-positive mutant of *S. thermophilus* LY03 shows improved galactokinase (GalK) and galactose 1-P uridylyltransferase (GalT) activities and even slightly higher HePS yields (Levander *et al.*, 2002). Similarly, absence of GalK activity in mutants of *Lb. casei* CRL 87 does not result in HePS production on galactose (Mozzi *et al.*, 2001). It is probable that activity of GalE, involved in both galactose breakdown and sugar nucleotide biosynthesis, is constitutively expressed.

There are indications that the GalU activity level controls the biosynthesis of intracellular UDP-Glc and UDP-Gal in wild-type cells of *L. lactis* (Boels *et al.*, 2001a), both of which are precursors for HePS biosynthesis as well as for cell or wall components. However, there is no effect on HePS production when over-producing *gal*U or *pgm*, indicating down regulation of HePS biosynthesis enzymes (Boels *et al.*, 2001a; Levander and Rådström, 2001). Higher levels of Pgm and GalU together lead to a proportional increase in the HePS yield of *S. thermophilus* LY03 (Levander *et al.*, 2002). HePS production increases considerably in a galactose-positive Pgm-negative mutant (Levander *et al.*, 2002). The highest level of HePS is seen if several genes (*pgmA* and *galU*) are over-expressed simultaneously in a galactose-positive mutant. Therefore, the interrelationship between galactose catabolism and HePS biosynthesis requires much more attention. Finally, data supporting that dTDP-rhamnose and UDP-GalNAc biosynthesis are important in the biosynthesis of rhamnose-containing or GalNAc-containing HePS, respectively, are reported for *Lb. sakei* 0-1 (Breedveld *et al.*, 1998; Degeest *et al.*, 2001a), *Lb. rhamnosus* (Mozzi *et al.*, 2003) and *S. thermophilus* LY03 and Sfi20 (Degeest and De Vuyst, 2000; Degeest *et al.*, 2001b).

Knowledge regarding the function of genes involved in HePS biosynthesis is crucial for future HePS and oligosaccharide engineering (van Kranenburg *et al.*, 1999a,b; Stingele *et al.*, 1999b; Jolly *et al.*, 2002a). In particular the genes involved in the biosynthesis of GTFs and chain length determination require detailed knowledge. The genetic pool of GTF genes (*gtf*) determines the HePS biosynthesis capacity of a particular strain. Functional analysis of GTFs revealed (i) the presence of a conservative, priming GTF (mostly a phosphoglucosyltransferase), homologous with an undecaprenyl-glycosyl 1-P transferase; (ii) the requirement of specific GTFs to incorporate particular sugar moieties in the repeating unit; and (iii) the possible exchange of GTFs with identical substrate specificity originating from different genera in Gram-positive bacteria. However, reaction mechanisms of these enzymes still remain unclear (Jolly and Stingele, 2001). The final stages of HePS secretion from the cytoplasmic membrane and finally excretion into the extracellular environment are less well defined (Jolly and Stingele, 2001). Currently, it is assumed that after the completion of the assemblage of the oligosaccharide repeating unit and according to CPS biosynthesis studies, a flippase is responsible for the translocation of the repeating units (Lamothe *et al.*, 2002). A (non-specific) polymerase would then polymerise the units to form high-molecular-mass HePS. Most probably several genes would regulate the number of units. Indeed, the LAB gene clusters are very conserved at the 5’ end and comparable to those of *S. pneumoniae* and *S. agalactiae cps* gene clusters directing CPS biosynthesis (Stingele *et al.*, 1996; van Kranenburg *et al.*, 1997). Most *eps* gene clusters contain homologues for the *O*-antigen polymerisation and export proteins and homologues to ExoP-like proteins, which function together in chain length determination and polymerisation. On the other hand, the high molecular masses of HePS found in LAB suggest that the regulation of polymerisation, if any, is different from the one for *O*-antigen or CPS biosynthesis (Jolly and Stingele, 2001).

Yield, structure and polymer length engineering

HePS engineering may be devoted to specific purposes, such as improving technological aspects of HePS such as texturising, stabilising, suspending and gelification functions, and targeted modification of HePS having health-beneficial properties such as immune stimulation.

Heterologous expression of a HePS in the non-ropy strain of *L. lactis* MG 1363 by transfer of a complete *eps* gene cluster has been attempted first for *S. thermophilus* Sfi6 (Stingele *et al.*, 1999a). The recombinant strain expresses a modified HePS in that the recombinant HePS does not include GalNAc, which requires a supplementary sugar nucleotide precursor that is probably absent from *L. lactis* MG1363. This result indicates that the major requirement for accurate heterologous expression is a transfer of the correct set of *eps* genes coupled with the presence, in the acceptor host, of the required activated sugar nucleotides. The recombinant HePS also lacks the galactose side-chain, probably because of the lack of a specific *S. thermophilus* side-chain galactosyltransferase. An unchanged heterologous

HePS expression has been accomplished in *L. lactis* MG1363 by introduction of the complete *eps* gene cluster from *S. thermophilus* Sfi39 (Germond *et al.*, 2001). This HePS is simply composed of the two most abundant monosaccharide precursors used in LAB HePS biosynthesis, namely glucose and galactose.

Interestingly, both experiments illustrate a certain broad selectivity in the HePS export and polymerisation system of the host. Such a lack of absolute specificity may also account for the natural evolution of bacterial HePS (Sutherland, 2001). In addition, whereas in the first case low amounts of recombinant HePS are obtained (Stingele *et al.*, 1999a), a similar yield as in the wild-type strain is achieved in the second case (Germond *et al.*, 2001). This suggests that the rate of biosynthesis of the repeating unit might be reduced by inefficient recognition by the sequential GTF enzyme machinery. Not much work has been done on engineered HePS overexpression. One single case has been reported, namely HePS production of a transformed *L. lactis* strain that is higher than that of a wild type (van Kranenburg *et al.*, 1999b). There are indications that both the level of sugar nucleotides and the *eps* gene expression levels are the bottlenecks for increased HePS production (Levander *et al.*, 2002). However, engineering HePS biosynthesis for overexpression does not seem straightforward from a biochemical, genetic and kinetic (thermodynamic) point of view. On the other hand, these food-grade bacteria show a clear potential for *in situ* HePS production of practical use in fermented foods that require improved textural properties. If the HePS is produced during processing of the food product, it is incorporated in the product in a natural way and thus has not to be considered as an additive. It might also be cost effective if no additional thickeners need to be used in the food.

Structure engineering aims to alter the physicochemical properties of the HePS by changing their primary structure. This can be achieved by means of enzymatic treatments (Boels *et al.*, 2001b). No such examples aiming to increase the textural properties of HePS have been reported up to now. Alternatively, gene shuffling and combinatorial approaches to individual GTFs can be exploited in constructing biosynthetic machinery introducing new or exchanging existing GTF genes in HePS-producing LAB strains for the production of oligo- and/or polysaccharides that have a predetermined structure with respect to sugar moieties or linkages, so-called designer polysaccharides. For instance, fructose-containing oligosaccharides, β-(2→1) linked, are specifically stimulating the growth of bifidobacteria in the human intestine (Gibson and Roberfroid, 1995), while phosphorylated oligosaccharides display good fibre-like properties in the protection of intestinal mucosa against oxidative damage (Kawaguchi *et al.*, 1999). A first example of such an approach is the possibility of functionally exchanging GTF with identical substrate specificity originating from different genera in Gram-positive bacteria (van Kranenburg *et al.*, 1999b). Today, several LAB GTFs have been identified and can be targeted as tools for both *in vivo* engineering and *in vitro* chemo-enzymatic synthesis of oligosaccharides and polysaccharides. Up to now, 17 different types of glycosidic linkages can be selectively created using these known GTFs (Jolly *et al.*, 2002a). Still, chain length engineering is not so evident given the lack of knowledge of HePS regulation, polymerisation and chain length determination, or polymerisation and export. At present the rapid developments in the generation and analysis of whole and partial genome sequences is expected to produce valuable information that should be followed up by further genetic biochemical and physiological analyses. Combined with metabolic control analysis (MCA) this information can be exploited for developing metabolic engineering strategies to produce tailor-made oligo- and polysaccharides for specific applications.

12.3 Producing stable polysaccharides

12.3.1 Limitations and kinetics

HePS production suffers from several limitations affecting their yield and stability. In general, LAB produce only small amounts of HePS (Degeest *et al.*, 2001c). For instance, in milk *S. thermophilus* strains generally produce between 20 and 100 mg of PDM per litre, being less than 1% of the amount of the energy source. Yields up to 2.8 g l^{-1} have been obtained with *Lb. rhamnosus* R-9595M in a medium based on whey permeate (Macedo *et al.*, 2002c). Although the amounts of HePS produced are species- and strain-dependent, variations in experimental yields may depend on the isolation and quantification methods employed, e.g. precipitation followed by colorimetric or gravimetric measurements (De Vuyst and Degeest, 1999).

Studies on HePS quantification evolve more in the direction of small-scale analysis, e.g. ultrafiltration (Bergmaier *et al.*, 2001; Levander *et al.*, 2001b), or open the possibility for on-line measurements in the fermentation liquor, e.g. near-infrared spectroscopy (Macedo *et al.*, 2002a). Further, growth conditions (temperature, pH, incubation time) and medium composition (carbohydrate and nitrogen sources, other nutrients) affect the HePS yield (Degeest *et al.*, 2001c). However, HePS are to be considered as minor products diverted away from glycolysis (Pham *et al.*, 2000). In general, HePS biosynthesis is growth-associated, which is supported by its requirement for large numbers of sugar nucleotides, and the necessary energy for building the repeating units, for membrane translocation and for polymerisation (Degeest *et al.*, 2001c). Hence, it needs to compete for the same energy source and sugar nucleotide precursors as for cell synthesis.

The limited energy generation through glycolysis by LAB is an additional obstacle for high cell and HePS yields. Also, both growth-associated and non-growth-associated production kinetics have been reported (Degeest *et al.*, 2001c; Torino *et al.*, 2001; Ricciardi *et al.*, 2002; Macedo *et al.*, 2002b,c). Suboptimal growth conditions may enhance HePS production. As Gram-negative bacteria (e.g. *X. campestris*), mesophilic LAB strains produce maximal amounts of HePS under suboptimal growth conditions that favour slower growth, this is at low temperature and high carbon/nitrogen ratios (Sutherland, 1990; Degeest *et al.*, 2001c). This effect has been explained by the fact that slow-growing cells exhibit slower rates of cell wall polymer biosynthesis, making more isoprenoid lipid carrier molecules available for HePS formation (Sutherland, 1972). Thermophilic

LAB show a direct relationship between optimal growth conditions and HePS yields (Degeest *et al.*, 2001c; Torino *et al.*, 2001; Ricciardi *et al.*, 2002).

Finally, HePS production is unstable, particularly in milk (Degeest *et al.*, 2001c), and the HePS-producing trait is often of a transitory nature (Cerning, 1990). HePS degradation upon prolonged fermentation has been attributed to the production of glycohydrolases (Pham *et al.*, 2000; Degeest *et al.*, 2001c, 2002). Specific polysaccharases, hydrolases or lyases may be responsible for degrading the HePS produced (Sutherland, 2001; Pham *et al.*, 2000). Loss of the HePS production capacity has been ascribed to the loss of HePS-encoding plasmids in mesophilic LAB strains, and to reversible DNA rearrangements as well as the presence of several insertion sequence (*IS*) elements in the genome of thermophilic LAB strains (Degeest *et al.*, 2001c).

12.3.2 Improvement of strains and processes

Their high thickening power and their food-grade origin has prompted the search for new HePS from stable strains and for processes with higher yields (Degeest *et al.*, 2001c). In that sense, several studies have reported that the yield of HePS produced by LAB can be influenced by optimising the culture conditions or the medium composition (Degeest *et al.*, 2001c; Torino *et al.*, 2001; Ricciardi *et al.*, 2002; Macedo *et al.*, 2002b,c). Kinetic models have been developed to study HePS production kinetics aiming at the estimation of HePS levels and functionality in fermented foods (Degeest *et al.*, 2001c).

Finally, metabolic engineering of HePS biosynthesis might result in the construction of genetically modified strains, producing higher levels of HePS, more stable HePS or new HePS structures (Kleerebezem *et al.*, 1999). However, the use of genetically modified microorganisms in the food industry needs acceptance by both the food processor and the consumer.

12.3.3 Media

Growth and HePS production is influenced by the carbohydrate and nitrogen source available in the medium. Concentrations of HePS produced by LAB cultures are generally very low, in particular in milk. The influence of casein supplementation is not clear (Degeest *et al.*, 2001c). Probably the combined effect of a high lactose content and addition of milk proteins is of importance (Hassan *et al.*, 2001a). Indeed, supplementation of complex nitrogen sources such as yeast extract increase both biomass and HePS production (De Vuyst *et al.*, 1998; Degeest and De Vuyst, 1999; Degeest *et al.*, 2001c; Macedo *et al.*, 2002c; Ricciardi *et al.*, 2002). Amino acids, minerals and vitamins present in yeast extract as well as their optimal supplementation may contribute to these enhancements (Mozzi *et al.*, 1995a, Gamar *et al.*, 1997; Grobben *et al.*, 1998; Petry *et al.*, 2000; Torino *et al.*, 2000; Macedo *et al.*, 2002c).

Complex media such as MRS interfere with the isolation and analysis of HePS (Cerning *et al.*, 1992; Kimmel and Roberts, 1998; Torino *et al.*, 2000; Degeest *et* *al.*, 2001b). In addition, the isolation of HePS from coagulated milks is tedious (De Vuyst *et al.*, 1998). Semi-defined media (Kimmel and Roberts, 1998; Levander *et al.*, 2001b) and chemically defined media (Grobben *et al.*, 1995; Gamar *et al.*, 1997; Looijesteijn and Hugenholtz, 1999; Dupont *et al.*, 2000; Petry *et al.*, 2000) circumvent the problem of interference, but growth in such media often gives low EPS yields. A useful chemically defined medium has been developed as an alternative to milk in studies of HePS production (Petry *et al.*, 2000).

12.3.4 Physical factors

Stringent control of temperature and pH is a prerequisite for high HePS production (Degeest *et al.*, 2001c; Macedo *et al.*, 2002b; Ricciardi *et al.*, 2002). The optimal pH for HePS production is often close to 6.0 (Degeest *et al.*, 2001c; Macedo *et al.*, 2002b,c; Ricciardi *et al.*, 2002). It may be that pH is a limiting factor for HePS production during milk acidification (Degeest *et al.*, 2001c). On the other hand, acidifying conditions may have a positive influence on the stability of the HePS produced (Degeest *et al.*, 2002; Mozzi *et al.*, 2003). There are indications that anaerobic conditions enhance HePS production (Degeest *et al.*, 2001c). On the other hand, aerobic metabolism may favour ATP production, which is very demanding, by HePS biosynthesis.

12.3.5 Carbohydrate sources

The carbohydrate source is a decisive factor in HePS production (Cerning *et al.*, 1994). HePS production is often stimulated by excess carbohydrate source; limitation of the energy source results in decreased HePS yields (Cerning *et al.*, 1994; Marshall *et al.*, 1995; Grobben *et al.*, 1995; Gamar *et al.*, 1997; Degeest and De Vuyst, 1999; Degeest *et al.*, 2001c; Hassan *et al.*, 2001a). *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 produces three times more HePS with glucose than with fructose as the sole carbohydrate source (Grobben *et al.*, 1997). The activity of GalU is higher in glucose-grown cells than in fructose-grown cells of this strain (Grobben *et al.*, 1996). Further, *L. lactis* subsp. *cremoris* NIZO B40 produces nine times more HePS with glucose than with fructose as the sole carbohydrate source (Looijesteijn and Hugenholtz, 1999). Apparently, during growth on fructose, the metabolic flux in the direction of sugar nucleotides is less than during growth on glucose in this strain. The production of HePS on fructose is elevated by the overproduction of Fru 1,6-bisphosphatase (Looijesteijn *et al.*, 1999). This enzyme is required for the conversion of Fru 1,6-diP into Fru 6-P. Hence, fructose metabolism is an essential step for the generation of Glc 1-P, which is the central sugar nucleotide precursor for B40 HePS biosynthesis in *L. lactis* subsp. *cremoris* NIZO B40 grown on fructose. Fructose is not common in dairy fermentations, but in *L. lactis* fructose 1,6-bisphosphatase is required for cell synthesis and HePS precursors from galactose, as galactose is phosphorylated at C6 during transport via the galactose–PTS or lactose–PTS systems.

Mannose or a combination of glucose and fructose is the most efficient carbohydrate source for HePS production by *Lb. rhamnosus* C83 (Gamar *et al.*, 1997). For *Lb. casei* CG11, glucose is the most efficient carbohydrate source for HePS production, whereas lactose is an inefficient carbohydrate source (Cerning *et al.*, 1994). *Streptococcus thermophilus* LY03 produces more HePS with lactose than with glucose (Degeest and De Vuyst, 2000), while *S. thermophilus* Sfi20 does the opposite (Degeest *et al.*, 2001b). Similarly, *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* strains produce larger capsules when grown on lactose or galactose than on glucose (Hassan *et al.*, 2001a). Mozzi *et al.* (1995b) also found that a strain of *Lb. casei* produces larger capsules in the presence of galactose. In both free pH and pH-controlled fermentations galactose is preferred to glucose for HePS biosynthesis (Mozzi *et al.*, 2001, 2003).

In general, the carbohydrate source does not influence the monomer composition of the HePS produced (van den Berg *et al.*, 1995; Escalante *et al.*, 1998; Gamar-Nourani *et al.*, 1998; Degeest and De Vuyst, 1999, 2000; Looijesteijn *et al.*, 2000; van Calsteren *et al.*, 2002). *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 synthesises HePS with a different composition when grown on glucose or fructose (Grobben *et al.*, 1996). The relative proportions of the individual monosaccharides of the HePS produced by two other strains of *Lb. delbrueckii* subsp. *bulgaricus* (CNRZ 1187 and CNRZ 416) also differ according to specific carbohydrate sources or pH (Petry *et al.*, 2000). Similarly, changes in monomer composition of the produced HePS are observed during different stages of the fermentation in milk and chemically defined medium without pH control (Petry *et al.*, 2000). Also, a different composition is found for the HePS of *Lb. delbrueckii* subsp. *bulgaricus* CNRZ 1187 in single-strain cultures compared with mixed-strain cultures including HePS-negative *S. thermophilus* (Bouzar *et al.*, 1997). Alternatively, lactose availability has been found to influence both the kinetics of HePS production and composition (Petit *et al.*, 1991).

However, none of these cases analysed the primary structure of the HePS repeating unit in detail. Only for *Lb. rhamnosus* RW-9595M it was shown that the culture conditions (carbohydrate source, temperature, fermentation time) do not influence the primary sequence of the HePS produced (van Calsteren *et al.*, 2002). Therefore, only when certain enzymes involved in sugar nucleotide biosynthesis are inactivated or lacking, production of a HePS with an altered composition might be possible, owing to a reduced availability of specific precursors for HePS biosynthesis. In addition, it may be possible that HePS production enhances when the pool of sugar nucleotides is increased. Finally, the presence or absence of side groups such as phosphate or acetate might be influenced by culturing conditions, as is known for instance for xanthan (Becker *et al.*, 1998).

12.3.6 Molecular mass

It has been found that the type of substrate limitation influences the molecular mass of the HePS produced (Looijesteijn *et al.*, 2000). For instance, the molecular mass of the HePS produced by *L. lactis* subsp. *cremoris* NIZO B40 and NIZO B891 is reduced under glucose limitation without changes in chemical composition of the HePS backbone. A reduced intracellular ATP concentration could be responsible for this phenomenon because one of the gene products involved in HePS biosynthesis contains an ATP-binding domain and is homologous to ExoP-like proteins, which are involved in chain length determination (van Kranenburg *et al.*, 1997). Moreover, the efficiency of HePS production is highest under glucose limitation (Looijesteijn *et al.*, 2000). This is probably due to the coupling of the biosynthesis of cell wall sugars and HePS. For *L. lactis* subsp. *cremoris* LC330 (Marshall *et al.*, 1995), *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2772 (Grobben *et al.*, 1997) and *S. thermophilus* LY03 (Degeest and De Vuyst, 1999), which produce both a high-molecular-mass and a low-molecular-mass HePS, a change in the ratio between them is observed when the fermentation temperature or the medium composition (energy source, carbon/nitrogen ratio) is changed. This is important in view of potential chain length modifications through medium engineering.

12.3.7 Process technology

Efficient production and recovery costs of HePS to be applied as food additives require not only knowledge of biosynthesis and genetics but also adoption of the appropriate bioprocess technology (Degeest *et al.*, 2001c). Classical batch and fed-batch as well as immobilisation technologies have been studied (Degeest *et al.*, 2001c; Bergmaier *et al.*, 2002). However, no remarkable improvements of the HePS yields have been achieved. Other difficulties are linked to the recovery and purification of HePS (De Vuyst and Degeest, 1999).

Alternatively, for the large-scale production of HePS-producing starter cultures, HePS production may limit cell propagation, in particular when using ultrafiltration processes either during cell recycling (biomass production with cell recycle reactors) or during downstream processing (biomass separation with micro- or ultrafiltration). Here, suboptimal environmental conditions for HePS production that still allow sufficient biomass formation can be applied to limit membrane fouling caused by EPS.

12.4 Producing functional polysaccharides

12.4.1 Physiological role of exopolysaccharides

Many investigators are interested in the ecological role of EPS (Roberts, 1995, 1996; Costerton *et al.*, 1999; De Vuyst and Degeest, 1999; De Vuyst *et al.*, 2001; Looijesteijn *et al.*, 2001). Cell-surface polysaccharides are involved in pathogenesis determining the immunological properties of the cell, in symbiosis, in bacteria-to-bacteria adhesion (which may aid in quorum sensing), in biofilm formation and in stress resistance. In general, the physiological functions of EPS are thought to be of a protective nature such as prevention against desiccation, phagocytosis and bacteriophage invasion, protozoan predation, toxic compounds such as metal ions, antibiotics, cell wall degrading enzymes and ethanol, and environmental stresses such as low temperature and high osmotic pressure. Also, EPS play a role in cell recognition, host–pathogen interactions, adhesion to surfaces and formation of biofilms. Therefore, EPS can be expected to be rather resistant to biodegradation since easily degradable EPS will not offer much protection to the cells. On the other hand, it has been shown that the biological breakdown of EPS can differ greatly, implying that the physiological roles of these compounds may vary a lot (Ruijssenaars *et al.*, 2000).

The EPS produced by *Streptococcus salivarius* and *Streptococcus mutans* are involved in the colonisation during dental plaque formation (Cerning, 1990; Hillman, 2002). Further, EPS from LAB could constitute a way of colonising dairy habitats. EPS as a defence mechanism against phages by preventing their adsorption is supported by the relationship between a strong ropy character and low phage susceptibility of several strains (Forde and Fitzgerald, 1999; Lamothe *et al.*, 2002). However, this matter is still debated. For instance, studies demonstrate that loosely cell-wall bound HePS from *L. lactis* does not play a role in the phage infection process (Deveau *et al.*, 2002). Except for *S. mutans*, which can utilise the oligosaccharides derived from dextran degradation, EPS are not used as energy sources by the producer microorganisms (Cerning, 1990; Colby and Russell, 1997).

12.4.2 Exopolysaccharides of technological importance

From a technological point of view, it is interesting to know if there are structure–function relationships among EPS from LAB. However, there is a gap between our knowledge of the structure of polysaccharides and the ability to predict their physical properties, their interactions with other components of food products, and thus their potential applications. Currently this knowledge is becoming available. Interest in glycobiology and the application of new analytical methods will certainly stimulate research in this area.

Commonly, viscosity is a simple function of temperature, shear, EPS concentration and molecular mass. For instance, the HePS from *S. thermophilus* Rs has a molecular mass of 2.6×10^6 Da and that of *S. thermophilus* Sts is 3.7×10^6 Da. Although it has an identical repeating unit structure and similar amounts of these HePS are produced in milk, the strain producing the HePS with a greater chain length produces a more viscous milk product. An interesting property of many viscous polysaccharide solutions is the fact that they exhibit pseudoplastic flow (shear-thinning properties); this is a (reversible) decrease in apparent viscosity upon increasing shear rate. Most HePS are random coils with no fixed shape; they have a randomly fluctuating tertiary structure (Tuinier *et al.*, 1999a,b; Higashimura *et al.*, 2000; Faber *et al.*, 2002a). For ideal random coils, the thickening efficiency depends on the squared Kuhn length, which is the length of the repeating unit per unit of mass (Tuinier *et al.*, 2001). The relative viscosity of EPS solutions is a function of (i) the intrinsic viscosity, which is the key parameter that describes the general concentration dependence of the viscosity of any flexible polysaccharide in solution; (ii) the specific volume occupied by the dispersed polymer; and (iii) the concentration of the dispersed polymer.

The specific volume of EPS in solution is determined by its molecular mass and its radius of gyration which is a measure of the size of the polymer in solution. The relationship between the radius of gyration and the molecular mass is determined by the chain stiffness which depends on monomer composition, type of linkages, charged groups and degree of branching (Tuinier *et al.*, 1999b). To obtain a higher (intrinsic) viscosity either the molecular mass (chain length) or the stiffness of the EPS polymer must increase. For instance, the average molecular mass of the HePS produced by *Lb. sakei* 0–1 is in the same order of magnitude as that of xanthan gum, but its intrinsic viscosity is higher (van den Berg *et al.*, 1995). Based on the stiffness of the HePS, viilian from *L. lactis* subsp. *cremoris* is classified as an intermediate, rigid–flexible system. It is thus comparable with polysaccharides such as carrageenan, pectin or alginate, but it is less flexible than amylose and dextran (Higashimura *et al.*, 2000). Microbial polysaccharides such as xanthan are more rigid.

Currently, the complex relationships between HePS chemistry and chain stiffness are not clear. Some general features can be distinguished: (i) monosaccharides connected by $\beta(1\rightarrow4)$ bonds result in stiffer chains compared with $\beta(1\rightarrow2)$ or $\beta(1\rightarrow3)$ bonds, or compared with the much more flexible α-bonds in dextrans and the flexible consecutive $(1\rightarrow6)$ links within the repeating unit of, for instance, the HePS produced by *Lb. helveticus* 766 (Tuinier *et al.*, 1999b; Faber *et al.*, 2002a); (ii) some side groups influence chain stiffness, thereby influencing the thickening efficiency, or the hydrodynamic volume, then influencing the intrinsic viscosity (van den Berg *et al.*, 1995; Tuinier *et al.*, 2001; Vincent *et al.*, 2001; Ruas-Madiedo *et al.*, 2002b); (iii) special branching patterns may influence the viscosity of the EPS in aqueous solution (Yang *et al.*, 2000).

Beyond these structure–function relationships, *in silico* molecular modelling will become an important tool for the development of atomic-scale models (Faber *et al.*, 2002a), making a link to macroscopic properties such as rheology and the capacity for interactions with other molecules present in the medium (Jolly *et al.*, 2002b). The interaction of EPS with other food components has not been studied into great detail (Skriver *et al.*, 1995; Tuinier *et al.*, 1999a). As an example, B40 HePS induces phase separation when added to skim milk above a certain concentration. When the continuous phase of skim milk is mixed with B40 HePS nothing happens. This indicates that the interactions between casein micelles and HePS are responsible for the observed segregative behaviour (Tuinier *et al.*, 1999c; Tuinier and de Kruif, 1999). Similarly, mixtures of high concentrations of aggregated whey protein colloid particles and HePS show such phase separation (de Kruif and Tuinier, 1999). The attraction between the protein particles increases the viscosity of the mixtures. However, one has to keep in mind that the properties of an EPS in a fermented food product may differ considerably from its properties in purified form in solution. Therefore, the next step will be the establishment of correlations between atomic structural properties and physicochemical properties of an EPS in the complex food matrix rather than in aqueous solution (Jolly *et al.*, 2002b).

12.4.3 Exopolysaccharides as therapeutic agents

It has been suggested that the health-promoting effects of EPS-producing strains are related to the biological activities of these biopolymers. Microbial polysaccharides that are capable of interacting with the immune system to up-regulate or down-regulate specific responses of the host can be classified as immunomodulators or biological response modifiers (Tzianabos, 2000). The use of polysaccharide immunomodulators as an alternative to classical antibiotic treatment for enhancing host defence responses seems very attractive.

The extracellular phosphopolysaccharide produced by *L. lactis* subsp. *cremoris* KVS20 has been shown to induce a B-cell dependent lymphocyte mitogenicity (Kitazawa *et al.*, 1992, 1993a,b,c), macrophage cytostaticity (Kitazawa *et al.*, 1991b) and cytokine production such as interleukin-1 and interferon-γ by macrophages in mice splenocytes (Kitazawa *et al.*, 1996). The phosphopolysaccharide produced by *Lb. delbrueckii* subsp. *bulgaricus* OLL1073R-1 also enhances lymphocyte mitogenicity (Kitazawa *et al.*, 1998) and macrophage functions (Kitazawa *et al.*, 2000). In both cases, the phosphate group together with the conformational structure may play an important role in these processes. Similarly, whole cells and cell walls of cells of different strains from the *Lb. acidophilus* group display a B-cell dependent mitogenic activity on murine spleen and Peyer's patch cells (Takeda *et al.*, 1997; Hosono *et al.*, 1997). An acidic polysaccharide is probably responsible for this activity. Also, specific monosaccharide constituents such as *N*-acetyl-glucosamine in the peptidoglycan may play a role.

The HePS from *Lb. rhamnosus* RW-9595M appears to elicit bioactive interferon-γ in splenocytes on a murine macrophage-like cell line as well as tumour necrosis factor (TNF), and interleukin-6 and -12 in human- and mouse-cultured immunocompetent cells (Chabot *et al.*, 2001). However, polysaccharides from LAB with specific activity for modulation of the immune system have not been examined in detail for both structure–function relationships and mechanism of action. The immunomodulating properties of EPS may depend on branching pattern, molecular and higher-order structure, three-dimensional conformation, and overall spatial charge organisation (Falch *et al.*, 2000). Interestingly, *L. lactis* shows potential as a host for the safe production of capsule antigens and as a vaccine delivery vehicle for polysaccharide antigens (Gilbert *et al.*, 2000).

Beside their interesting immunostimulating properties some EPS exert other health benefits such as anti-ulcer activity (Nagaoka *et al.*, 1994), anti-mutagenic, anti-tumour and anti-carcinogenic activity (Oda *et al.*, 1982, 1983; Matsuzaki *et al.*, 1990; Kitazawa *et al.*, 1990, 1991a; Ebina *et al.*, 1995; Sreekumar and Hosono, 1998) and cholesterol-lowering activity (Nakajima *et al.*, 1992b). Kefiran also displays an anti-tumour activity (Shiomi *et al.*, 1982; Murofushi *et al.*, 1983). Also, the homopolymeric β-D-glucans possess biological activity as immune stimulants or tumour-suppressive agents (Tzianabos, 2000).

Anti-tumour activity is probably mediated through immune activation. For instance, it can be assumed that the stimulation of lymphocytes and macrophages by EPS explains the increased production of antigen-specific antibodies *in vivo* when HePS from *L. lactis* subsp. *cremoris* SBT 0495 is used as an adjuvant

(Nakajima *et al.*, 1995). It has been reported that the potentiation of an immune response with LAB seems to arise predominantly from the conformation of the carbohydrate moieties of the bacterial cell walls as CWP or CPS, and of EPS. Alternatively, acidic moieties in bacterial polysaccharides may bind mutagens (Sreekumar and Hosono, 1998). All these characteristics open up possible applications in the rapidly expanding field of functional foods. These are foods that beneficially affect one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved state of health and well-being and/or reduction of risk of disease (Roberfroid, 2000).

12.4.4 Exopolysaccharides and probiotics

Studies on bacterial adhesion have shown that CPS might be one of the promoters for the adherence of bacteria to biological surfaces, thereby facilitating the colonisation of various ecological niches. Interestingly, the well-known probiotic strain *Lactobacillus rhamnosus* GG, displaying prominent adhering properties, produces a HePS, while the non-adhesive *Lb. rhamnosus* LC-705 strain does not (Landersjö *et al.*, 2002). Hence, it may be assumed that the HePS of *Lb. rhamnosus* GG is involved in the adhesiveness of the organism, despite the small amounts of HePS produced. Similarly, the phosphopolysaccharide of *L. lactis* subsp. *cremoris* ARH 74 is responsible for its adhesion to cultured intestinal epithelial cells, making the strain a candidate probiotic (Elina and Salminen, 1997). Probiotics are live microbes of human origin, used as food supplements or pharmaceutical preparations, that survive passage through the upper gastrointestinal tract (GIT), transiently colonise the gut by adhesion to the intestinal mucosa, and are beneficial to health (Salminen *et al.*, 1998). EPS may facilitate this adhesion. It is further speculated that the increased viscosity of EPS containing fermented foods may increase the residence time of the ingested food product in the GIT and, coupled to a low biodegradability of EPS, might therefore be beneficial to a transient colonisation by probiotic bacteria (German *et al.*, 1999).

12.4.5 Exopolysaccharides as prebiotics

Some of the LAB HePS as well as levans have become of interest as potential prebiotics (Roberfroid, 2001). Prebiotics are defined as non-digestible food ingredients that affect the host beneficially by selectively stimulating the growth and/or activity of a limited number of bacteria in the colon, and thus improve host health (Gibson and Roberfroid, 1995). Non-digestible carbohydrates in general, and fructo-oligosaccharides in particular (e.g. inulin and inulin-type fructans), are the most common prebiotics (Roberfroid, 1993). They selectively stimulate the growth of bifidobacteria. The establishment of metabolically active bifidobacteria in the human colon is considered to improve the gut flora, to stabilise the gut mucosal barrier and to prevent diarrhoea. The end-products of carbohydrate metabolism (lactate and short-chain fatty acids like acetate) result in a lower luminal pH and inhibit pathogens. Prebiotic properties further include vitamin production, increasing mineral absorption, immunostimulating effects, anti-carcinogenic activity, cholesterol-lowering effects, decreasing the possibility of constipation and protection against infections.

One of the prerequisites of a prebiotic is that it is neither hydrolysed nor absorbed in the upper part of the GIT. In this respect it has been found that some EPS are resistant to several enzymes including digestive enzymes, and to passage through the gastrointestinal tract (van Casteren *et al.*, 1998; Ruijssenaars *et al.*, 2000; Looijesteijn *et al.*, 2001). A bifidogenic property of fructans produced by two strains of *Lb. sanfranciscensis* has been shown (Dal Bello *et al.*, 2001). Comparison of an intestinal microflora incubated in media with EPS and media without an energy source showed that the EPS stimulate the growth of bifidobacteria by more than log2. The high-molecular-mass fructan of the levan-type produced by *Lb. sanfranciscensis* TMW1.392 (Korakli *et al.*, 2001; Dal Bello *et al.*, 2001) is metabolised by several bifidobacteria *in vitro* as well (Korakli *et al.*, 2002).

Interestingly, the trisaccharide sequence β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p* present in the repeating unit of the HePS from *S. macedonicus* Sc136 corresponds to the internal backbone of the lacto-*N*-tetraose and lacto-*N*-neotetraose units, which serve as a structural basis for the large majority of human milk oligosaccharides (Vincent *et al.*, 2001). Human milk has been reported to contain several hundreds of oligosaccharides based on lactose and tetraose backbones substituted by fucosyl and sialic acid residues bound by several types of linkages. This provides human milk with several nutritional and health benefits, including bifidogenicity and the ability to inhibit the adhesion of pathogenic bacteria to the infant gut mucosa. In this sense EPS containing such structures offer an important potential for the development of improved infant nutrition properties or consumer-specific nutrition in general. In addition, EPS or oligosaccharides can be modified into biologically active carbohydrates to treat and even prevent infections by pathogenic bacteria by interacting with the binding through carbohydrate interactions of the latter to receptors of the intestinal mucosa.

During HoPS biosynthesis an acceptor (e.g. maltose, cellobiose, maltotriose) can be added to the reaction mixture to direct glycansucrase-mediated polymerisation towards the biosynthesis of oligosaccharides containing α-(1→2)-linked branches (Monsan *et al.*, 2001). Other acceptors may be useful as well (Demuth *et al.*, 2002). These polymers resist the attack of digestive enzymes and present interesting prebiotic properties. Gluco-oligosaccharides are presently marketed for human nutritional and derma-cosmetic applications (Monsan *et al.*, 2001).

12.5 Applications

Viscosity and shear-thinning behaviour as well as elasticity are important in food applications. Viscosity contributes to thickening. It is the property of a product to resist deformation. In the context of fermented dairy products, this attribute can be described as slimy and fluid. Shear-thinning as a rheological property (the decrease of viscosity with increasing shear rate) has been associated with the texture/consistency of a food product. For example, in a fluid food (e.g. chocolate milk or drinking yoghurt) the viscosity has to be high at zero-shear rate to prevent particle sedimentation, whereas during pouring and drinking (invoking higher shear rates) it is desirable that the viscosity decreases. Elasticity is the property of a product to recover after a deformation occurred. This attribute corresponds to a firm body and gum-like fermented product. Viscosity (and shear-thinning behaviour) and elasticity are important for the organoleptic quality of a food product and for its appealing appearance and pleasant mouthfeel.

Many HePS produced by LAB show shear-thinning properties. Some of them even display higher viscosities at all shear rates compared with xanthan (van den Berg *et al.*, 1995; Bubb *et al.*, 1997). Unfortunately, large-scale production of HePS from LAB in a (semi-)purified form is currently not interesting, because of the low yields compared with other commercial bacterial HePS such as xanthan (Degeest *et al.*, 2001c). However, small amounts of HePS produced in fermented milk and during cheese production have a strong impact on the texture (and properties) of these products. Therefore, HePS-producing strains that produce HePS *in situ*, that is in the food matrix, are of particular interest for applications in fermented food products. Meanwhile they promote the ropiness, mouthfeel, taste and stability of the product.

12.5.1 Nordic ropy fermented milks

Nordic ropy milk is the generic name for milks fermented with mesophilic cocci which produce EPS, mainly *L. lactis* and *Leuconostoc* species (Roginski, 1999). A typical example is Finnish viili. Homolactic fermentation leads to a very sour taste, while concomitant HePS production influences the texture (Toba *et al.*, 1990; De Vuyst and Degeest, 1999). Viilian, the polymer responsible, has been isolated from a strain of *L. lactis* subsp. *cremoris*. It is a phosphorylated HePS composed of rhamnose, glucose and galactose in the molar ratio 1.00:1.45:1.75 (Nakajima *et al.*, 1992a). It displays both interesting rheological (Oba *et al.*, 1999; Higashimura *et al.*, 2000) and health-promoting properties (Nakajima *et al.*, 1992b).

12.5.2 Yoghurt

HePS-producing LAB strains are used in yoghurt, drinking yoghurt, sour cream and whipped toppings, to improve rheological properties, to prevent syneresis and to replace stabilisers (Duboc and Mollet, 2001). Also, ropy milk has been proposed as stabiliser in the production of ice cream (Christiansen *et al.*, 1999).

Traditionally, yoghurt is produced by inoculation of fortified milk with *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* as starter culture in a 1:1 ratio. Yoghurt is marketed in two forms: set-type and stirred-type, and the rheology of the two systems differ. Set yoghurts have the properties of a gel whereas stirred yoghurts have flow properties that are characteristic of a pseudoplastic, thixotropic, weakly viscoelastic fluid. To reduce the amount of added milk solids, to increase yoghurt viscosity, to improve body, texture and mouthfeel, and to avoid syneresis and gel fracture during fermentation or upon storage of the yoghurt, stabilisers such as gelatin, starch, pectin, alginate and guar gum are used. This is important to help maintain good textural and visual properties during transportation and storage. However, this is not allowed in all countries. To replace added stabilisers, EPS-producing, functional starter cultures are interesting (Wacher-Rodarte *et al.*, 1993; Bouzar *et al.*, 1997; Sebastiani and Zelger, 1998; Marshall and Rawson, 1999; Hassan *et al.*, 2001b; Jaros *et al.*, 2002). Both *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* strains are known to produce HePS *in situ* (Cerning, 1990, 1995; De Vuyst and Degeest, 1999; Marshall and Rawson, 1999; De Vuyst *et al.*, 2001).

In addition, texture and stability are affected by the culture conditions as well as by the manufacturing process (milk composition, heat treatment, acidification) (Laws and Marshall, 2001). The most important textural characteristics of yoghurt are firmness and the ability to retain water. These properties are related to the gel structure and might be influenced by the type of culture and HePS. In general, increases in apparent viscosity are at the expense of the gel strength of the yoghurt (Vlahopoulou and Bell, 1993; Hess *et al.*, 1997; Vlahopoulou *et al.*, 2001). Therefore, the firmness and cohesiveness of yoghurts made with ropy strains decrease with the presence of increased amounts of HePS, while the water-holding capacity increases, which in turn decreases the susceptibility to syneresis (Zoon *et al.*, 1990; Rohm and Kovac, 1994; Hassan *et al.*, 1996b; Rawson and Marshall, 1997; Marshall and Rawson, 1999). HePS could contribute to adhesiveness, while firmness and elasticity are probably due to the milk protein matrix. This has been explained by the fact that ropy bacteria surround themselves by a HePS layer capable of binding the protein matrix and hence modifying the protein network structure of the yoghurt gel (Schellhaass and Morris, 1985; Bottazzi and Bianchi, 1986; Teggatz and Morris, 1990; Rohm and Kovac, 1994; Skriver *et al.*, 1995; van Marle and Zoon, 1995; Hassan *et al.*, 2001b, 2002a).

The interaction between HePS and bacteria is disrupted when yoghurt is sheared, but, after the HePS is separated from the cell surface, HePS continues to interact with the denatured caseins and influences apparent viscosity (Teggatz and Morris, 1990; Rohm and Schmid, 1993; Skriver *et al.*, 1993; Rohm and Kovac, 1994; Hess *et al.*, 1997). However, no clear correlation between the viscosity of fermented milk and the HePS concentration has been found (Wacher-Rodarte *et al.*, 1993; van Marle and Zoon, 1995; Bouzar *et al.*, 1997; Sebastiani and Zelger, 1998; Ruas-Madiedo *et al.*, 2002b). Yet, viscosities of milks fermented with HePS-producing strains remain much higher than those obtained with HePS non-producing strains, showing the advantage of using HePS-producing strains in the manufacture of stirred yoghurt (van Marle and Zoon, 1995; Hassan *et al.*, 1996a). Furthermore, the viscosity of non-ropy yoghurt continuously decreases with increasing shear rate compared with ropy yoghurts, again indicating that HePS play a significant role in the rheology of stirred yoghurt (van Marle *et al.*, 1999). Therefore, it is suggested that the spatial structure of the protein networks influences the apparent viscosity of stirred yoghurt (van Marle and Zoon, 1995). Where the protein gels obtained with the ropy strains show a homogeneous structure with randomly distributed small cavities, gels obtained with non-ropy strains show larger cavities filled with bacteria and serum (Duboc and Mollet, 2001). Indeed, HePS occur in pores within the protein network, their size determining its microstructure. However, HePS and proteins appear to be increasingly segregated with the amount of HePS (Hassan *et al.*, 2002b). Moreover, ropy cultures have a more pronounced effect on the protein network than capsular non-ropy cultures. Stirring fermented milk breaks the protein network, hence facilitating aggregation of HePS into more extended structures.

The more ropy the culture, the longer and larger the strands formed during stirring (Hassan *et al.*, 2002b). Moreover, the HePS are assumed to form a gel-type structure, this is a three-dimensional network in which the polysaccharide chains are cross-linked, instead of filaments attached to casein micelles (Hassan *et al.*, 2002b). The earlier reported filamentous structure has been recognised as an artefact resulting from the sample preparation process for conventional scanning electron microscopy.

Differences observed in the microstructure of HePS may explain differences in ropy characteristics of different strains. The network structure of HePS formed in yoghurt will be substantial given that only small amounts of HePS are isolated from fermented milk. Furthermore, it is supposed that various strains enhance the viscosity differently owing to differences in the intrinsic viscosity of the HePS produced. To obtain a high viscosity the molecular mass should be high and the chain should be relatively stiff (Faber *et al.*, 1998; Tuinier *et al.*, 1999a, 2001; Ruas-Madiedo *et al.*, 2002b). However, interactions have to be kept in mind. For instance, preliminary experiments indicate that linear neutral HePS contribute to the viscosity but not to the elasticity of yoghurt, because they interact only weakly with the positively charged proteins but dissolve well in the serum phase. Negatively charged HePS may contribute to the elasticity but not to the viscosity, since they can interact with the positively charged casein particles by electrostatic interactions, reinforcing the strength of the network (Duboc and Mollet, 2001). To conclude, a complex interaction exists between bacterial cells (all or not surrounded by HePS, encapsulated or not), milk proteins (degree of denaturation, colloid diameter, protein network, interactions), microstructure (gel network, pH, ionic strength) and HePS (molecular mass, monomer composition, degree of branching, concentration, stiffness).

Finally, oligosaccharides that have only limited thickening properties could be used in yoghurt as bodying agents with a high water binding capacity and as a non-digestible food fraction (Jolly *et al.*, 2002b).

12.5.3 Kefir

Kefir is a traditional, slightly alcoholic, fermented milk from Eastern Europe, produced by a mixture of LAB, lactose-fermenting yeasts and acetic acid bacteria.

The cells are embedded in kefiran, a type of HePS composed of glucose and galactose in equimolar proportions (Micheli *et al.*, 1999). Kefiran is the matrix of the kefir grains and confers a slimy texture to the product.

12.5.4 Cheese

Texture characteristics of cheese include firmness, hardness, springiness, cohesiveness, chewiness and gumminess. The fat and moisture contents play a determining role in texture development. For instance, for use on pizza, mozzarella cheese should exhibit good shredding, melting and stretching properties and be free of textural defects. The removal of fat in low-fat mozzarella cheese can result in cheese that is low in moisture because the casein fibres are tightly bound, giving the cheese poor melting and stretching properties. Starters producing HePS (*S. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. helveticus*) have been tested for the production of low-fat mozzarella cheese to enhance moisture retention without losing typical sensory and rheological characteristics (Perry *et al.*, 1997, 1998; Low *et al.*, 1998; Bhaskaracharya and Shah, 2000; Petersen *et al.*, 2000; Broadbent *et al.*, 2001). Further, the ability of some strains of *S. thermophilus* to use galactose in capsule production could reduce browning of mozzarella cheese during baking by removing a source of reducing sugar (Hassan *et al.*, 2001a). The microstructure of such cheeses displays a porous structure in which large pores are filled with HePS (Hassan *et al.*, 2002b).

A compact protein matrix with fewer and small pores is seen in cheese made with HePS non-producing cultures. HePS and protein appear to be segregated. The HePS form a network-like spongy structure, thus mimicking the milk fat. The spongy texture of the milk gels could be a result of decreased dominance of the structural matrix and increased water holding in such low-fat products.

The production of other low-fat cheeses such as low-fat feta may profit from the use of HePS-producing starter cultures as well. Similarly, using confocal laser microscopy Hassan *et al.* (2002b) observed that feta cheese made with HePS-producing cultures contained larger casein aggregates than those in cheese made with HePS non-producing cultures, and that the HePS formed thick sheets filling pores in the protein network. It is important to mention that the use of HePS-producing starter cultures for the production of low-fat cheeses implies that the HePS remain in the cheese matrix and are not removed with the whey during cheese manufacturing. In that view encapsulated HePS-producing strains can be utilised to increase the moisture level of cheese and to improve the melt properties of mozzarella cheese without adversely affecting whey viscosity and hence without altering whey processing and properties (Petersen *et al.*, 2000).

12.5.5 Sourdough

Cereal grains are predominantly composed of starch. In addition, non-starch polysaccharides such as β-glucan (composed of glucose), polyfructan (composed of fructose) and arabinoxylan (composed of xylose and arabinose) are present

(Belitz and Grosch, 1999). Some of these polysaccharides are partially digestible, for example starch, and some of them are believed to serve as dietary fibre, such as arabinoxylan. In addition to polyfructans, wheat and rye flours contain kestose, nystose and other fructo-oligosaccharides of the inulin-type (Campbell *et al.*, 1997). Finally, rye and wheat flours contain a little sucrose, which is also added to dough formulas. Therefore, the application of EPS-producing sourdough lactobacilli is promising to optimise the technological and nutritional properties of sourdough and bread, respectively.

During sourdough fermentation, an important step in bread production from rye or mixtures of wheat and rye, strains of *Lb. sanfranciscensis* produce EPS (Korakli *et al.*, 2000, 2001). EPS production during sourdough fermentation has the potential to affect the rheological properties of the dough as well as the volume, texture and keepability of bread. For instance, dextrans may improve texture build-up by softening the gluten content of the dough, and extending the shelf-life and increasing the specific volume of the resultant bakery product. Fructans are known to improve dough machinability as well as the sensory properties of bread (Yasushi and Akifumi, 1995). In addition, EPS produced by sourdough LAB will improve the nutritional properties of sourdough fermented bakery products because of the prebiotic properties of the EPS produced (Korakli *et al.*, 2002). Whereas the water-soluble polysaccharides in wheat and rye are also degraded by bifidobacteria *in vitro*, polyfructan and the starch fraction, which possess a bifidogenic effect, are degraded by the cereal enzymes during dough fermentation, while the EPS are retained. The stability of the EPS should enable them to withstand the baking process as well (Cummings *et al.*, 2001). Hence, EPS in sourdough bread are of particular importance for the consumer.

12.6 Future trends

To summarise, future trends for developing new polysaccharides are to be sought in:

(1) large screening programmes for novel EPS structures, in particular HePS and inulin-type HoPS produced by strains used as starter cultures in the production of several fermented foods;
(2) structural and enzymatic characterisation of the vast pool of GTF present in LAB;
(3) increased knowledge of the regulation mechanisms involved in HePS production to obtain higher HePS yields, and to enable engineering of structure and chain length of designer polysaccharides;
(4) elucidating structure–function relationships through genetic engineering and molecular modelling;
(5) structure and chain length engineering of HePS through enzymatic modifications and genetic engineering strategies;
(6) development of kinetic models to predict EPS production kinetics and functionality in fermented foods;

(7) development of new techniques to monitor on-line HePS production accurately during fermentation processes;
(8) the use of new fermentation strategies and the development of cheap isolation protocols to increase EPS yields and make EPS more interesting as food additives;
(9) validation of the use of EPS as prebiotics; and
(10) comparative genomics, transcriptomics and metabolomics to clarify the possibilities of these approaches towards the production of tailor-made oligo- and polysaccharides.

12.7 Sources of further information and advice

For recent reviews on HePS from LAB, the reader is referred to the papers of Cerning (1990, 1995), Cerning and Marshall (1999), De Vuyst and Degeest (1999), Ricciardi and Clementi (2000), De Vuyst *et al.* (2001), Degeest *et al.* (2001c) and Ruas-Madiedo *et al.* (2002a). Also, the special issue of the *International Dairy Journal* edited by De Vuyst and Marshall (2001) on the occasion of the 'First International Symposium on Exopolysaccharides of Lactic Acid Bacteria: from Fundamentals to Applications', held in Brussels (Belgium) in May 2001, summarises a state-of-the-art of EPS production by LAB.

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13

The rheology and textural properties of yoghurt

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13.1 Introduction

Starting with comments on the historical background and the economic importance of yoghurt, the first part of this chapter deals mainly with some basic biochemical and microbiological aspects of yoghurt production. An outline of different manufacturing methods, resulting in various types of yoghurt, is followed by a description of the main technological factors, which are known to influence rheology and textural properties. These factors comprise the preparation of the base milk, including dry matter enrichment, homogenisation and pre-heat treatment, as well as incubation conditions and post-incubation treatments, which vary largely, depending whether set-style or stirred yoghurt is produced. Interrelations between technological factors and physical parameters are given and, where possible, are explained on the basis of microstructure. The following section gives an overview of rheological methods commonly used for the testing of set-style and stirred yoghurt, and presents some tools for the numerical treatment of response data. Some empirical methods, which are the basis for simple product testing in routine laboratories or manufacturing plants, are then presented. Prior to an outline of presumable future trends and sources of additional information, a few comments deal with sensory procedures used for the evaluation of physical properties of yoghurt.

13.2 The principles of yoghurt manufacture

Fermentation is one of the oldest procedures for transferring raw materials of plant or animal origin into products with extended shelf-life, and it is assumed that the fermentation of milk dates back approximately 10 000 years (Stanley, 1998). The term ‘fermented milk’ or ‘cultured milk’ refers to products such as yoghurt, sour milk, cultured butter milk and sour cream, which are usually made from cow’s milk by pure lactic acid fermentation. Additionally, some products are made from milk from other species such as sheep, goats or horses, and combined fermentation (by, e.g. lactic acid bacteria and yeasts) results in products known as kefir or koumiss.

Yoghurt represents the most popular fermented milk product worldwide and originates from countries around the Balkans and the Eastern Mediterranean Sea (Staff, 1998; Walstra *et al.*, 1999). Generally, yoghurt is manufactured from preheated milk, with fat and dry matter content varying with respect to region and legislation, either in the plain form or with added material such as fruits or fruit premixes, sugar, cereals, or additives such as gelling agents, flavourings or colorants. Legislation and codex regulations differ widely around the world; in some countries, the use of additives is prohibited, or the presence of a certain number of viable starter bacteria in yoghurt is required (e.g. 10^7 bacteria per gram in the USA; Mistry, 2001). Consumption statistics for fermented milks show highest per caput-consumptions throughout Europe and a continuous growth in nearly all major markets. Exceptions are countries with an already existing high consumption level, such as the Netherlands and Iceland (Table 13.1).

Table 13.1 Consumption of fermented dairy products including yoghurt

Country	Consumption (kg/caput)				
	1980	1990	1993	1998[a]	2000[a]
Australia	1.8	3.5	4.8	5.6	5.7
Austria	9.8	10.4	13.0	16.4	21.4
Belgium	7.7	9.6	11.9	20.5	21.1
Canada	2.3	3.7	3.5	3.7	4.9
Czech Republic	–	–	–	10.6	13.8
Denmark	26.7	21.6	20.7	25.9	26.2
Finland	41.0	38.3	38.1	38.8	40.7
France	9.3	16.4	17.3	27.8	28.5
Germany	–	–	–	24.7	26.5
Netherlands	27.3	32.5	29.7	45.0	44.8
Norway	10.1	14.9	–	19.3	16.6
Slovakia	–	–	–	5.4	6.7
Spain	6.0	8.0	9.8	14.7	15.7
Sweden	24.0	29.1	28.6	37.9	38.0
USA	6.2	–	3.5	2.4	2.7

[a] Data include milk drinks and fermented milk products.
Source: Compiled from various sources (Anonymous, 1982, 1992, 1995, 2001).

Table 13.2 Proximate composition (g kg^{-1}) of cow, goat and sheep milks

Component	Cow	Goat	Sheep
Protein	34	29	55
Casein	28	25	46
Fat	37	45	74
Lactose	46	41	48
Ash	7	8	10

Source: Jensen (1995).

Generally speaking, cultured or fermented milk products are made by inoculation of milk with a specific combination of microorganisms, which are able to convert lactose into lactic acid. Milk is a complex fluid containing relatively high amounts of proteins and minerals, which, as it is intended to nourish young mammals, varies in composition according to the species' needs (Table 13.2). The major part of the milk proteins, the casein, which occurs in conjunction with calcium phosphate in the form of colloidal particles with 100–500 nm diameter and approximately 10^8 Da (Buchheim and Welsch, 1973), is of great importance for the functional behaviour of the final acidified product. The colloidal calcium phosphate (CCP) plays an important role in maintaining the integrity of the casein micelles, which are in dynamic equilibrium with their surroundings. Therefore, a lot of structural research has been undertaken to explain the mechanisms of the stability of casein micelles and their sub-units, irrespective of whether these are present in the form of sub-micelles or not (Holt, 1993; Holt and Horne, 1996; Rollema, 1992; Schmidt and Both, 1982; Visser, 1992; Walstra, 1990; Walstra *et al.*, 1999).

During fermentation of yoghurt, the milk sugar in the base milk is partially converted into lactic acid by the action of various enzymes, originating from the growth of thermophilic lactic acid bacteria. This causes a sufficient decrease in the pH, resulting in a dissociation of the CCP, a destabilisation of the casein micelles and even some liberation of individual casein molecules, accompanied by reaching a maximum in voluminosity (Dalgleish and Law, 1988; Lucey and Singh, 1998). Below a pH of 5.5 the casein micelles begin to swell and, as almost all CCP is dissociated, start to precipitate. This precipitation leads to a sufficient decrease in the voluminosity of casein micelles (van Hooydonk *et al.*, 1986) and to the formation of clusters and chains that link together to form a gel, composed of a continuous three-dimensional network with the milk serum containing whey proteins, lactose and salts entrapped as liquid phase (the amount of whey proteins depends on heat treatment, see below). Electron microscopy shows the particulate character of acidified milk gels with empty spaces or pores in the network where the serum was entrapped (Kalab, 1979, 1993; McManus *et al.*, 1993).

The classical yoghurt starter culture is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, usually with a cocci–rods ratio of 1:1 (Hassan and Frank, 2001; Hutkins, 2001). These organisms grow in a proto-cooperative relationship, resulting in rapid acidification by stimulating each other.

Depending on type and activity of the starter cultures, other metabolites such as carbon dioxide, acetic acid, diacetyl, acetaldehyde, large molecular weight exopolysaccharides or several other compounds are produced besides lactic acid, resulting in the characteristic properties of the products regarding flavour, texture and aroma. Since *Streptococcus thermophilus* is weakly proteolytic its growth is stimulated by the rods, which liberate free amino acids and small peptides from casein. The cocci in turn encourage the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* by producing formic acid and carbon dioxide (Matalon and Sandine, 1986; Rajagopal and Sandine, 1990). Nowadays, microorganisms such as *Bifidobacterium* spp. and *Lactobacillus acidophilus* are often added for therapeutic purposes (Mistry, 2001; Yucuchi *et al.*, 1992). (Generally, and based on the accumulating knowledge from well-defined, randomised and placebo-controlled studies (Fondén *et al.*, 2000), health-promoting effects of some strains used for yoghurt fermentation become more and more evident). Because of their slow acid production, these bacteria are usually used in combination with classical yoghurt starters, resulting in so-called 'yoghurt-like products'; depending on local legislation, this distinction might be of great importance (Hassan and Frank, 2001; Marshall and Tamime, 1997). Lactic acid bacteria that produce high molecular weight extracellular polysaccharides (EPS) are now commonly used in the yoghurt industry to improve product texture, partly replacing the addition of stabilisers and gelling agents, by enhancing yoghurt viscosity, independent of the fat content.

Manufacturing methods vary considerably and, for example, depend on the country, the type of product manufactured, raw materials used and product formulation. However, a number of common principles are generally applied (Staff, 1998):

- the total solids content of the base milk is increased to enhance the water-holding capacity of the product;
- a heat treatment of the base milk, usually > 80 °C for some time, is applied to achieve a proper denaturation of the whey proteins, also increasing the water-binding capacity;
- the inoculation with a specific starter culture and subsequent incubation with a time–temperature profile depends on the properties of the starter, and on technical requirements;
- cooling and addition of appropriate ingredients (fruit premixes, flavours);
- packaging and chilled storage.

Yoghurt types are usually distinguished according to their physical state in the retail container, which results from differences in the manufacturing process. Apart from set yoghurt and stirred yoghurt (Fig. 13.1), with production figures varying from country to country, there is a generally increasing demand for yoghurt drinks consisting of yoghurt mixed with skimmed milk, whey or water, and of yoghurts with increased shelf-life such as frozen or thermised yoghurt.

Incubation of set yoghurt takes place in retail containers (plastic cups or glasses of different sizes) until the required pH (around 4.4–4.7) is reached, leading to an

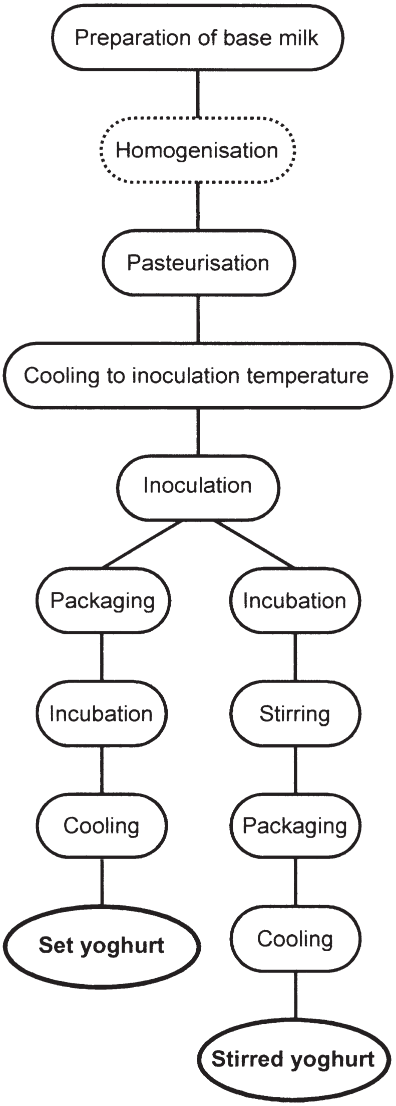

Fig. 13.1 Basic steps in the production of set and stirred yoghurt.

undisturbed gel. The continuous, viscoelastic gel network consists of aggregated spherical casein particles forming a continuous structure, and enclosing fat globules and serum. From a structural point of view, yoghurt belongs to particulate gels with disordered structures (Horne, 1999; Walstra *et al.*, 1999). Stirred yoghurt is inoculated and incubated in large fermentation vessels. The gel formed is then gently stirred to obtain a smooth and viscous, but still pourable product, which is finally packed. By breaking up the gel, a highly viscous, non-Newtonian liquid is formed, which shows a strong shear rate and time-dependent flow behaviour.

Drinking yoghurt is produced from low-solid milk on the basis of the stirred manufacture process, or regular stirred yoghurt is diluted to some extent. Increased shelf-life of yoghurt may be achieved either by freezing or by thermisation of the fermented product. Whereas the thermisation process is designed to reduce the number of potential spoilage microorganisms and, therefore, results in a partial inactivation of the starter culture, the freezing procedure, provided that appropriate methods are applied, leaves the culture bacteria viable. In frozen yoghurt, higher amounts of sugar and stabilisers are required to maintain the air bubble structure during the freezing process (Tamime and Deeth, 1980).

13.3 The main factors affecting yoghurt texture

It is generally accepted that three main factors determine the physical properties of yoghurt: the preparation of the base milk, comprising several possible treatments, the fermentation process and the post-fermentation treatment.

13.3.1 Preparation of the milk

Dry matter enrichment

The amount of total solids in the base milk, to a large extent, determines the physical properties of the final yoghurt product. The dry matter content of the base milk typically ranges from 9% in case of skim milk yoghurt without fortification up to more than 20% for certain types of 'concentrated yoghurt', with the most usual values of commercial products ranging between 13 and 17%. There are several possibilities for the fortification of the base milk (Kulkarni *et al.*, 1990), either procedures which increase milk solids proportionally, e.g. evaporation of the base milk to the desired dry matter level, particular membrane processes (i.e. reverse osmosis), the addition of skim milk powder or the addition of evaporated milk concentrates, or procedures that increase milk solids disproportionally. These methods comprise the addition of certain types of protein powders, either whey protein or casein-based, the addition of whey protein concentrates, or the application of several types of membrane processes, such as ultrafiltration or nanofiltration. The selection of a particular method is largely determined by the availability of raw materials (e.g. whey for concentration), and the equipment of the production plant.

Independent of the type of fortification, the protein content of the base milk is the most important factor which influences rheological and physical properties of yoghurt. An increase of the protein content increases the amount of bound water and, consequently, the firmness of the resulting gel. According to Snoeren *et al.* (1982), milk casein is able to immobilise as much as 2.82 g H_2O per g protein and shows a voluminosity of 3.57 m g^{-1}. Corresponding hydration values for undenatured and denatured whey protein are 0.32 g H_2O per g protein and 2.34 g H_2O per g protein, respectively. Data on both quantitative and qualitative effects of dry matter enrichment on physical parameters such as apparent viscosity, gel firmness or susceptibility to syneresis are available from various authors (Table 13.3).

Table 13.3 Effects of various fortification methods on physical properties of yoghurt

Reference	Fortification method[a]	Results
Becker and Puhan (1988)	SMP, UF, EV	Increase in gel firmness, decrease in syneresis
Guirguis *et al.* (1987)	RO (compared to milk powder addition)	Increase in firmness, less syneresis
Jaros *et al.* (2002a,b)	Reconstituted SMP to 10, 12, 14% dry matter with two different starters (non-ropy, ropy)	Increase in gel firmness, viscosity and serum-holding capacity; Oscillatory measurements of EPS yoghurt gels showed almost no effect of the protein content
Kulkarni *et al.* (1990)	Whey protein concentrate	Decrease of viscosity and firmness with increasing whey protein content
Lankes *et al.* (1998)	Comparison of SMP, VEV, UF techniques	Higher firmness and viscocity for UF (higher protein content) due to membrane characteristics
Modler *et al.* (1983)	NaC, UF-MPC, SMP, UF-WPC	Increase of firmness, decrease of syneresis; WPC less firm than casein-based products
Rohm and Schmid (1993); Rohm (1993a)	SMP, NaC, UF	Increase in viscosity, most for NaC fortification; decrease in syneresis
Savello and Dargan (1995, 1997)	SMP, UF	UF yoghurts have higher firmness and viscosity than SMP
Schkoda *et al.* (2001)	Increase of protein content from 3.5 to 7% by nanofiltration of skim milk	Increase in gel firmness, viscosity and serum-holding capacity
Tamime *et al.* (1984)	RO (compared to milk powder addition)	Increase in firmness, less syneresis

[a] RO, reverse osmosis; SMP, skim milk powder; UF, milk protein concentrate from ultrafiltration; (V)EV, (vacuum)evaporation; NaC, sodium caseinate; UF-WPC, whey protein concentrate from ultrafiltration.

The use of concentrated whey proteins in yoghurt manufacture is of increasing interest, both for utilisation purposes but also from a nutritional point of view; increasing the whey protein–casein ratio improves the biological value of the mixture (Meisel, 1989). Results on firmness and syneresis of yoghurt with a modified whey protein–casein ratio are somewhat contradictory. There is, however, a tendency towards less viscous, softer gels for products with an increased whey protein content, compared with products showing a similar protein content on casein basis (Buchheim *et al.*, 1986; Jelen *et al.*, 1987; Modler *et al.*, 1983; Morris *et al.*, 1995). In any case, it was suggested the increase in the whey protein–casein ratio should be limited because of the potential off-flavour effects (de Boer, 1996).

Homogenisation

Whole milk is homogenised at pressures between 10 and 20 MPa in a temperature range of 55–65 °C, usually prior to heat treatment, to prevent creaming during fermentation. The process results in the disruption of the milk fat globules, which are stabilised by a specific fat globule membrane consisting mainly of proteins, phospholipids and neutral glycerides, from native size (approximately range 1–5 μm) into much smaller ones. Commonly used homogenisation techniques and the mechanism of the fat globule size reduction are described in detail by Kessler (1996) and Walstra (1995). The covering of the homogenisation-induced, enlarged fat globule surface with fragments of milk proteins leads to the development of a secondary fat globule membrane, which is of great importance for the characteristics of fermented dairy products (Schkoda, 1999). Electron microscopic investigations (Buchheim and Dejmek, 1990) confirmed the model of the fat globule membrane of homogenised milk as proposed by Walstra and Jenness (1984), showing casein micelles and whey proteins as part of the layer: depending on the homogenisation temperature either casein micelles (at 40 °C), or micelle fractions (or sub-micelles) and whey proteins (> 60 °C) represent the main part of the newly built membrane.

Large fat globules as present in unhomogenised milk may decrease firmness of fermented products by interrupting the gel network (Aguilera and Kessler, 1988). It is generally accepted that the partial replacement of the native fat globule membrane with other milk proteins allows the fat globules to be incorporated into the gel by cross-linking them to the matrix (van Vliet and Dentener-Kikkert, 1982). However, the effects of homogenisation depend on the layout of the production process. Schkoda (1999) stated that aseptic homogenisation, with the pre-heat treatment preceding the mechanical treatment, causes a higher load to the membrane because of the aggregation of denatured whey proteins to each other and to casein; on the other hand, heating after homogenisation (septic homogenisation) leads to a partial aggregation of membrane proteins to each other, resulting in the formation of aggregates of linked fat globules. Studies of physical properties of high-fat yoghurt made from whey protein-enriched base milk homogenised either aseptical or septical revealed large differences, with higher viscosity and firmness values for products where homogenisation was performed after heat treatment (Kulkarni *et al.*, 1990). The authors dedicated the different effects of heating to different mechanisms of membrane loading, enhanced by the relatively high amount of whey proteins, and concluded that casein micelles incorporated in the secondary fat globule membrane built during homogenisation will be covered with whey proteins. Their denaturation during subsequent heating might prevent the active contribution of the fat phase to the gel properties (Sharma and Dalgleish, 1994; Tamime and Marshall, 1997).

The effects of incorporating the fat globules into the gel network also depend on the globule size and, consequently, the homogenisation procedure. After homogenising whole milk at different conditions, Plock *et al.* (1992) found a linear increase in gel firmness with decreasing fat globule diameter, and achieved a higher efficiency by multiple stage homogenisation at low homogenisation pressures.

Pre-heat treatment

Heating of the base milk is essential in yoghurt manufacture, and temperature–time conditions may be varied to adjust physical properties of yoghurt products. Generally, heating conditions are much more intense than necessary for preservation purposes, causing a sufficient denaturation of whey proteins, which are then able to associate with casein micelles (Law, 1996; Pearce, 1995). Heating increases voluminosity and water-binding capacity of whey proteins (Snoeren *et al.*, 1982) and decreases their solubility. Reactive side groups of globular whey proteins, especially thiol groups, are exposed due to unfolding, resulting in an oxidation to disulphide linkages and associations between whey proteins and casein. Additionally, denatured whey proteins may associate with casein micelles via hydrophobic interactions with κ-caseins (Smits and van Brouwershaven, 1980). All possible interactions result in a significant contribution of the denatured whey proteins to the properties of the yoghurt gels.

In commercial yoghurt production temperature–time profiles usually ranging from 80–85 °C for 30 min to 90–95 °C for 5 min are applied (Lucey and Singh, 1998). Dannenberg and Kessler (1988a,b) demonstrated the close relationship between the degree of β-lactoglobulin (β-lg) denaturation, which was linked to heating conditions by a kinetic approach (Dannenberg and Kessler, 1988c), and selected physical properties of yoghurt. Within a β-lg denaturation range of 60–99%, susceptibility to syneresis decreased linearly, whereas yoghurt gel firmness showed an optimum at a residual β-lg level of 10%. Lucey *et al.* (1997) compared acid gels made of reconstituted skim milk from powder subjected to different heat treatments during manufacture, by further subjecting reconstituted milk to several heating conditions. Increasing heating temperature and time led to higher denaturation of β-lactoglobulin and α-lactalbumin (α-la), with β-lg being more heat sensitive (i.e. 95% denaturation after heating at 90 °C for 15 min). Dynamic rheological measurements showed a marked increase in the storage modulus (G') for heating conditions higher than 80 °C for 15 min, indicating higher gel firmness. The authors suggested that denatured whey proteins in heated milk become susceptible to aggregation during acidification, as the isoelectric points of whey proteins are approached.

However, details of the mechanisms of the heat-induced interactions of α-lactalbumin and β-lactoglobulin with casein micelles are not yet clear. Since native whey proteins, added to the milk after heat treatment, do not contribute to the gel matrix (Lucey *et al.*, 1998a, 1999; Mahaut and Korolczuk, 1992), it appears that denatured whey proteins build associations with casein micelles during heat treatment then act as bridging material by interacting with other denatured whey proteins. Corredig and Dalgleish (1999) performed heating procedures between 70 and 90 °C on skim milk after addition of different amounts of purified α-la and β-lg and reported on two main interaction mechanisms: a direct interaction of β-lactoglobulin with casein micelles, via κ-casein binding, and the formation of soluble aggregates of both types of whey proteins as an intermediate before reacting with the casein. Furthermore, the presence of β-lg was necessary for the occurrence of any association with the casein micelle, and binding sites seem to be limited.

13.3.2 Incubation conditions

Cultures

The selection of the starter culture also determines the physical properties of stirred yoghurt to a large extent. As described above, some extracellular polysaccarides (EPS) produced by lactic acid bacteria are known to cause an increase in apparent viscosity, thus leading to improved physical stability of the fermented product.

A lot of work has been done in the field of isolating and characterising the composition of EPS produced by various strains of lactic acid bacteria (e.g. Faber *et al.*, 2002; Grobben *et al.*, 2000; Petry *et al.*, 2000; van Calsteren *et al.*, 2002). Yet, the functionality of the EPS in fermented milk is still not completely clear. It seems not that the amount of polysaccharide, but rather type, charge and molecular mass of EPS are important for rheological properties (Bouzar *et al.*, 1997; Laws and Marshall, 2001; Marshall and Rawson, 1999; Pleijsier *et al.*, 2000; Ruas-Madiedo *et al.*, 2002). The amount of EPS is, however, correlated to viscosity properties when a particular type of EPS is considered (Sebastiani and Zelger, 1998). Some authors suggested that the effects of EPS on yoghurt texture arrive from a possible attachment to the casein matrix (e.g. Domínguez-Soberanes *et al.*, 2001; Skriver *et al.*, 1995), which has been shown in micrographs obtained by conventional SEM. From these figures, it was concluded that the junction between polysaccharides and casein strands are responsible for increased viscosity values of yoghurt made with EPS-producing starters. However, Kalab (1993) referred to some previous work done by himself (Tamime *et al.*, 1984) and stated 'In micrographs of samples which had been dehydrated prior to electron microscopic examination, the mucus appears in the form of filaments. This appearance is an artifact; since the polysaccharides cannot be fixed chemically, they shrink on drying and form filaments.' It is more likely that EPS are either excreted outside the cell walls and remain there, thus forming a type of capsule (this was shown using confocal laser scanning microscopy by Hassan *et al.*, 1995), or are loosely attached to the cells or even excreted into the serum phase (Cerning, 1995).

Oscillatory measurements on intact yoghurt gels revealed no effects of non-charged EPS on the storage modulus of the gel network at a dry matter level of approximately 12%, whereas an improved stiffness of products with lower dry matter contents was found (Jaros *et al.*, 2002a; Pleijsier *et al.*, 2000). It may be assumed that non-charged EPS are dissolved in the serum phase in the pores of the network and, therefore, do not contribute to the strength of the protein network. This might be the reason for the significant effect they show on the viscosity of stirred yoghurt, as measured either in thixotropic loops in large deformation measurements or by empirical funnel flow measurements (Jaros *et al.*, 2002b). Permeability measurements showed lower values for yoghurt gels fermented with EPS-producing cultures compared to non-EPS gels, indicating a larger resistance to capillary flow (Jaros *et al.*, 2002b; van Marle and Zoon, 1995). Van Marle *et al.* (1999) also distinguished between two different types of EPS on the basis of steady-shear viscosity measurements, with viscosities of yoghurt serum being five times higher for starter cultures producing EPS which are released into the aqueous phase.

Temperature–time regimes of fermentation

The conditions of incubation may additionally influence the properties of the final product. Generally, thermophilic lactic acid bacteria show an optimum temperature ranging around 40–43 °C. In the dairy industry two different fermentation procedures are usually applied: the short incubation method and the long incubation method. By providing the microorganisms with their optimum temperature range, incubation times of approximately 2.5–4 h can be achieved. When incubation should take place overnight owing to technical requirements, temperature has to be reduced to 30–32 °C, leading to a fermentation time of 10–12 h to reach the desired end pH. This retarded activity of the starter organisms results in a slower rate of acidification and leads to a difference in the kinetics of protein network formation. It was generally accepted that, the lower the fermentation temperature, the longer it takes to reach a certain pH and, therefore, firmness, but the final product is much firmer (Walstra *et al.*, 1999). However, Lankes *et al.* (1998) compared yoghurts manufactured either at 30 °C or 42 °C and found higher gel firmness and higher viscosity for products fermented at 42 °C. Haque *et al.* (2001) found a systematic increase in gel strength for set-type yoghurt and viscosity for stirred yoghurt with increasing temperature of fermentation between a range of 37–46 °C. A possible explanation for these contradictory results was suggested by Horne (1998), who recently introduced a model of the casein micelle as a complex balance of hydrophobic attraction and electrostatic repulsion.

13.3.3 Post-incubation treatments

Set-style yoghurt

Since set-style yoghurt is fermented directly within the retail container, no further treatment other than cooling is necessary after fermentation. Usually, incubation is stopped at a pH slightly above the desired pH of the product, as a sufficient period of time is necessary for cooling the cups in ventilated chambers. When using separated incubation and cooling chambers, it is essential to avoid any vibrations of the packages during transportation as the gel is still weak and susceptible to local fracture with subsequent syneresis. Generally, rapid cooling is important to diminish the continued growth of the lactobacilli, otherwise leading to excessive acid production. Furthermore, enhanced acidification to pH values below 4 may lead to body and texture defects such as gel shrinkage and syneresis.

Stirred yoghurt

After fermentation of yoghurt in large vats, the gel is broken by stirring, thus forming a viscous non-Newtonian liquid, which is strongly shear-rate thinning. Defining the stirring regime is a crucial process, which induces considerable changes in the rheological properties of the final product. At a given shear rate, the apparent viscosity of stirred yoghurt depends on the firmness of the gel before stirring, giving higher viscosity with higher firmness. Additionally, higher gel firmness allows a more vigorous stirring, consequently leading to smoother products that do not become too thin. Higher firmness of the gel in the vat also lowers the risk of syneresis, which might lead to less viscous and more lumpy products.

There are no generally accepted rules for the layout of the time–temperature profile during stirring and cooling, and the applied procedures vary from manufacturer to manufacturer. However, it is generally accepted that the stirred product needs some time after stirring to rebuild some structure. After reaching a particular pH, the product may be slowly stirred in the fermentation vat to achieve a homogenous temperature distribution during cooling. Upon reaching 22–24 °C, the product may then be pumped to the filling and packaging unit, where relatively high shearing forces are applied. During the subsequent cooling process of the packed product, the desired increase in viscosity will be achieved.

13.4 Measuring the rheological and textural properties of yoghurt

13.4.1 Rheological and other physical methods

Set yoghurt

It is obvious that rheological properties of intact yoghurt gels can only be accessed when the fermentation process is performed *in situ*, i.e. within a specific geometry of any rheometer. Measurements may either be performed after the gel forming process is finalised, or even during fermentation provided that some basic requirements are fulfilled. These include, for example, the choice of an appropriate geometry, a sufficient protection against evaporation during fermentation (this can be achieved by avoiding a milk–air interface using a low-viscosity oil), and an appropriate instrument set-up. It is essential that the strain applied to the gelling system is kept very small to minimise any disturbance of the gelation process and to achieve reliable results. This can easily be achieved by using strain-controlled rheometers but might be difficult in the case of stress-controlled instruments where the smallest applicable stress, particularly in the early stage of fermentation when almost liquid milk is within the system, will result in a sufficient angular deformation (Hemar *et al.*, 2000). By comparing gelation profiles, Haque *et al.* (2001) recently concluded that slippage along supporting surfaces occurs easily when using horizontal geometries (i.e. cone-and-plate and plate–plate), whereas cup-and-bob systems are much more robust towards artefacts.

Rheological studies on intact yoghurt gels fermented within a rheometer system have been performed by, among others, Fiszman *et al.* (1999), Haque *et al.* (2001), Rohm (1993b), Rohm and Kovac (1994), Rönnegard and Dejmek (1993), van Marle and Zoon (1995) and Vlahopoulou and Bell (1995). Generally, mechanical spectra obtained within the linear viscoelastic region, i.e. a strain lower than approximately 1–3%, reveal a response typical for biopolymer gels (Ross-Murphy, 1994), with the storage modulus G' (Pa) exceeding loss modulus G'' (Pa) by a factor of about 5–7, little dependency of either G' or G'' on angular frequency ω (rad s^{-1}) with power law slope exponents in the range of 0.1–0.2 and, consequently, another power law relation between complex viscosity η^* (Pa s) and ω (here, slope exponents are $1–n$). As experimental conditions (base milk, heat treatment, starter, analytical conditions, etc.) vary to a large extent, a G' of 10^3 Pa can only be taken as a rough estimate of the gel firmness of set yoghurt.

Stirred yoghurt

From the rheological point of view stirred yoghurt is a complex viscoelastic fluid, which exhibits shear-thinning and time-dependent properties. A complete characterisation of flow properties of yoghurt therefore requires a large set of experiments, considering both the dependency on shear rate and time effects. It is somewhat complicating that, under practical conditions and especially when using stress controlled instruments, a stress region is observed below which no flow takes place. The existence of a yield stress has been demonstrated on the basis of several different rheological methods (Dimonte *et al.*, 1998). However, Barnes and Walters (1985) and Barnes (1999) insisted on finite viscosity values for almost all materials, with the response at small stresses to be treated as a Newtonian plateau, and considered the yield stress as a mathematical constant for modelling purposes.

Applying constant shear rate for a specific period of time results in typical decay curves for viscosity versus time, and viscosity usually decreases at any time when the experiment is repeated with increased shear rate. Although an equilibrium viscosity was not achieved within one hour (Butler and McNulty, 1995; O'Donnell and Butler, 2002; Ramaswamy and Basak, 1991; Schellhaass and Morris, 1985), the decrease of viscosity diminishes with increased time of shear, and quasi-equilibrium values were assumed for shearing times ranging between 10 and 20 min (Benezech and Maingonnat, 1993; van Marle *et al.*, 1999). Although these differences might not be large when expressed in figures, it is important for modelling purposes to decide whether steady-shear conditions at a specific time are met or not.

For the latter condition comprising stress decay *ad infinitum*, analytical data may be fitted to the logarithmic time model originally described by Weltman (1943):

$$\tau = A + B \ln \left(\frac{t}{t_m}\right) \qquad [13.1]$$

where τ (Pa) corresponds to shear stress at time t (s), t_m (s) is the time at which maximum stress is measured, and A and B (Pa) refer to intercept and slope in the Weltman model, respectively (consequently, A corresponds to τ for $t = t_m$).

Whereas Weltman coefficients have been related linearly to the applied shear rate $\dot{\gamma}$ (s^{-1}) ranging from 100 s^{-1} to 500 s^{-1} by Ramaswamy and Basak (1991), O'Donnell and Butler (2002) used a power law fit and a logarithmic equation to describe the shear-rate dependency of A and B, respectively.

Assuming an approach to equilibrium viscosity η_e (Pa s) at finite time the model of Tiu and Boger (1974), based on the theory of Cheng and Evans (1965), may be used:

$$\tau = \lambda\tau_m \tag{13.2}$$

Here, shear stress is related to the maximum shear stress τ_m (Pa) times a dimensionless structural parameter λ ($0 < \lambda \leq 1$). Depending on the model to be used for relating shear stress to shear rate one will find that:

$$\tau_m = \lambda K\dot{\gamma}^n \tag{13.3}$$

or

$$\tau_m = \lambda(\tau_0 + K\dot{\gamma}^n) \tag{13.4}$$

for the power law equation (O'Donnell and Butler, 2002) and the Hershel-Bulkley model (Butler and McNulty, 1995), respectively. In Eqns 13.3 and 13.4, τ_0 (Pa) corresponds to a yield stress and K (Pa s^n) and n are fitting coefficients, with K referring to the apparent viscosity at $\dot{\gamma} = 1$ s^{-1}, and $0 \leq n \leq 1$ as yoghurt is shear-thinning.

The time dependency of λ at constant shear rate is given by:

$$\frac{1}{\lambda - \lambda_e} = \frac{1}{\lambda_m - \lambda_e} + k_1 t \tag{13.5}$$

with λ_m corresponding to λ at t_m and λ_e being the structural parameter at equilibrium conditions. Substituting Eqn 13.2 into $\eta = \tau/\dot{\gamma}$ yields:

$$\lambda = \frac{\eta\dot{\gamma}}{t_m} \tag{13.6}$$

which also holds for maximum and equilibrium conditions and can be further substituted into Eqn 13.5 giving:

$$\frac{1}{\eta - \eta_e} = \frac{1}{\eta_m - \eta_e} + \frac{k_1\dot{\gamma}}{\tau_m}\ t \tag{13.7}$$

A plot of $1/(\eta - \eta_e)$ versus time results in a straight line with a slope equal to ($k_1\ \dot{\gamma}/\tau_m$) and, if repeated for a number of shear rates, the relation between k_1 and $\dot{\gamma}$ can be established. A similar treatment can be applied for the Hershel-Bulkley model by using Eqn 13.4 instead of Eqn 13.3 to express the denominator in Eqn 13.6, τ_m. With the above-mentioned tools it will be possible to model initial maximum stress and equilibrium stress as well as λ_e by a power law (or whatever is intended) function of shear rate, thus completely describing shear-thinning and thixotropic behaviour.

Several authors used a more qualitative approach to study stirred yoghurt, either by increasing shear rate stepwise or by increasing shear rate linearly with time, followed by a decrease until $\dot{\gamma} = 0$ s^{-1}. Flow curves were fitted by means of the power law (Abu-Jdayil *et al.*, 2000; Geraghty and Butler, 1999; Keogh and

O'Kennedy, 1998; Parnell-Clunies *et al.*, 1986; Schellhaass and Morris 1985), the Casson equation (Parnell-Clunies *et al.*, 1986; Skriver *et al.*, 1993) or the Hershel Bulkley equation (Hassan *et al.*, 1996; Ramaswamy and Basak, 1991, 1992; Rohm, 1993a; Rohm and Schmid, 1993). However, it has to be noticed that any equation coefficients obtained by regression analysis will depend heavily on the set-up of the test, i.e. the acceleration of shear rate, caused by the time-dependent viscosity decay of yoghurt (Rohm, 1992). Some additional information can be drawn from the area included between the upward and downward curves when applying the 'thixotropic loop technique'. The area is given in terms of ($N/m^2 \times s^{-1}$) or, if related to the volume of the sheared sample, can be treated in terms of the power necessary for structure degradation.

A number of studies dealt with oscillatory methods applied to stirred yoghurt (Jaros *et al.*, 2002b; Ozer *et al.*, 1998, 1999; Rohm and Kovac, 1995; Skriver *et al.*, 1999, Steventon *et al.*, 1990). Qualitatively, mechanical spectra resemble those of set yoghurt, with the moduli being 8–10 times lower. Afonso and Maia (1999) and Skriver (1995) compared the results of dynamic measurements of stirred yoghurt with apparent steady shear viscosity and observed that the empirical Cox–Merz rule, which should result in identity of η^* versus ω and η_{app} versus γ-curves (Cox and Merz, 1958), was only obeyed after introducing a horizontal shift factor as has been suggested by Bistany and Kokini (1983) and Rao and Cooley (1992). More recently, Haque *et al.* (2001) demonstrated the importance of the sample loading procedure by showing a sufficient (approximately 25%) increase of the modulus following the first few minutes after loading, attributable to some structure recovery.

Figure 13.2 provides some interesting detail of a comparison of two samples of stirred yoghurt produced at laboratory scale under identical conditions (i.e. base skim milk: 14% total solids, pre-heat treatment: 90 °C/30 min; incubation temperature: 43 °C, final pH: 4.5). Whereas the mechanical spectra showed no noticeable differences, yoghurt A inoculated with a highly viscous starter revealed a completely different response to an up-and-down shearing cycle (shear rate acceleration: $1\ s^{-2}$) than sample B fermented with a standard starter, with a much smaller area enclosed between the upwards and the downwards curve. This means that, in both cases, the breakdown of structure is exactly compensating the strain rate increase that would result in a higher shear stress response if the sample remained unchanged.

Permeability

A special method, originally described for rennet milk gels but later adopted for acidified milk gels (Lucey *et al.*, 1997; van Marle and Zoon, 1995) has been introduced by van Dijk and Walstra (1986) and Roefs *et al.* (1990). Small glass tubes containing *in situ* fermented gels are placed in whey, with the gel surface below the whey level, thus causing a pressure difference. The whey flux through the gel network is usually observed as a function of time and may serve as an indicator of the occurrence of microsyneresis, i.e. some rearrangement and condensation in the casein network, leading to an increase in the size of micropores,

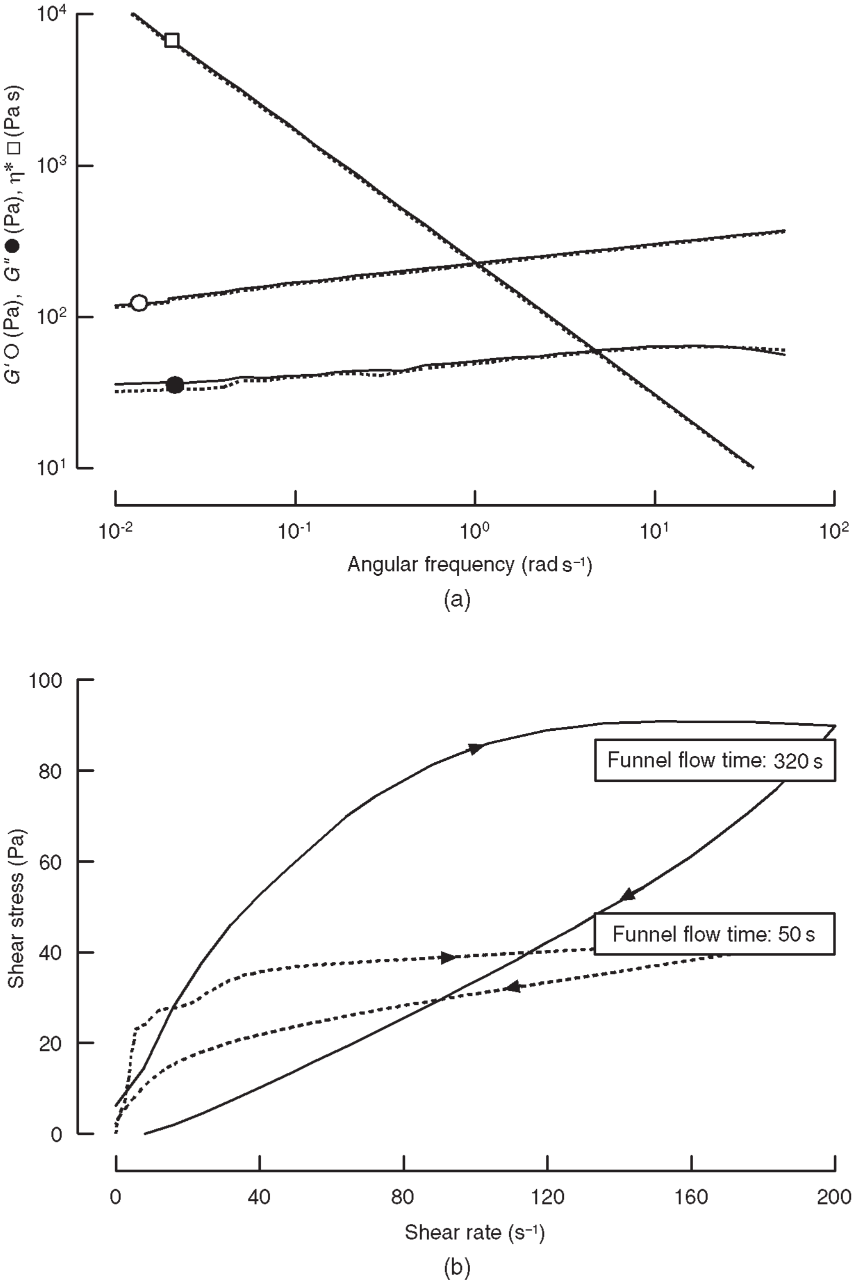

Fig. 13.2 Mechanical spectra (a) and flow curves of stirred yoghurt (b). Full lines show yoghurt A inoculated with a highly viscous starter; dotted lines show yoghurt B inoculated with a standard starter. For further explanations see text.

or might be helpful in the detection of interactions between milk constituents and polysaccharides produced by starter cultures.

13.4.2 Empirical methods

Gel firmness

Gel firmness measurements of set yoghurt are usually performed by means of constant speed penetration on universal testing machines or similar instruments, using cylindrical plungers (15 mm < d < 40 mm) and crosshead speed values ranging between 10 mm/min and 100 mm/min, usually below room temperature. With up-to-date equipment the force response, which is affected by plunger size and penetration speed, is monitored as a function of penetration depth. Several authors (e.g. Barrantes *et al.*, 1996; Ferragut *et al.*, 2000; Hassan *et al.*, 1996; Lorenzen *et al.*, 1999; Tamime *et al.*, 1991, 1996) used force values (or force values related to plunger diameter) at a predefined penetration depth to express gel firmness.

Another way to evaluate firmness from penetration data is based on the work of Schmidt and Ahmed (1972), who proposed expressing firmness as the elastic response from the undisturbed sample by calculating the initial slope of the force–penetration curve. The advantage of applying regression analysis to the response curve (Fiszman and Salvador, 1999; Fiszman *et al.*, 1999; Jaros *et al.*, 2002a; Rohm, 1995; Rohm and Kovac, 1994) is that the parameter, denoted as apparent firmness modulus (N mm^{-1}), mostly resembles the elastic response of the yoghurt gel and is not influenced by upward shear forces occurring after sufficient penetration of the plunger. These shear forces along the circumference of the plunger obviously cause some fluctuations in the force–penetration curve and, finally, may result in poor repeatability (Fig. 13.3).

The use of cones with varying dimensions on constant mass penetrometers, which read penetration depth after a predefined period of time, has been described by, among others Davi and Shah (1998), Mistry and Hassan (1992) and Ozer and Atamer (1999).

One-point measurements of viscosity

Although apparent viscosity of stirred yoghurt depends heavily on shear rate, shearing time (at constant rate) and shear history, single point measurements have been performed in a number of studies to achieve appropriate indicator values. Most commonly, shear rate in the case of rotational viscometers (Abu-Jdayil *et al.*, 2000; Daubert *et al.*, 1998; Moreira *et al.*, 2000; Schkoda *et al.*, 2001) or rotations per minute in case of the Brookfield-type instruments (Fernandez-Garcia and McGregor, 1997; Fernandez-Garcia *et al.*, 1998; Skriver *et al.*, 1999; Trachoo and Mistry, 1998) of the shearing device were set to a predefined value, varying to a large extent from study to study, and torque, shear stress or apparent viscosity were recorded after a predefined period of time. In some other cases, flow curves comprising a particular shear rate-time regime were recorded, and apparent

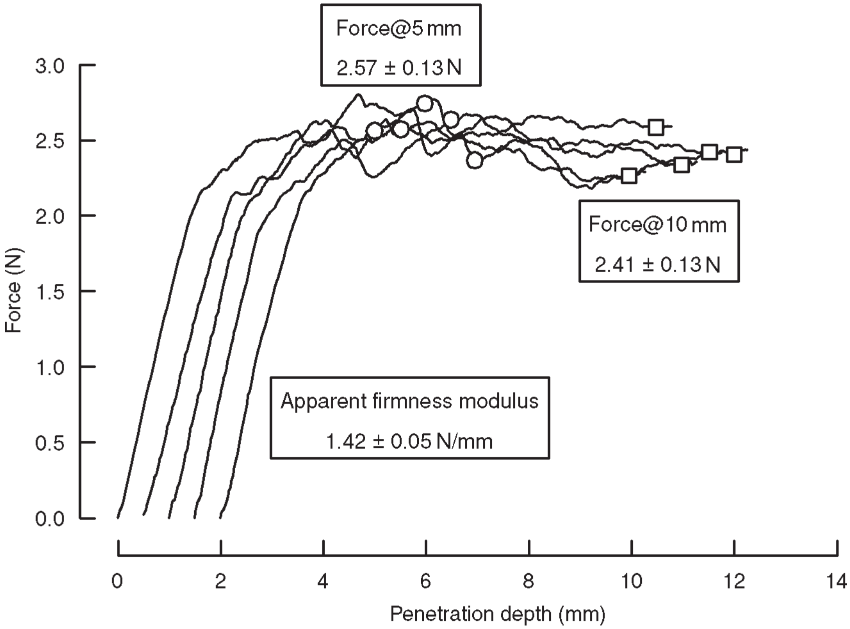

Fig. 13.3 Evaluation of different parameters from force penetration curves applied to set yoghurt.

viscosity was calculated from the shear stress at a predefined shear rate (Hassan *et al.*, 1996; van Marle and Zoon, 1995).

Another indicator for apparent viscosity can be obtained from the flow time necessary for a predefined amount of stirred yoghurt to pass through devices such as the Posthumus funnel, which represents a cup with a narrow efflux tube. Funnel flow can be considered as an easy-to-perform and inexpensive method for viscosity evaluation, provided that the entire sample preparation procedure (e.g. prestirring of yoghurt, transfer into the funnel) is thoroughly defined (Beal *et al.*, 1999; Martin *et al.*, 1999). Although Hellinga *et al.* (1986) showed in a theoretical approach that shear rates occurring during the flow of the product through the orifice are much higher than shear rates expected during sensory evaluation of yoghurt, Skriver *et al.* (1999) reported on a sufficient interrelation between funnel flow time and oral examination of viscosity, thus allowing the prediction of sensory properties with the Posthumus device.

Syneresis

The stability of set yoghurt towards syneresis or whey drainage can be evaluated either under the influence of regular gravity or by applying additional gravitational forces by centrifugation. The aforementioned principle can easily be applied to both pilot plant or laboratory-scale products as well as to commercial yoghurts, whereas the procedure based on centrifugation requires the fermentation of yoghurt in special containers in case of set yoghurt. A third, but rarely used method, which can only be applied to set yoghurt, is based on gel shrinkage after wetting.

Measurements at normal gravity

Generally, a certain amount of yoghurt supported by a container or moulded with a specific device is placed on a sieve, and the amount of separated whey is measured after a predefined period of time. Prior to measuring whey drainage, Modler *et al.* (1983) used a Cherry-Burrell knife to determine firmness of yoghurt gels. The containers were then inverted and placed on a stainless steel sieve supported by a funnel. The amount of whey was collected after 2 h of draining at 3 °C and taken as a measure of syneresis. Although originally intended for enzymatic coagulation, a special device based on this principle was proposed by Pompei *et al.* (1994). Guirguis *et al.* (1987) used a container to apply this method to stirred yoghurt. Several authors formed a hemisphere of yoghurt gel by means of an ice cream scoop and collected the drained whey (Dannenberg and Kessler, 1988a; Hassan *et al.*, 1996; Hoffmann *et al.*, 1997; Lorenzen *et al.*, 1999).

Several authors evaluated spontaneous whey separation in yoghurt containers after a predefined storage period (Augustin *et al.*, 1999; Fiszman and Salvador, 1999; Fiszman *et al.*, 1999; Moreira *et al.*, 2000). However, this method shows some disadvantages, especially in firm yoghurt, as the amount of spontaneously released whey is usually very small.

Centrifugation methods

Harwalkar and Kalab (1983) subjected yoghurt gels fermented in centrifuge tubes to centrifugal forces ranging between 30 and 2000*g* (6 °C, 10 min), then observed a sigmoid relationship between separated whey and *g*-value, and proposed the *g*-value at the inflection point of the curve as a measure of susceptibility to syneresis. However, this method is laborious as it requires a number of centrifugations per sample, and several authors used the work of Harwalkar and Kalab (1983) as a basis for a simplified procedure, covering only one centrifugation step and taking into account the relative amount of separated whey (Ferragut *et al.*, 2000; Haque *et al.*, 2001; Lucey, 2001; Rohm and Kovac, 1994; Schkoda *et al.*, 2001) . Both the magnitude of the *g*-values as well as centrifugation time and temperature vary considerably, e.g. Lucey *et al.* (1998b) used 100*g* whereas Parnell-Clunies *et al.* (1986) applied as much as 13 500*g*.

The centrifugation method can be applied to set yoghurt (as long as the gel is fermented within the centrifugation tubes) as well as to stirred yoghurt. Generally, the amount of whey separated from the capillary space decreases with increasing dry matter content of yoghurt and is influenced by technological conditions such as pre-heat treatment of the base milk, milk homogenisation or the type of starter culture used.

Gel shrinkage measurement

Van Dijk and Walstra (1986) used the fact that milk gels in containers only start to shrink either after cutting or after wetting of the surface, and established a method for measuring the height of a defined slab of the gel after spraying whey or water onto the surface. This method was adopted for acid milk gels by Lucey *et al.* (1997).

13.4.3 Sensory assessments

Apart from simple difference testing (de Ancos *et al.*, 2000), issues related to quality perception and acceptance (Grunert *et al.*, 2000; Laye *et al.*, 1993; Muir and Hunter, 1992) as well as consumer studies using free-choice profiling and Procrustes analysis (Gacula, 1997), most of the studies (e.g. Barrantes *et al.*, 1996; Biliaderis *et al.*, 1992; Faergemand *et al.*, 1999; Kneifel *et al.*, 1992; Martin *et al.*, 1999; Rohm *et al.*, 1994; Skriver *et al.*, 1999) dealing with sensory assessments of physical properties of yoghurt were performed by using the quantitative descriptive analysis (QDA) originally described by Stone *et al.* (1974). Simply speaking, QDA is based on vocabulary and sensory procedures selected by means of test products and established in a group process by subjects. In repeated measurements, data are collected by interval scales, and after applying analysis of variance, multivariate procedures (e.g. principal component analysis, factor analysis) may be performed to eliminate redundancy.

Descriptors for texture properties of yoghurt extracted from the literature differ to a large extent; additionally, some studies lack definitions and procedures of the assessed sensory parameters. A collection of descriptors and definitions is summarised in Table 13.4.

13.5 Future trends

Continuously increasing consumer health consciousness is responsible for the expanding worldwide interest in functional foods. Fermented dairy products such as yoghurt have, for a long time, been known for their use in managing intestinal disorders such as lactose intolerance or acute gastroenteritis. There are three different approaches in the dairy industry, which are aimed at modifying the intestinal microflora and thereby beneficially influencing the health of the host. These include the fermentation of milk with probiotics, such as various strains of lactic acid bacteria and *Bifidobacterium* spp., which inhabit the human gut; the addition of prebiotics, which are non-digestible food ingredients, supposed to stimulate the growth of various health-promoting bacteria in the human colon; and the use of synbiotics, defined as a mixture of probiotics and prebiotics. The application of each treatment potentially influences rheology and texture properties, as different starter cultures are used, or conventional starter cultures show other modified fermentation patterns.

Although technology has been almost successfully adopted to produce low-fat, low-calorie yoghurt with sufficient rheological properties, there is still a need for product optimisation. The addition of processed dairy ingredients (e.g. microparticulated whey proteins) might be a promising way of mimicing fat properties and of improving physical properties and sensory characteristics.

Table 13.4 Descriptors applied in sensory analysis of yoghurt

Reference	Texture descriptor	Sensory definition and/or procedure
Barrantes *et al.* (1996)	Perceived whey separation	Not specified
	Firmness	Not specified
	Lumpy/coarse	Not specified
	Gummy	Not specified
	Body and texture	Not specified
	Creamy	Not specified
Biliaderis *et al.* (1992)	Thickness	Not specified
	Graininess	Not specified
Faergemand *et al.* (1999)	Whey drainage	'Visual determination of liquid on top of yoghurt'
	Firmness	'When cutting the yoghurt gel with a spoon'
	Flakiness	'By cutting yoghurt surface with a spoon'
	Grittiness	'Oral determination'
	Creaminess	'Oral determination'
Kneifel *et al.* (1992)	Whey drainage	'Visual observation of gel surface after inserting a spoon into the gel'
	Texture	'Visual observation after stirring the product with a spoon'
Martin *et al.* (1999)	Smoothness	'Quantity of particles in the gel quantified by visual inspection of the spoon's back'
	Sliminess	'Product's ability to flow in a continuous way from the spoon'
	Thickness (non-oral)	'Product's ability to flow from the spoon'
	Thickness (oral)	'Product's flowing resistance assessed by crushing one spoonful of the product between the tongue and palate'
	Mouth coating	'Product's ability to form a film lining the mouth'
Rohm *et al.* (1994)	Texture	'Gel firmness perceived by penetrating the gel with a teaspoon and removing an appropriate amount of yoghurt without exerting any shearing force'
	Mouthfeel	'Degree of smoothness perceived by squeezing yoghurt between tongue and palate'
	Viscosity	'Perceived resistance against stirring with a teaspoon'
	Ropiness	'Perceived cohesiveness of the stirred product after pouring it from a teaspoon'
Skriver *et al.* (1999)	Non-oral viscosity	'Penetrating the yogurt gel with a teaspoon, placing approx. 5 ml on the surface of the yoghurt gel and observing how fast it disappeared'
	Oral viscosity	'Perceived degree of thickness when eating the yogurt'

Whereas membrane processes, providing selective cut-offs of particular ingredients, may be used to enhance the quality of traditional products, new techniques such as high-pressure treatment might result in microstructural modifications, presumably leading to the development of completely new products.

13.6 Sources of further information and advice

Many different research groups around Europe and the USA are involved in studies concerning dairy products in general or yoghurt in particular. Apart from many publications in the scientific literature, a good overview of current or recently completed international projects is available on the internet pages of the Community Research & Development Information Service of the European Union (http://www.cordis.lu). Currently, research on fermented milks is mainly focused on lactic acid bacteria, dealing with topics ranging from molecular biology and genetics to genetically engineered food products, which are supposed to improve product quality, and to consumer attitudes towards these food systems. Additionally, extensive work has been done on the isolation of new starter cultures from fermented dairy products and on human probiotics for fermented milks. The mechanisms and the controlled production of exopolysaccharides from lactic acid bacteria as natural thickeners and the improvement of texture properties of yoghurt are still under investigation. In particular, a project finalised recently was dealing with the relationship between composition, processing conditions and gel texture of particle gel systems by means of modern technologies such as Brownian dynamics simulation of real and simulated systems.

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14

Controlling the texture of spreads

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14.1 Introduction

Bread is an important part of the diet in many parts of the world (McGee, 1984). As with many of the basic dietary ingredients, it is seldom eaten in a completely pure form, but usually in combination with other foodstuffs or top layers that provide the meal with some extra (variable) flavour such as meat products, cheese or marmalade. Furthermore, bread tends to be a relatively dry product that is rather difficult to swallow without any additional lubrication of the meal. Spreads are mainly applied to perform for these two functions: adhesion of the 'top layer' to the bread and lubrication. Moreover, some spreads are designed to serve specific nutritional purposes and to contribute to a healthy diet. This chapter concentrates on the texture of spreads and on the tools that are available to vary the texture of these food emulsions. First, however, it seems useful to consider spreads in the context of food emulsions.

An idealised model food emulsion contains two components, usually oil and water, which are immiscible on a molecular length scale, forming distinct continuous and disperse phases. These phases are mixed homogeneously on a much coarser length scale, typically longer than 10 or 100 μm. There are two main classes of food emulsions. Oil-in-water (O/W) emulsions hold a dispersed oil or fat phase in a continuous water phase. In contrast, water-in-oil (W/O) emulsions contain water dispersed in oil or fat.

These classical emulsion types should be regarded as textbook examples of systems that can be encountered in practice. The dispersed phase in an archetypal model emulsion is considered to consist of isolated, non-interacting spherical droplets surrounded by a liquid continuous phase, whereas droplets in many food emulsions may form aggregates, partially coalesce (e.g. in butter or ice cream), or even contribute to structures that are partly bi-continuous. In addition, the dispersed phase and/or the continuous phase may not be liquid, but a complex mixture of solid and liquid material instead (Dickinson, 1992).

Typical food O/W emulsions include milk (~3% fat dispersed), fresh cheese (~15–40% fat) and mayonnaise (70–80% fat). These three examples illustrate that the amount of dispersed phase may vary considerably in such emulsions, contributing to significantly different mechanical properties of the resulting products. Butter, margarine (16–20% water) and halvarine or low-fat spreads (60% water) are well-known W/O food examples. It is noteworthy that their mechanical properties are quite similar, despite having very different amounts of dispersed phase. Liquid margarine (16% water) on the other hand, behaves completely differently from normal margarine, illustrating that the physical properties of the final product depends on the characteristics of the continuous phase as well, and not only the amount of dispersed phase. Schematic representations of some of these examples are shown in Fig. 14.1.

Most food emulsions can be viewed as composite materials for mechanical properties such as firmness. A typical composite consists of a matrix phase and a filler phase. Depending on the relative properties and volumes of both phases, their behaviour is dominated by one of either phase. For example, the properties of a firm matrix with a modest amount of soft filler will be determined mainly by the properties of the matrix, as is the case for a wrapper margarine (a foam could be considered as an even more extreme example, if the air is taken as the filler phase). On the other hand, a soft matrix filled with a reasonable amount of firm filler may derive much of its properties from the filler phase, as is the case for some fresh cheese-type products, provided that the adhesion between matrix and filler is sufficient. Furthermore, the amount of filler is important: mayonnaise obtains its firmness from the volume of the filler phase, although both the matrix and filler phase are essentially soft materials.

Apart from type of matrix and filler material, the spatial distribution of the filler particles in the product is of importance. The mechanical properties of a food emulsion are expected to be much more sensitive to filler phase characteristics if attractive forces between filler phase particles are important (e.g. in acid protein-stabilised emulsions), than when such interactions are much weaker (as in W/O emulsions, where the weaker van der Waals forces dominate).

The present chapter will focus on spreadable food emulsions. Whether or not an emulsion can be considered to be spreadable depends on the rheological properties of the product. A spreadable material is characterised by a more or less plastic rheology. Very loosely stated, a plastic material is a solid-like material for which the deformation history (e.g. flow experienced) does not affect its current mechanical properties. In this sense, a plastic material is reminiscent of a liquid. A typical solid (e.g. a chocolate bar) would break upon application of sufficient deformation and never regain its original properties. Few materials are *really* plastic, but spreads such as margarine and butter are reasonable approximations (de Bruijne

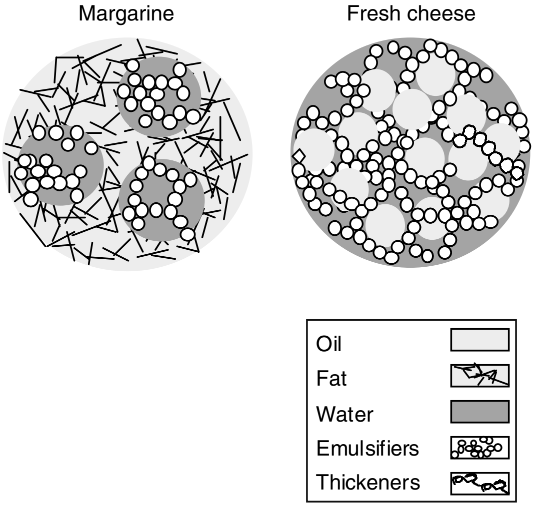

Fig. 14.1 Schematic representation of a number of food emulsions.

and Bot, 1999). A spreadable texture can only be obtained if the product is built from microstructure elements that interact weakly and reversibly, qualities which usually come together. In full-fat mayonnaise, for example, these interactions arise from the fact that the fat droplets are being pressed together in the product, being present at concentrations that exceed the random close packed fraction for hard spheres. Later in this chapter, it will be described how van der Waals interactions between fat crystals will generate a similar weak reversible interaction in margarine.

The outline of this chapter is as follows. First, the functional properties of the ingredients that are used to build up these food emulsions are considered. Subsequently, the processing that is required to achieve the desired microstructure and related product properties is discussed for a selection of products. Next, the stability of the product is described during storage, transport and product shelf-life. Finally, textural changes in the mouth during spread consumption are discussed.

14.2 Emulsion microstructure: ingredients

A stable food emulsion requires a formulation containing at least three components, a continuous phase, a dispersed phase and an emulsifier. For typical spreads, this boils down to having a lipid phase, a water phase and an interface. Understanding the properties of these phases requires a basic understanding of their composition and physical chemistry. The present section outlines the most important physical properties of the main ingredients in these phases.

14.2.1 Fats and oils

The lipid phase is typically composed of a mixture of triacylglycerols (TAGs, or triglycerides) referred to as fats and oils. Oils do not show any crystallised TAGs at ambient temperature, whereas fats do. Fats and oils are usually classified in terms of the source from which they are retrieved (e.g. soy bean oil, sunflower oil, butter fat), but the fundamental differences between lipids can best be explained in terms of the constituting TAGs. For example, the amount of crystalline fat ('solid fat content' or SFC) is related to the level of saturated fatty acids (SAFA) in the specific TAG mixture. The SFC of a TAG mixture is routinely measured using nuclear magnetic resonance (NMR) techniques, and is often referred to as the N-line of the fat (Gribnau, 1992). Designing the solidification behaviour of the lipid phase requires an understanding of the crystallisation behaviour of pure TAGs and their mixtures (Garti and Sato, 1988; Wesdorp, 1990; Larsson, 1994; Marangoni and Narine, 2002). The TAG composition of the lipid phase can be modified through simple blending of fats and/or the use of oil modification techniques. These oil modification techniques include hydrogenation, dry or wet fractionation, and chemical interesterification (Bockisch, 1993).

Primarily, fat crystallisation is not much different from any other kind of crystallisation in foods (Hartel, 2001; Garti and Sato, 2001). However, two special characteristics give fat crystallisation an air of mystery, even after years of study. These characteristics are first monotropic polymorphism of the TAG crystals, and secondly the effect of the presence of a wide range of chemically different TAGs in any fat reflecting the complex fatty acid composition of the raw materials (Garti and Sato, 1988, 2001; Wesdorp, 1990; Marangoni and Narine, 2002; Hartel, 2001). The chemical complexity of the TAG mixture originates both from the many different combinations of fatty acids that can be attached to a glycerol backbone, as well as from the isomeric variation in the position of the fatty acids at the glycerol backbone.

Monotropic polymorphism (Turner, 1971; Hageman, 1988; Sato, 1999) is the ability to appear in different solid states, of which only a single one is stable. In fats there are many polymorphs or sub-polymorphs. In general three main polymorphs are distinguished (Sato, 2001a). These are the so-called α state, a hexagonal crystal structure with relatively loose packing, the β' state, which has been characterised to be orthorhombic (van Langevelde *et al.*, 2000; van de Streek, 2001; Sato, 2001b), and finally the stable β state, a monoclinic crystal structure. The driving force for converting an α crystal to a β' crystal and ultimately to a β crystal structure is the energy gain due to the increasing packing density of the TAGs in the crystal lattice throughout the range α, β', β. The intermediate states α and β' have a significant lifetime only by virtue of Ostwald's rule of stages (Ostwald, 1897), which states in short that 'it is easier to convert to an energetically similar state than to the energetically most favourable'.

The normal rules for crystallisation processes in terms of nucleation and growth apply to simple systems composed of a single TAG (Garti and Sato, 2001). In mixed TAG systems recrystallisation behaviour is more complex, however, because foreign TAG molecules have to be incorporated in the crystal lattice. This

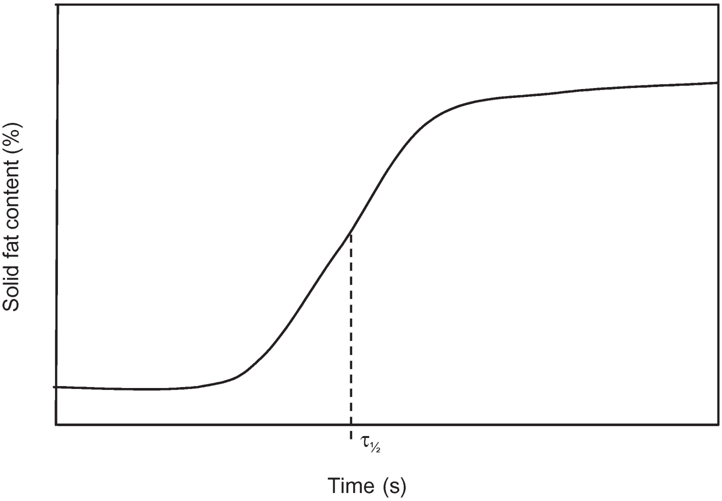

Fig. 14.2 Schematic representation of crystallisation behaviour. The transition time is indicated by $\tau_{½}$.

incorporation occurs less readily at higher packing densities of the crystal structure, which implies that the transition process from a less stable to a more stable polymorphic form has to involve a change in composition of the crystalline phase. It is established empirically that the rate of a polymorphic transition is a function of temperature and in particular of the molecular composition of a fat mixture. It is expressed as the transition time, $\tau_{½}$, the time at which a maximal conversion rate is found (see Fig. 14.2). However, the details of this process at a molecular level are still unclear, and may involve either melt mediation or a solid–solid transition, or a simple competitive crystallisation process. The final shape of a crystal reflects the different growth rates per lattice direction. These growth rate differences are governed by the anisotropy of the molecular interactions (Hollanders, 2001; van de Streek, 2001).

The consistency of a lipid phase is based on the structuring effect of fat crystals in the lipid phase, which in turn depends on the amount of crystals, their size, morphology (shape) and polymorph, and on the strength of the interactions between the crystals. The SFC of a fat mixture at a given temperature is primarily a function of molecular composition and the polymorph (see solid lines in Fig. 14.3 below, further explanation will follow later). A space-filling network within a lipid phase is achieved for SFC levels as low as 1% (w/w) (Kloek, 1998). SFC, crystal size, shape and morphology are again functions of the TAG composition of the fat and the crystallisation conditions. Generally, crystal morphologies with a high surface to volume ratio are best to obtain maximum texture: platelets and needles are more effective than cubes or spherulites.

The strength of the interaction between the single crystals is of key importance for the strength of the network and its plasticity or brittleness. For a plastic structure, crystals should predominantly be linked through secondary bonds based on van der Waals forces (Haighton, 1965; Kloek, 1998). When crystals are more or less sintered together through solid bridges, also referred to as primary bonds (Haighton, 1965), the resulting network is usually harder but less plastic. This type of bond is typically produced with fat mixtures that crystallise in distinguishable steps, so that delayed precipitation of solid material preferentially takes place in corners and holes of the primary structure (Johansson, 1995; Johansson and Bergenståhl, 1995a).

14.2.2 Proteins

Proteins are polypeptide chains built from (L-)amino acids, which are involved in biological processes such as catalysis, transport and storage. Proteins are often folded to form complex three-dimensional structures. This structure determines their properties, usually critical for their biological function, which are lost when the conformation of the protein changes sufficiently. In spreads, (bovine) milk protein is the most common protein source, and further discussion will therefore be limited to milk proteins.

Milk proteins are mainly used in foods as emulsifiers or as structuring agents. Broadly speaking, the emulsifying capacity is similar between milk protein fractions. However, it should be borne in mind that small entities (e.g. single proteins) tend to diffuse faster and adsorb more rapidly at the interface, whereas more bulky entities (e.g. aggregates) tend to be more sluggish and sterically hindered. Thus smaller emulsifiers tend to facilitate the emulsification process more efficiently than bigger particles. However, long-term stability of aggregated emulsions is better ensured with the bulky aggregates. The use of proteins to structure products usually requires a change in their colloidal state, since most native proteins are soluble (or at least dispersable) and do not aggregate. Proteins can start to aggregate upon changes in solvent quality (acidification, addition of salt), or as a result of temperature-induced conformational changes (heat treatment).

Milk consists of butterfat, milk protein (mainly casein and whey protein), lactose, colloidal calcium phosphate and some minor components (Walstra and Jenness, 1984). Casein is present in milk in the form of small protein aggregates, called casein micelles, of typically 50–300 nm diameter. Each micelle consists of a mixture of α-, β- and κ-caseins. Various models exist for the structure of these micelles, but currently the most explanatory appears to be the version where α-casein forms the structural backbone of the micelle, supplemented by β-casein and colloidal calcium phosphate, and where most of the κ-casein can be found at the surface of the micelle (Holt and Horne, 1996). κ-Casein is a block-polymer-like protein consisting of a relatively hydrophobic part and a relatively hydrophilic part. The hydrophobic part anchors itself in the micelle, whereas the hydrophilic part (known as the glycomacropeptide, GMP, or casein macropeptide, CMP) acts more or less as a salted polyelectrolyte polymer brush (de Kruif and Zhulina, 1996; Tuinier and de Kruif, 2002). This brush stabilises the micelles against aggregation because the charged carboxylic acid groups in the hydrophilic part of the κ-casein repel each other weakly. The stabilisation is retained as long as the κ-casein remains negatively charged.

For unheated milk, the globular whey protein is in its native form and is relatively unimportant for the aggregation behaviour of the milk. Heat treatment at sufficiently high temperatures and long duration will denature (i.e. partly unfold) the whey protein, triggering the formation of disulphide-links between whey proteins and between whey proteins and micelles. Depending on the conditions (e.g. pH) under which this heat treatment is applied, the whey protein may form separate aggregates or cover the casein micelles (Creamer *et al.*, 1978; Heertje and Pâques, 1995). Such a heat treatment will modify the pH below which milk gel formation will start to occur (Vasbinder *et al.*, 2001). The two main types of milk protein, micellar casein and whey proteins, are both able to form a gel upon processing, caseins upon acidification/renneting and whey proteins upon heat treatment.

The behaviour of unheated milk upon acidification is determined mainly by the properties of casein micelles in the milk. The κ-casein brush stabilises the micelle as long as it remains negatively charged, but the brush collapses when the pH of the solvent drops. Acidification of milk therefore results in aggregation of the micelles and therefore in the formation of an opaque milk gel, a so-called curd, which contracts by a process called syneresis. A related effect can be attained at neutral pH by removing the hydrophilic glycomacropeptide from the casein micelle by an enzymic reaction involving chymosin, the active enzyme in rennet, which is the basis for the preparation of soft and hard cheeses.

The gelation behaviour of whey proteins is determined predominantly by the behaviour of the main component, β-lactoglobulin. Native β-lactoglobulin does not gel, but upon denaturation (or during, depending on the solvent conditions) a network is formed once the electrostatic interactions between proteins have been screened sufficiently. Gels formed at neutral pH and at low ionic strength are translucent, dense and elastic. Gels formed under acidic conditions (pH close to the isoelectric point of β-lactoglobulin) or at high ionic strength form coarse, brittle and opaque networks. Detailed studies on β-lactoglobulin and whey protein gel structures as a function of pH were performed by Langton and Hermansson (1992).

In heated milk, both whey protein and casein micelles will participate in the gel formation. The incorporation of the whey protein in the milk gel will increase the firmness of the gel. This is caused by the increased availability of insoluble protein for network formation and by the presence of covalent interactions that contribute relatively efficiently to gel firmness.

The pH at which heating is done is critical to the building blocks of the gel: Vasbinder (2002) showed that heating at neutral pH results in gels which contain both whey protein aggregates and whey-protein coated micelles, whereas heating at slightly acid pH (<6.5, approximately) results in whey-protein coated micelles only. The balance between coated micelles versus micelles + whey aggregates affects the pH of aggregation and the coarseness of the resulting gel network, which subsequently affects the stability of the protein network against syneresis (Schorsch *et al.*, 2001).

14.2.3 Emulsifiers

A good emulsifier should adsorb quickly at the oil–water interface to lower the interfacial tension, thus facilitating the creation and stabilisation of a new interface and avoiding re-coalescence. Emulsifiers are molecules with an affinity for both the lipid and the water phase, which therefore accumulate at the emulsion interface (but the emulsifier is usually also soluble to some extent in one of the bulk phases). The type of emulsion that is promoted depends on the preferred phase by the emulsifier: a predominantly hydrophilic emulsifier stabilises an O/W emulsion, whereas a predominantly hydrophobic emulsifier stabilises a W/O emulsion (Dickinson, 1992). This phenomenon is known as the Bancroft rule (Bancroft, 1913). It applies not only to low molecular weight emulsifiers and single proteins, but also to supra-molecular emulsifying agents: O/W emulsions are stabilised by casein micelles and W/O emulsions are stabilised by fat crystals (also known as Pickering stabilisation; Pickering, 1907). In terms of required surface loading, stabilisation through protein aggregates or fat crystals is more efficient for smaller particles than for their larger counterparts as a result of their smaller volume to cross-section ratio. This effect was shown for the comparison between casein micelles and whey proteins by, for example, Pelan *et al.* (1997).

Food emulsions may contain three classes of low-molecular weight emulsifiers: non-ionic emulsifiers (e.g. saturated and unsaturated mono- and diglycerides) anionic emulsifiers (e.g. esters of monoglycerides with various food acids) and amphoteric emulsifiers (phospholipids, e.g. lecithin). Fat crystals could be classified as non-ionic emulsifiers, and proteins as amphoteric emulsifiers. The distinction is important, because it determines whether the emulsifier will retain its functionality if the emulsion is acidified. Non-ionic emulsifiers are not sensitive to changes in pH in the product.

14.3 Emulsion microstructure: processing

The major ingredients required for making a spreadable food emulsion were discussed above. However, the final product properties will also depend to a large extent on processing, which should give the desired product microstructure. The most critical aspects of spreads processing tend to be optimal emulsification and control of the structuring process (fat crystallisation for margarine and acidification for fresh cheese), and these will be discussed below more extensively.

Before doing so, however, it is important to mention that in practical situations, the desired product microstructure is just one of many considerations that play a role when designing a process. Requirements of microbiological safety, hygiene of process line and of the product during shelf-life necessitate processing steps such as pasteurisation, and restrictions on water droplet size distribution in non-preserved W/O products. Taste stability usually implies a constraint on extreme temperature conditions or residence times in order to avoid undesired chemical process in the formulation as much as possible. Finally, economic considerations favour industrial processes that involve relatively little complexity and that allow for high line throughputs.

The present section will focus on the microstructural aspects, however, as this is most related to spread texture. First, the main processing operations will be addressed (fat crystallisation, emulsification, acidification). Secondly, typical processes for the production of two products, margarine and fresh cheese will be discussed.

14.3.1 Fat crystallisation

Fat crystallisation in an industrial context is done in two ways. In applications where controlled crystallisation is not so critical (e.g. if maximum crystal size is already limited by droplet size, as is the case for many O/W emulsions), one can simply use a tubular heat exchanger or even a cooling tunnel. However, to achieve a good and well-defined margarine texture, controlled crystallisation is critical. Therefore, a precisely defined time–temperature profile is applied, for example in a scraped surface heat exchanger or 'A-unit' (in combination with other processing units). An A-unit consists of rows of scraper blades mounted on a rotating axis which is immersed in the liquid mixture to be emulsified. Cooling fluid, typically liquid ammonia, is pumped through an outer wall during the passage of the liquid emulsion. The typical diameter of the outer cylinder wall in a factory-scale unit is 50 cm, and the split between rotating axis and cylinder wall is about 1 cm. Typical rotational speeds and cooling temperatures are 200–500 rpm and –5 to –20 °C.

The crystalliser, also called C-unit, consists of a hollow tube with a row of pins on the inside. The rotor with pins mounted on it, is rotating at ~100–300 rpm. The C-units provide residence time for crystallisation of the fat that was supercooled in the A-units, and the crystal mass is worked in order to give the required product consistency/hardness. Crystalliser volume (~50–100 l) and rotor speed are crucial factors, both in relation to throughput of the process line.

Processing is used for W/O emulsions as the main tool to manipulate crystal sizes, mainly by balancing crystal nucleation and growth processes during crystallisation. This balance can be influenced through changes in supersaturation, typically by the degree of undercooling, or changes in flow conditions. Other means to stimulate nucleation, though less common in spreads manufacturing, are ultrasound radiation or seeding. In general it is favourable to steer processes towards small crystal sizes, since these are more effective in terms of structuring and result in quicker melting. Furthermore, Pickering stabilisation in W/O emulsions is most effective in terms of the utilisation of the crystalline fat if small platelets are present (Johansson and Bergenståhl, 1995b; Johansson *et al.*, 1995a,b). Oil exudation, either on increased temperature or external pressure, decreases strongly with decreasing crystal size. Crystal sizes above 30 μm are definitely to be avoided because crystals of these dimensions can be perceived orally as particles.

In processing, the formation of small crystals is primarily stimulated through the application of high driving forces, very quick cooling and high local shear rates. A peculiar consequence of such process conditions is the fact that at first dendrite-like crystals in the α polymorph grow on the cooling surface. By scraping the cooled surface these crystals are mixed back into the bulk phase and broken into many nuclei. In the subsequent steps of the manufacturing process these α crystals convert into β′ crystals in the bulk phase, given that sufficient residence time is provided in a crystalliser. A very important process parameter is the level of completeness of this transition while the product is still being sheared, because it determines the relative amounts of primary and secondary bonds in the structure, and thus the plasticity of the fat crystal network.

14.3.2 Emulsification

Since spreads are emulsions it is necessary to mix the dispersed phase thoroughly into the continuous phase using some sort of mechanical shearing equipment, usually a votator or a homogeniser. The best results are obtained if these processing units are fed with a coarse pre-emulsion (droplet size less than ~100 μm). The pre-emulsion is typically made under low shear from separate lipid and water phases in which the minor ingredients are added to the phase in which they dissolve.

The efficiency of emulsification depends predominantly on the power input during the emulsification process and to a lesser extent on the interfacial tension between lipid and water phases. Reduction of the dispersed liquid droplet size becomes increasingly more difficult for finer droplets, because the surface area in an emulsion with droplets of radius R increases as $1/R$. Emulsifiers are therefore added to lower the interfacial tension and to facilitate the emulsification process. Low-power mixers, e.g. shear mixers, can only be used to create a coarse pre-emulsion. The use of a colloid mill already results in a finer emulsion.

In factory-scale spread production emulsification is achieved in the A-units or by using homogenisers. In the A-unit the kinetic energy of the rotating blades is transferred into the liquid causing droplet break-up and a finer emulsion typically having an average dispersed phase droplet diameter of 5 μm. For W/O emulsions, small droplet sizes increase microbiological stability of the product dramatically (Verrips and Zaalberg, 1980; Verrips *et al.*, 1980). Droplet sizes can now be measured routinely through nuclear magnetic resonance (NMR) techniques in both W/O and O/W emulsions (van den Enden *et al.*, 1990; Goudappel *et al.*, 2001), or by static light scattering in diluted O/W emulsions (Gelin *et al.*, 1994; Bot *et al.*, 2003).

For a finer droplet size, it is necessary to apply much higher shear during processing using, for example, a high-pressure homogeniser. A homogeniser is in essence an orifice or a valve that blocks the fluid flow, causing intense shearing forces as the liquid is forced through the narrow gap, if necessary in combination with a suitable recirculation pump. Dispersed phase droplet sizes below 1 μm can be obtained with such equipment when operating at a pressure of above ~100 bar.

14.3.3 Acidification

Acidification of a water phase can either be achieved by fermentation or by addition of an acidulant. The latter process has the disadvantage that, usually, concentrated acid has to be mixed with a large batch of neutral mix, which requires precautions to ensure homogeneous mixing throughout the process. The addition of concentrated acid can be avoided through the use of glucono-delta-lactone, a slowly dissociating acid.

For most fresh cheese-type products, however, fermentation is preferred over chemical acidification because fermentation generates a wide range of taste and flavour components which enhance the dairy connotation. Fermentation can either be done by thermophilic microorganisms, which show optimal activity at ~40 °C, or by mesophilic microorganisms, which prefer temperatures of ~25 °C. Thermophilic organisms tend to give relatively acidic yoghurt-type flavours, whereas mesophilic organisms generate a wider, more subtle range of flavours. The latter process tends to require longer fermentation periods.

Fermentation is usually performed in a batch process, but alternatives have been considered. For example, it is possible to design a continuous fermentation process in which neutral mix is added continuously to the fermentation tank, and acid mix is tapped from the tank. However, such processes have not been introduced as a standard because in-line control of the pH in an industrial process is notoriously difficult, and because the stability of commercial multi-strain fermentation cultures over prolonged periods is not guaranteed as a result of competition among the strains.

Disadvantages of fermentation are the long periods required for acidification, the fact that the period required for fermentation is never completely predictable and the sensitivity of the fermentation cultures to phage infections.

14.3.4 Margarine processing

As stated above, emulsification and fat crystallisation occur simultaneously during margarine processing. The actual margarine production process starts with the preparation of a pre-emulsion from molten lipid phase and water phase. This pre-emulsion is pasteurised, and subsequently fed into the actual margarine processing unit, usually a votator (Hoffmann, 1989).

The votator consists of combinations of A-units together with B- or C-units, both of which allow crystallisation of the continuous fat network under either shear through viscous flow and optionally additional sieve plates in B-units (in practice additional piping) or through agitation in C-units (a pin-stirrer, kneading unit) (Bockisch, 1993). The combination of cooling and shearing of the supercooled oil phase in the A-unit is necessary in order to promote the formation of fat crystals around and between the water droplets that are a prerequisite for Pickering stabilisation, in addition to creating a small dispersed phase droplet size.

Since fat crystallisation is not instantaneous within the A-unit, some residence time is desirable during processing and thus the cooled margarine is given time in the B- or C-units to allow further development of the crystal network. This is

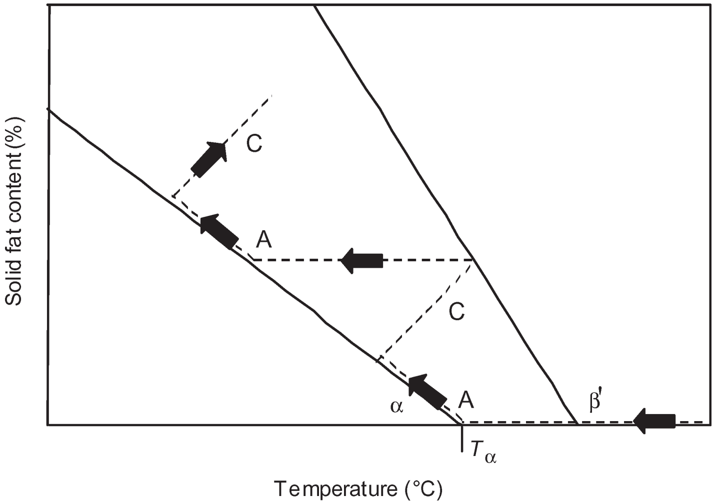

Fig. 14.3 Schematic representation, of the path of a crystallising fat during margarine processing conditions, in an SFC versus temperature diagram. The solid line labelled with α refers to the conditions under which α crystallisation occurs. The dashed path describes a system that is cooled below the α crystallisation temperature T_α in an A-unit. Subsequently, adiabatic recrystallisation to the β′ modification in a C-unit results in an increase in temperature, but new crystallites are formed as long as the system remains below the β′ crystallisation temperature. The A–C sequence is repeated once more in this diagram.

illustrated in Fig. 14.3, which shows an SFC-temperature diagram that describes a typical margarine manufacturing process. First, the mix is cooled until the α crystallisation point, T_α, at which crystallisation in the α modification occurs. Crystals are formed upon further cooling. After leaving the A-unit, adiabatic (re)crystallisation from α to β′ modification leads to an increase in temperature. Since it is usually impossible to extract all the thermal energy from the crystallising emulsion in a single A-unit, repeated cooling (A) and resting steps (C) can be combined, as is illustrated in Fig. 14.3 as well. The optimal process will depend always on the fat blend used, to give a final product with the desired final microstructure, plasticity and droplet size distribution. An example of the microstructure of a typical product is shown in Fig. 14.4.

As mentioned earlier, emulsifiers are used as processing aids and to confer properties to the final product. For W/O emulsions, typically a low molecular weight emulsifier of either a mono- or diglyceride of a fatty acid is used, or a lecithin (phospholipid). These hydrophobic fat-soluble emulsifiers quickly adsorb at the interface and thus lower the interfacial tension. Monoglycerides also help to stabilise the newly created water droplets by forming a shell around them which also facilitates contact with the stabilising TAG network.

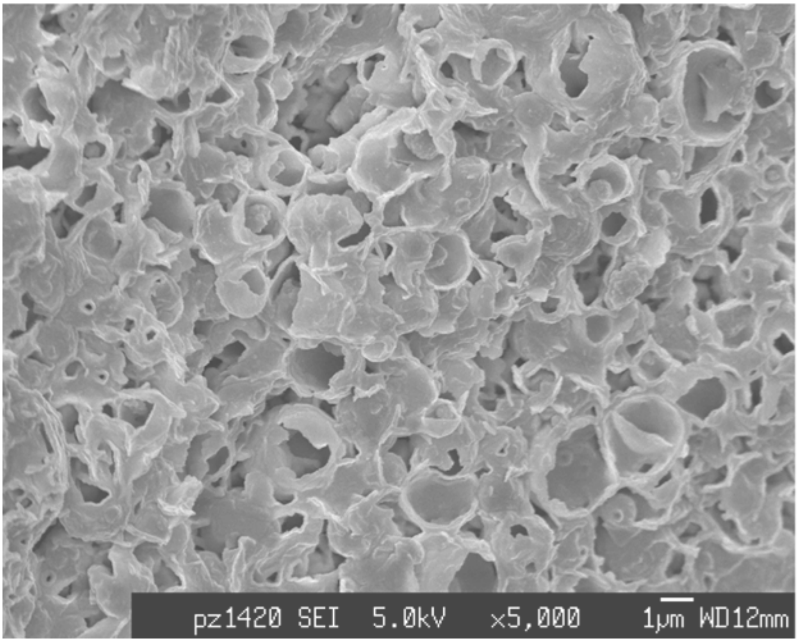

Fig. 14.4 Cryo-scanning electron microscopy image of a fat crystal network in a 60% fat-continuous spread; oil and water have been removed from the sample. (Image by P. Zandbelt, URDV.)

14.3.5 Fresh cheese processing

In broad outline, the traditional fresh cheese production process consists of the following steps: pasteurised, homogenised milk is destabilised through acidification via fermentation using lactic acid bacteria, which leads to aggregation of the milk protein. The aggregated milk protein is concentrated by separating a liquid phase through mechanical treatment (usually centrifugation followed by cutting of the concentrate). This concentrated phase, the curd, can be pasteurised to inactivate the lactic acid bacteria and is usually homogenised once more before the product is filled in the final packaging (Guinee *et al.*, 1993). Usually a sealed container is used for this purpose to increase shelf-life of the product. The production process is complex from a physicochemical point of view owing to the presence of two irreversible steps, milk protein aggregation during acidification and separation of the whey from the curd. In addition, acidification is performed by live organisms, introducing variability in the process.

The microstructure of a fresh cheese-type product can best be characterised as an acid protein-stabilised O/W emulsion, in which attraction between the proteins at the surface of the droplets causes them to aggregate into clusters of droplets. Surplus protein tends to aggregate onto these clusters as well, leading to a protein network around the protein aggregates. Intense flow (shear, elongation, turbulence),

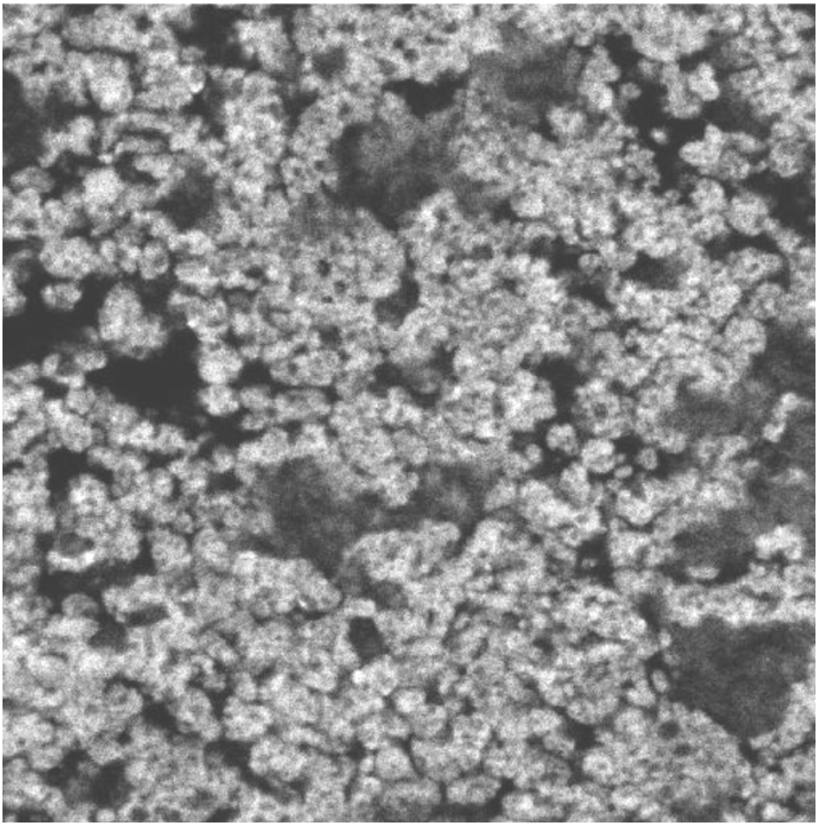

Fig. 14.5 Confocal scanning laser microscopy image of a protein-stained commercial fresh cheese. (Image by H. van Aalst, URDV.) The majority of the fat is in small ~1 µm protein-stabilised droplets, which is reflected in the coarseness of the protein structure. Image width 65 µm × 65 µm.

as for example applied during homogenisation, may lead to a (partial) collapse of this protein network onto the droplet aggregates. A microscope image of a typical commercial cheese is shown in Fig. 14.5.

Usually, the droplets in the fat–protein aggregates remain present as individual droplets, and thus the overall structure of the product can best be characterised as a composite, a protein matrix filled with fat droplets. The properties of the filler phase do affect the final product properties because there is an interaction between droplet and filler phase. These effects become more important at higher filler fractions. When the interaction between filler and matrix becomes less, the contribution of the filler phase to the hardness becomes less important (van Vliet and Dentener-Kikkert, 1982; Reiffers-Magnani *et al.*, 1999).

14.4 Spread stability during transport and storage

During storage, transport and use of spreads, a number of undesirable textural changes may occur. These changes are partly due to the fact that spreads are not completely in thermodynamic equilibrium when leaving the production line, and partly due to temperature fluctuations during storage and transport. These changes may result in product defects, caused by modifications in the fat crystal network, and emulsion instability. Some examples are listed below.

Margarine leaving the votator is still considerably supercooled, a significant portion of the fat crystallising quiescently (in the tub, i.e. under 'unworked' conditions). This may lead to the development of brittleness. Other defects may develop as a result of recrystallisation processes, which change the fat crystal network owing to polymorphic transitions and/or de-mixing of the mixed crystals. These processes typically take place slowly and can be stimulated by exposure to shear or increased temperatures. They result typically in reduced product hardness, coarser crystals, or the development of very distinct crystal agglomerates of sizes sometimes as large as millimetres.

Coarsening of the crystals in the fat crystal network may occur spontaneously or because of temperature cycling, e.g. during transport or during use, as a result of the lower surface free energy for bigger crystals. The phenomenon is known as Ostwald ripening (Ostwald, 1897). The process is greatly accelerated by temperature cycling, because the redissolution of crystallised fat in oil at higher temperatures followed by subsequent crystallisation at lower temperatures helps the transport of fat molecules from the smaller crystals to the bigger crystals.

The product stability is largely governed by the properties of the continuous phase. A W/O emulsion will not suffer from recoalescence unless the margarine is severely abused during storage, for example by extreme temperature cycling. The emulsion can undergo some initial destabilisation by Ostwald ripening since water is slightly soluble in oil and the difference in Laplace pressure between large and small droplets is enough to drive the process. However, such changes are minimal if process control (droplet size distribution) is good and a short tempering step is included in the storage step. For O/W emulsions, hydrocolloids are often used to thicken or gel the water phase to give stability against coalescence and creaming, or to reduce syneresis (i.e. exudation of the water phase). Very small dispersed phase droplet size can also provide stability to both these unwanted processes.

For a fresh cheese-type product, microbiological stability during storage and transport is usually the most critical property. To extend the shelf-life of the product before opening by the consumer ('closed shelf-life'), chilled transport and storage are preferred. Transport and storage conditions may also affect taste and (off-)flavour of a spread, for example by promoting oxidation processes in the product.

14.5 Analysing spread texture

The texture and stability of a spread are in general determined by the microstructure of the emulsion, which involves aspects such as the mechanical properties of both continuous and dispersed phases, the volume fraction and droplet size of the dispersed phase, and the properties of the interface between continuous and dispersed phase. A logical consequence is to consider the relation between microstructure and product properties under consumer use. First, one can consider the properties of the spread as it would be in the tub or during spreading. This involves properties such as firmness, spreadability and plasticity. Secondly, one can consider the behaviour of the spread in the mouth. This involves properties such as melting behaviour and emulsion destabilisation. Last but not least, one should consider product applications, which involves interactions with bread in the mouth. This is the most complicated and will be treated in a schematic way only.

14.5.1 Spreads in the tub and during spreading

The firmness of a spread is usually measured as the force needed to penetrate a standardised rod into the product, using a Stevens penetrometer or a Texture Profile Analyzer (de Bruijne and Bot, 1999). The firmness of high-fat margarine is primarily determined by SFC, and the strength and number of interactions between the particles in the fat crystal network. Keeping everything else the same, smaller crystals give firmer products, and processing is the tool to achieve this. For typical products, the rule of thumb is that the firmness increases roughly proportionally to SFC squared (Haighton, 1976), for a given process line at constant processing conditions. The proportionality constant depends strongly on the degree of formation and disruption of bonds between the crystals. Because SFC is a function of temperature, the relation also explains the strong decrease of the firmness with increasing temperature.

Firmness is indicative of the spreadability of a product, and can be modified in margarine through changes in the composition of the vegetable fat blend as a consequence of the relation between SFC and firmness. This flexibility is a clear advantage of a vegetable fat product such as margarine over a traditional product such as butter. In practice, the fat blend is adapted to the specific conditions needed for the application of the product. This is illustrated in Fig. 14.6 for a tub margarine that should be spreadable at fridge temperature, and for a wrapper margarine suitable for kitchen use. Very firm products are poor for spreading. This is illustrated by butter, which tends to be too hard for spreading when used directly from the fridge (although special spreadable butters are available from blends of butter fat fractions). For higher dispersed volume fractions, such as in low-fat spreads, the dispersed phase may contribute significantly to product firmness. This applies particularly to very fine emulsions (droplet size <5 μm), which tend to be firmer, and products containing water phase structured with hydrocolloids. In fresh cheese the properties of the dispersed phase are important as well. However, because the composition of

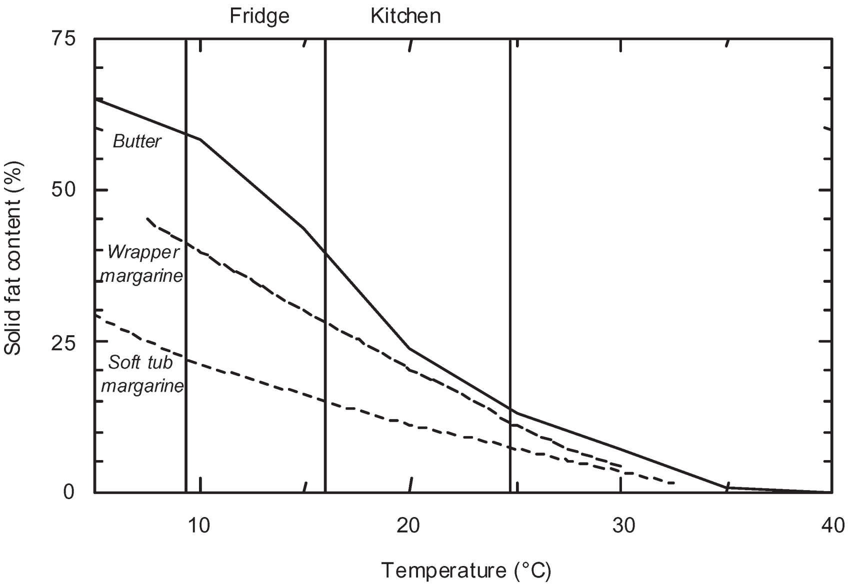

Fig. 14.6 SFC as a function of temperature for a number of spreads for two temperature ranges that are typical for their application. Since SFC is a reasonable indication of firmness, the graph indicates that the wrapper margarine has acceptable firmness at kitchen temperature, the tub margarine at refrigerator temperature. Butter is expected to be quite firm at refrigerator temperature, although differences in processing make a direct comparison somewhat difficult. Fresh cheese has the same N-line as butter, but is softer nevertheless because the amount of fat is lower than butter or margarine.

dairy fat is essentially fixed, the product properties can be changed most easily by changing the amount of dispersed phase, i.e. the amount of fat in the product.

Air as a filler phase also changes the product properties. Aeration of spreads with nitrogen (5–10% v/v) is sometimes applied to give the product a soft and smooth texture. Very low amounts (about 0.1% v/v) of non-dissolved air bubbles included during processing in firm margarine disturb the fat crystal network, giving rise to brittleness and sometimes torn structures.

The loss of firmness upon deformation is a criterion for the plasticity of a spread (Haighton, 1965), which is investigated in a large deformation test which imitates working and kneading (de Bruijne and Bot, 1999). If the stress required to deform the spread at large deformations is the same as at small deformations, the spread is considered to be plastic. If the stress at larger deformations is smaller, the material is considered to be work softening. Crystal networks with a high SFC, relatively large crystals and strong bonds between the crystals show more work softening than products with small fat crystals, and predominantly weak van der Waals bonds between crystals. Depending on the molecular composition of a margarine fat matrix it is found that after kneading of a product and subsequent relaxation time, up to 60% of the original product hardness is lost. Butter approaches the desired plastic deformation better than margarine, and work softening is therefore often used as a criterion for butter-likeness.

A product property that is only indirectly related to firmness is exudation of the continuous phase. Water exudation from an O/W product (also known as syneresis) and oil exudation from a W/O product are very similar phenomena, with explanations involving the viscosity of the exuding phase and the geometry of the continuous phase through which it flows (Lucey *et al.*, 1997; de Bruijne and Bot, 1999; Mellema *et al.*, 2002). The mechanisms promoting exudation of the continuous phase are thought to be rearrangements of the structuring agent in the continuous phase ('endogeneous' exudation) and buoyant forces on continuous phase (caused by density differences between exuding phase and network). In specific geometries, drainage is a possible mechanism too, e.g. when a product is positioned on a grid. Since all these exudation phenomena involve flow through a porous network, it is possible to describe these phenomena – at least qualitatively – by models based on the Darcy equation (Darcy, 1856; Bird *et al.*, 1960). Important parameters in such a description are the magnitude of the force driving exudation, the time over which exudation takes place, the surface area through which exudation occurs, the viscosity of the exuding fluid and the geometry (esp. porosity and pore size) of the microstructure through which the fluid flows. For margarine, oil exudation is sometimes tested by stacking (wrapper) product and evaluated by the observation of either the critical duration or stacking height for which exudation does not occur.

14.5.2 Textural changes during mastication of spread

The fate of a food emulsion may involve it being eaten without any further processing, as in the case of ice cream, but more often the emulsion has to be applied to some other host foodstuff, as in the case of margarine or mayonnaise. This involves processes such as spreading or topping. For emulsions with a plastic rheology, spreading does not tend to change the emulsion structure dramatically, unless the volume fraction of the dispersed phase is very large and spreading induces coalescence of the emulsion droplets. Low-fat spreads may suffer from this defect unless the water droplets are structured with biopolymers to retard re-coalescence under shear.

Processing in the mouth will result in more dramatic microstructural changes. First we will consider the behaviour of a food emulsion in the mouth during mastication when it is used pure, i.e. not on bread. In this situation, a typical piece of food emulsion undergoes a chain of events as sketched below, although some stages may occur simultaneously or may be unnecessary for a specific food emulsion (de Bruijne and Bot, 1999; Bot and Pelan, 2000).

The *comminution* of a food emulsion in the mouth is largely mechanical and involves the formation of millimetre-size pieces by the action of the teeth. The efficiency of the process is known to vary widely in duration and intensity among individuals.

The *softening* stage involves making the pieces soft enough for efficient break-up in the subsequent stage. The mechanism (temperature rise, saliva uptake) depends on the type of food emulsion, but some general observations can be made. The first observation is that the appropriate main mechanism to soften a food emulsion depends strongly on the structural property that is responsible for the hardness of the product. If the continuous phase of the emulsion is solid, it should become liquid; if the effective dispersed volume is above the close packing fraction, it should be dispersed. Softening of the pieces nearly always requires transport, for example of heat or of saliva.

Softening of a margarine involves heat transport from the mouth to the product, which contributes to both heating of the spread and melting of the fat crystals. For a given heat flow from the mouth to the product, softening of the product will be delayed somewhat by the presence of crystallised fat. Therefore products containing more crystallising fat will be perceived to melt a bit slower than products containing more oil. For relatively steeply melting fat blends, such as those containing coconut fat or palm kernel fat, this may give rise to perception of coolness. Complete melting of the fat phase will result in a very thin product, but TAGs that do not melt at or below mouth temperature may cause a residual thickness in the mouth. Such differences can be demonstrated also in viscosity measurements in which a typical temperature profile in the mouth is imitated.

Softening of fresh cheese products rely to a larger extent on dispersion of the product in saliva, although the product consistency reduces considerably as a result of the melting of fat at mouth temperature as well. A completely molten fresh cheese will be perceived to have much more residual thickness in the mouth than a margarine.

When the emulsion has softened sufficiently to a yield stress below ~50 Pa, the (intermittent) stress level in the oral cavity tends to be high enough to allow further *break-up* of the pieces in the elongational flow of saliva in the mouth (de Bruijne *et al.*, 1993). Note that the validity of this statement is restricted to cases in which the food emulsion is broken down to a liquid: the stress level in the mouth will be much higher than 50 Pa if other food components thicker than saliva are present in the mouth.

The break-up of a food emulsion can be very important for flavour release. For flavours that are soluble in both the lipid and water phases, diffusion should be fast enough to allow flavours to move from the product to the saliva within the typical residence time in the mouth.

The release of water-soluble taste components such as salt and acid is determined primarily by the rate of mixing of the water phase with the saliva, which explains why the taste experience for an O/W emulsion such as a fresh cheese is so much different from that of a W/O emulsion such as a margarine. Mixing occurs through rapid dilution and diffusion processes for O/W emulsions, whereas W/O emulsions require an intrinsically slow coalescence process. The presence of protein in a margarine, which acts as a O/W emulsifier, accelerates the coalescence process considerably. Margarine product development involves balancing the hydrophobic emulsifiers needed for processing and their hydrophilic emulsifiers contributing to good eating qualities. Finally, it is important that coalescence is not prevented by any structuring agents present in the water phase (hydrocolloids or gelatin). The mouthfeel of a fat-containing emulsion depends also on the properties of the fat and water phases at mouth temperature after break-up of the emulsion.

Secondly, we address the behaviour of a spread when it is applied on bread. Although on a microscopic scale many of the processes described above for pure food emulsions still occur, the ultimate outcome is quite different. Above, a situation was described in which a semi-solid material was transformed in the mouth into a thin liquid. However, this does not happen when a spread is eaten in combination with bread.

Food such as mixtures of bread and spread cannot be diluted into a liquid in the mouth and form a so-called bolus, an aggregate of small comminuted particles stuck together by cohesive forces (Heath and Prinz, 1999). Bolus formation ensures that when the food mass is swallowed, it will pass the pharyngeal region safely without risk of inhaling small particles into the lower respiratory tract. The properties of the bolus can be controlled by the average food particle size, and by the relative amounts of food and saliva. Smaller particles will lead to greater cohesiveness (Prinz and Lucas, 1997), and excess saliva will significantly decrease the viscosity of the dispersion (cf. Bot and Pelan, 2000). These parameters can be controlled by varying the time during which the bolus is formed, and by modifying the pressure applied by the tongue to compact the bolus. The optimum moment for swallowing is thought to coincide with the maximum cohesive force between food particles (Prinz and Lucas, 1997), which in turn can be expressed as criteria for particle size and lubrication (Hutchings and Lillford, 1988; Lillford, 2000).

During the later stages of mastication, much of the microstructure of a food emulsion is already broken down, and it is largely the individual ingredients of the spread that play a role. These ingredients are more likely to affect cohesiveness of the bolus than the particle size of the food. Oil, which is not absorbed much by the bolus, may be more effective in lubricating dry foods such as bread than aqueous agents. Thus, swallowing of bread is greatly facilitated by the presence of a spread such as margarine.

14.6 Future trends

While the use of traditional margarine seems to be declining, a number of new trends are emerging. The growth of the ‘functional foods’ market segment stimulated traditional margarine manufacturers to develop this market further. As a result, cholesterol-lowering spreads have been introduced, containing phytosterols or phytostanols. These products can be seen as the next generation healthy margarines, building on the products containing high levels of polyunsaturated fatty acids that have been on the market for 40 years now. Another recent development is the fortification of margarine with minerals such as Ca and vitamins. Healthy zero-fat spreads appear to remain niche products.

Another trend involves increased convenience, such as in liquid margarine for kitchen use: tailor-made products that are intended for specific applications in baking and shallow frying. Furthermore, the distinction between various product categories seems to be vanishing slowly. The introduction of sweet W/O spreads and fresh cheese taste varieties move the typical 'underlayer', a spread used to adhere, e.g. marmalade to bread, in the direction of a 'top layer'.

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15

Factors affecting texture of ice cream

C. Trgo, Danone, France

15.1 Introduction: the structure of ice cream

The basic composition of typical ice cream consists of the following components: 10–17% milk fat, 8–11% fat-free milk dry mass (lactose, proteins, mineral salts), 13–17% sugar, 0.2–0.5% stabilizers/emulsifying agents and the main component water. During the freezing procedure of this mixture in production a complex structuring process takes place. Apart from freezing the water, the impact of air with the associated formation of foam is of much importance. The model represented in Fig. 15.1 shows the arrangement of the individual components in the finished ice cream. The ice crystals are suspended with average diameters of approximately 40 μm in an aqueous remainder solution. The water-soluble materials (sugar, salts, proteins) are concentrated in this non-frozen remainder solution. Air is present in the form of finely divided bubbles with diameters in the range between 40 and 100 μm. At its boundary surfaces they are partly covered with fat. The volume percentage of air in ice cream amounts to about 50%.

Min (1994) showed that the ice crystal size distribution can be regarded as a reliable standard for evaluating the sensory quality. In his test findings ice cream with average crystal diameters larger than 56 ± 21 μm in number size distributions were felt as rough. The structure stability and the melting behaviour represent further important aspects of quality. An unstable foam structure also has a clear influence on the sensory occurrence of the ice crystal structure. Rohenkohl and Kohlus (1999) show that the rheological behaviour, which is determined particularly by the air bubble size distribution, clearly influences the mouthfeel.

It is of great interest to the ice cream manufacturer to understand the procedures

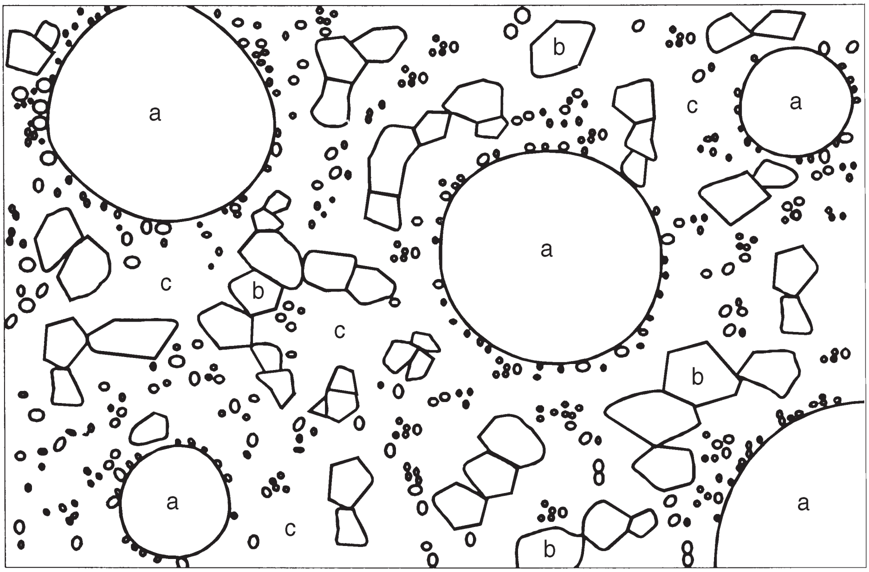

Fig. 15.1 Model of ice cream structure: (a) air bubbles; (b) ice crystals; (c) non-frozen solution with fat globules. (After Timm, 1985.)

during the structuring as well as to know the decisive, influencing factors for stabilizing texture. Owing to the significance of texture this chapter will deal particularly with the principles of the formation of ice crystal distribution as well as of the foam structure.

15.2 Influences on textural properties: ice crystallization

The manufacturing of ice cream begins with preparing a mixture, containing as main components water, fat, fat-free milk dry matter, sugar, emulsifying agents and stabilizers. This mixture designated as an ice cream mix is beaten by a rotating dasher with air into a micro-foam structure. Simultaneously the mix is frozen in the scraped surface heat exchanger with high dasher rotational speed and attached surface-scraping knives. In this rapid freezing procedure, in the so-called freezer, minute ice crystals are forcibly formed, while being distributed extremely finely between the air bubbles, in fat and in a very viscous, non-frozen remainder solution. The ice cream is taken from the freezer at temperatures between –3.5 and –7 °C and has a soft-ice-like, plastic consistency. The product is filled in. Afterwards it continues to freeze in a deep temperature freezing process, usually in freezing tunnels or in plate freezers, down to a core temperature of at least –18 °C.

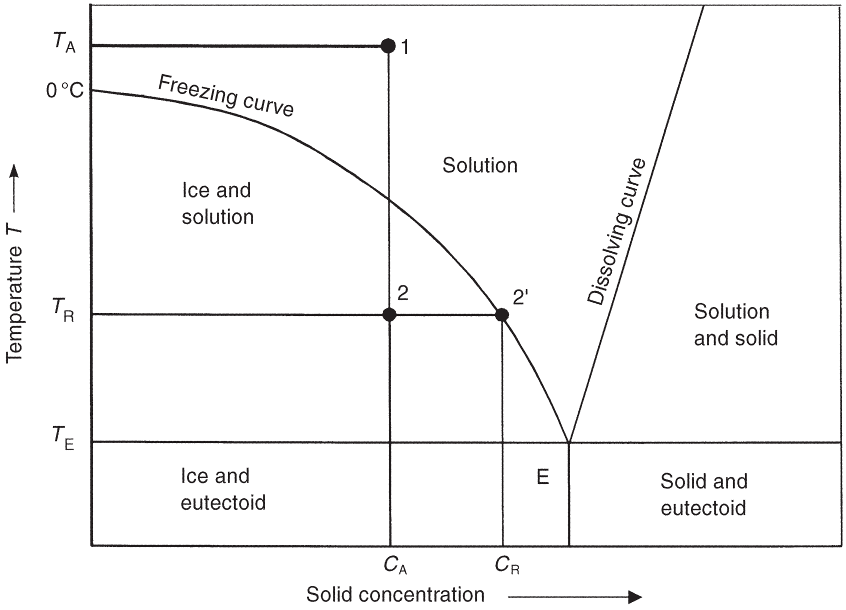

Fig. 15.2 Phase state diagram of a liquid solution.

15.2.1 Phase transition of water

The liquid/solid equilibrium is usually described by a temperature–concentration diagram, as Fig. 15.2 shows for a solution with one solved component. The thermodynamic equilibrium between the pure ice phase and the concentrated remainder solution gives the freezing curve. If a solution with the initial concentration *A* and the initial temperature T_A (condition 1) is cooled down to the temperature T_R (condition 2), then a pure ice phase and a concentrated remainder solution with the concentration *R* (condition 2′) develop. The cooling can be carried out to the eutectic temperature T_E. Below this point, specific to the respective component, the solution is supersaturated, and the ice as well as the solved material crystallize at the same time. Harz (1987) could show that the phase behaviour of many aqueous solutions corresponds to the qualitative diagram shown in Fig. 15.2. However, it has to be taken into account that the crystallization of some solved components, in particular carbohydrates, is kinetically restrained. No possible formation of mixed crystals was observed by Harz.

In solutions that contain polymers, e.g. proteins, no eutectoid arises, since these materials do not crystallize. Thus, a small part of supersaturated solution remains. This concentrated, but still liquid phase solidifies below a certain temperature T_g to a glass. Glass is an amorphous solid, in which the molecules are not arranged in a crystal lattice, but rather presented in a distribution typical for a liquid. The transition to the glass-like condition arises when the viscosity of the liquid phase becomes so high that diffusion of the water molecules is no longer possible. During sufficient cooling in systems with kinetic inhibition, i.e. the eutectoid has not formed yet, despite falling below the eutectic temperature, the viscosity of the remainder solution becomes so high that this solution solidifies to a glass. The glass transition temperature for ice cream lies between –23 and –45 °C depending upon composition (Goff *et al.*, 1993).

The beginning of the phase change of water to ice is characterized by the ice crystallization. Mullin (1993) differentiates between primary homogeneous nucleus formation, primary heterogeneous nucleus formation as well as secondary nucleus formation in regard to inducing the mechanisms. Homogeneous nucleus formation is the spontaneous precipitation of nuclei from an ideally pure solution, when the undercooling, from the standpoint of fusing temperature, exceeds a critical level. When the nucleus formation is catalysed by the presence of foreign nuclei, one speaks of heterogeneous nucleus formation. Furthermore nuclei, which originate only from the undercooling supply, are primary nuclei in contrast to the secondary nuclei, which are induced by the crystals themselves. Since the secondary crystallization represents the most favourable, energetic way of forming crystals and clearly requires less undercooling, this mechanism is likely to dominate during the ice crystallization in the freezer after the formation of the first incipient crystals. Books by Franks (1982), Hobbs (1974) and Mullin (1993) give an overall view of these theories.

The second phase of the crystallization consists of the growth of the produced nuclei by accumulating further crystal building blocks. The velocity of this process is indicated in literature as linear crystal growth rate.

Since a polycrystalline structure does not represent any favourable, thermodynamic status, the recrystallization begins after the development of ice crystals. The recrystallization appears where molecules in the crystal lattice change their lattice sites. Here there are modifications into number, size, form, orientation or perfection of the crystals. According to Fennema (1973) the recrystallizing mechanisms prevailing in food are an isomass, a migration and an agglomeration process. In the isomass process the crystals are rounded off to a spherical form during storage. The migration process is, at constant temperatures, the result of different solubilities of large and small crystals. The small crystals with their smaller radii of curvature cannot bind their surface molecules as firmly as their large crystals. When crystals come into contact by growth, the touching ice crystals grow together to form a large ice crystal. This is called agglomeration.

Temperature fluctuations, which lead to melting and refreezing, likewise affect the ice crystal size distribution. Therefore Hartel (1998) also ranks these phenomena among recrystallization. If the temperature rises, then the ice crystals melt and the smaller ice crystals can dissolve completely. If the temperature sinks again, the water freezes preferentially on the larger crystal.

15.2.2 Process influences on ice crystal sizes

The process in the freezer represents the relevant step of the formation of ice

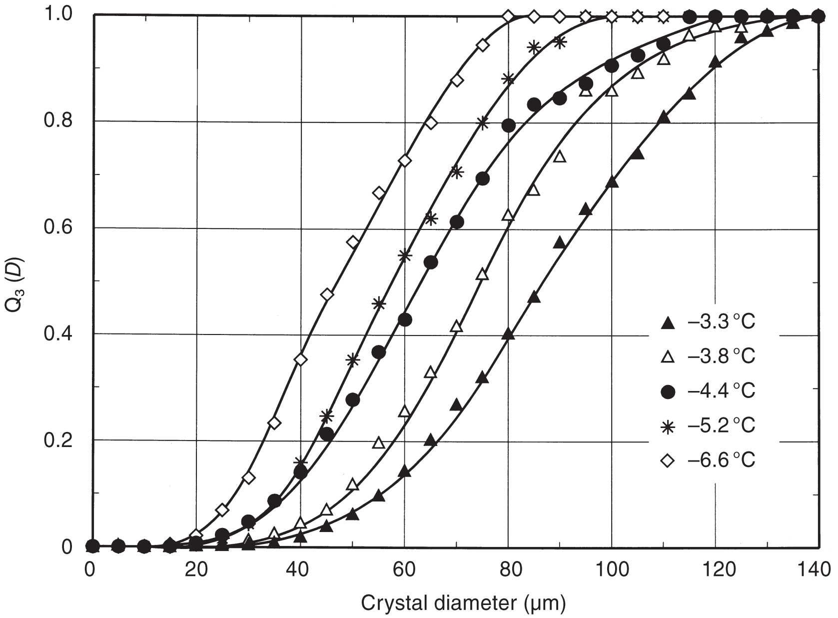

Fig. 15.3 Q_3–ice crystal size distributions of hardened ice creams which were filled in containers at various drawing temperatures (Trgo, 1996).

crystals, as experimental studies from Windhab and Bolliger (1998) show. During the hardening of ice cream the formation of new ice crystals is no longer expected. In the ice cream freezer a polycrystalline structure of minute crystals is formed. Owing to the intensive radial and axial mixing and, from that, the resulting dispersion of temperature, the crystallization takes place not only on the freezer wall, but also in the core current. The newly formed crystals are clearly smaller than the sizes observed at the freezer outlet. Trgo (1996) shows that the determining mechanism is already in the initial stage of freezing the recrystallization, which is strongly dependent on temperature. The higher the temperature is, the faster the crystals recrystallize.

Since recrystallization of crystals is more intensified at high temperatures, the temperature of the ice cream at the freezer outlet clearly influences the ice crystal size distribution. Consequently the process of hardening the ice cream does not only have to pass through a higher temperature range, but, more important, more warmth from crystallization has to be dissipated. Thus the residing time within the critical growth range increases, resulting in an enlargement of the ice crystals. Figure 15.3 clarifies this with ice cream samples which were filled in containers at various drawing temperatures and then hardened.

Discrepancy exists in literature concerning the influence of the revolutions of the dasher, which provides for intensive mixing in the freezer. While Windhab and

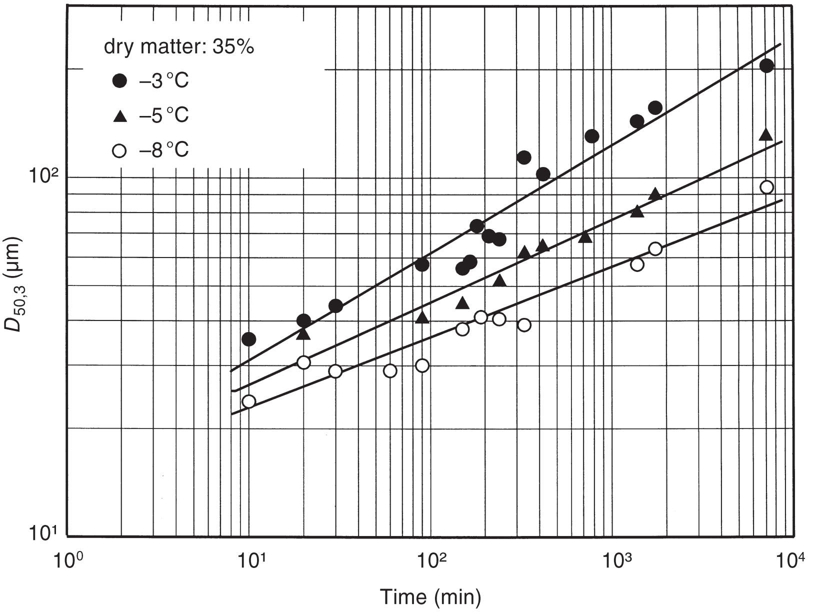

Fig. 15.4 Ice crystal medians $D_{50,3}$ of ice creams which were stored after freezer outlet at different temperatures (Trgo, 1996).

Bolliger (1995) determined that there are smaller crystals by increasing the rotation, Trgo (1996) and Rohenkohl (1997) could not observe significant effects by varying the number of revolutions. Cebula and Russell (1998) found out that there are even larger crystals with an increase in the number of rotations. They attribute this to the associated higher energy entry, which leads to an increased partial melting of the formed crystals. These discrepancies in the observations are because of the authors' different experimental sequences. The biggest difficulty is that neither the shear stresses on the ice crystals nor the diffusion in the ice mass in the freezer can be directly compared. Koxholt's (2000) experimental studies show that longer storage periods in the freezer lead to larger ice crystals.

After the partial freezing in the freezer and then filling, the ice cream undergoes a hardening process. Up to 80% of the water freezes out. The ice cream achieves temperatures down to –40 °C. Figure 15.4 shows the importance of the rapidity of hardening. The growth of the ice crystal medians $D_{50,3}$ from standard ice cream is represented in the range between 10 minutes and 7 days after passing the freezer outlet in double logarithmic plotting for temperatures of –3, –5 and –8 °C. The straight lines correspond to the respective regression lines. The diagram shows that apart from temperature, the recrystallization also depends on the size of the crystal. Growth slows when the size of the medians increases.

In order to attain an optimal end-product structure, it is necessary to strive for short storage in the freezer at very low temperatures as well as a rapid hardening.

15.2.3 Changes of the ice crystal matrix during storage

It is generally accepted that the texture of ice cream undergoes no damage in storage at temperatures below –18 °C. However, a clear influence is attributed at higher temperatures. Because of recrystallization the ice crystals can reach sizes which are sensorially felt as rough. Different authors describe mathematically the size increase of the ice crystals caused by recrystallization. The rate of recrystallization is slower at very low temperatures.

Min (1994) determined a power function:

$$x = kt^n \tag{15.1}$$

for the dependency of the ice crystal size x on time t (k constant, n reaction order). A kinetic reaction analysis according to Arrhenius resulted in an activation energy of approximately 62 kJ mol^{-1}. Trgo's kinetic reaction reflection (1996) is based on the recrystallization theory from Burke and Turnbull (1952) with the equation:

$$D^2_{50,3} - D^2_{50,3(0)} = 2kt \tag{15.2}$$

whereas $D_{50,3}$ is the median and $D_{50,3(0)}$ the median at time $t = 0$. The plotting of the natural logarithm of the kinetic constant k over the inverse absolute temperature in the Arrhenius diagram furnishes an activation energy of 225 kJ mol^{-1} for the temperature range above –8 °C. According to Trgo (1996) the recrystallization rate within this range is not determined by diffusion, but rather by reactions on the crystal surfaces. Sutton *et al.* (1994) also came to the conclusion through their model experiment that under certain circumstances the incorporation of water molecules into the existing crystal lattice is the limiting reaction. In Sutton *et al.*'s (1996) further kinetic reaction experiments the Williams–Landel–Ferry kinetics furnished quite good results. In their kinetic approach the driving force is the temperature difference between the glass transition and the actual storage temperature.

Changes in the sizes of ice crystal also occur during temperature fluctuations in storage, the so-called heat shocks (Caldwell *et al.*, 1992a,b). Donhowe and Hartel (1996) show the mechanism by microscopic photographs. Some of the small crystals melt during a temperature rise completely. The water refreezes on the rest of the larger crystals, when a cooling process follows. Trgo's experiments (1996) showed that with temperature variations in the cold storage chain of ice cream the total growth is composed of the respective growth phases additively.

15.2.4 Influences of ingredients on ice crystal sizes

Modifying milk dry mass by withdrawing or adding water influences the freezing point and the frozen quantity of water. Despite different dry masses of up to 20% in the ice cream mixtures tests Trgo *et al.* (1999) showed that at constant temperatures the recrystallizing rates are alike in the critical growth region, i.e. above –8 °C. It is of importance that pure ice crystals form in the complex ice cream system. The non-aqueous components are concentrated in the non-frozen water. In this remainder solution the concentration of the solved components that are mainly responsible for the freezing point is only temperature-dependent. Since the remainder solution is in equilibrium with the freezing point, the concentration of the solved components is irrespective of the original ice cream mix (see also Fig. 15.2).

Trgo *et al.* (1999) made the same observations by adding sucrose at the change of freezing point. Despite the shift of the freezing point to 3.3 °C in the mixture no change in the crystal growth was determined. Therefore it can be concluded that the temperature and not the concentration determines the recrystallization. It is important for production that a low freezing point with a constant-holding freezing level furnishes lower outlet temperatures and faster hardening. Consequently the ice cream has smaller ice crystals.

Buyong and Fennema (1988), Rohenkohl (1997) as well as Trgo *et al.* (1999) could determine no influence of air bubbles on the ice crystallization. The problem in these experiments was that the air bubbles, just like the ice crystals, are not constant in size. Electron micrographs from Caldwell *et al.* (1992b) clarify the increase in size of the air bubbles in ice cream. Because of the air bubble instability, the gaps between the bubbles, where the partially frozen solution is, achieve orders of magnitude that clearly exceed the ice crystal sizes.

Despite their small quantity in the recipe, stabilizers find special importance in literature as an influencing factor on the ice crystal sizes. By using stabilizers the icy mouthfeel decreases noticeably. This is often attributed to a reduced crystal growth. Min (1994) as well as Sutton and Wilcox (1998a,b) concluded that hydrocolloids represent an important aid in stabilizing the crystal structure. However, recrystallization processes have no connection with the viscosity of the freezing system.

According to Sutton and Wilcox (1998a,b) certain hydrocolloids could adsorb on the crystal surface. Thus they disturb water molecules integrating into the crystal lattice. It is assumed that this effect also occurs in so-called antifreeze proteins, which are present in Antarctic fish (Fenney and Yeh, 1998).

15.3 Influences on textural properties: foam stability and melting behaviour

The ice cream mix in a non-frozen condition represents an oil-in-water emulsion, in which sugars and salts are solved. Milk proteins and stabilizers are solved colloidally. Minute air bubbles are usually whipped in the freezer. The volume percentage can amount to 50% or more depending on the manufacturing process. The ratio between whipped air and the mix is characterized by the so-called overrun, which is calculated as follows (ρ stands for density):

$$\text{Overrun (\%)} = \frac{\rho_{\text{ice mix}} - \rho_{\text{ice cream}}}{\rho_{\text{ice cream}}} \times 100 \qquad [15.3]$$

15.3.1 Physical factors influencing foam stability and melting behaviour

Owing to the surface tension σ, the Laplace pressure p_k is higher in smaller gas bubbles than in larger ones. The following equation can be described when a spherical interface with the diameter d is assumed:

$$p_k = \frac{4\sigma}{d} \qquad [15.4]$$

The gas of a smaller bubble solves and diffuses into a larger one of two neighbouring different-sized bubbles. Thus, the large bubble grows at expense of the smaller one with the radius r. Provided that the radius of the larger gas bubble is to be regarded as infinitely large and the gas transport takes place through the liquid diffusively, this procedure can be represented by the following equation:

$$r^2 = r_0^2 - \left(\frac{4RT}{p_0}\right) \times \left(\frac{DS\sigma}{\delta}\right) t \qquad [15.5]$$

In this equation r_0 stands for the diameter of the gas bubble at time $t = 0$, R for the general gas constant, T for absolute temperature, p_0 for atmospheric pressure, D for diffusion coefficient of gas in liquid, S for solubility of gas in liquid and δ for thickness of the liquid layer through which diffusion takes place. Thus, monodisperse distribution of gas bubbles is favoured for a stable foam. Furthermore, a less soluble gas would slow down the diffusion. In addition to the so-called Ostwald ripening, the drainage, i.e. liquid draining from the foam structure owing to the force of gravity, represents a further destabilizing factor. When the drainage is approximately regarded as a flow of spheroids, the respective flow of mass m over time t through a sectional area A, according to Kessler (1996), is described by the following equation:

$$\frac{m}{t} = \frac{A\Delta p\rho}{23\eta} \times \frac{1}{\xi} \qquad [15.6]$$

ξ stands for flow resistance, ρ for density and η for viscosity of liquid when draining. The difference in pressure Δp corresponds to the hydrostatic pressure due to the gravitation in the liquid column. If the air bubbles and ice crystals are assumed to be seen as equal-sized spherical forms with diameter d, the flow resistance ξ can be calculated as follows:

$$\xi = \frac{9}{4} \times \frac{(1-\varepsilon)^2}{\varepsilon^2} \times \frac{\mu^* h}{d^2} \qquad [15.7]$$

Herein ε is porosity, e.g. the percentage of volume of the non-frozen solution in the ice cream foam, μ stands for the length of distance of the bubbles and ice crystals flowing around and h is the height of the flowing liquid. The following statements can be derived from Eqns 15.6 and 15.7:

- The driving force Δp rises with increasing density.
- The drainage rate sinks with increasing viscosity.

- Higher percentages of the volume of gas lead to decreasing drainage, when the diameters of the bubbles remain the same.
- Smaller air bubbles and smaller ice crystals in diameter lead to slower drainage.
- Longer flow ways cause slower drainage.

Ice cream is primarily stabilized by frozen water and the associated high viscosity of the non-frozen remainder solution at storage temperatures below –18 °C. By supplying heat the ice crystals begin to melt. This leads to a reduction of viscosity of the non-frozen remainder solution. At the beginning of the drainage, the ice crystals contribute to the flow resistance. They become less resistant with increased melting. The lamellas get wider and the drainage of the serum is promoted. Therefore, ice cream with larger ice crystals melts faster. If the air bubbles are sufficiently stabilized, the drainage is brought to a standstill by decreasing hydrostatic pressure and by narrowing drainage canals.

15.3.2 Functionality of milk fat, emulsifiers and milk proteins

Milk fat is considerably involved in structuring foam. During freezing and whipping in the freezer's cylinder, the fat globules are exposed to a shear force by the rotation of a dasher. The fat particles burst like a shattering eggshell giving a partial coalescence of fat globules. Keeney (1958) has already pointed out that a certain portion of fat emulsified in the ice cream mix should be destabilized, in order to get a good texture. Experiments made by Berger and White (1971) confirm this so-called fat destabilization as an influencing factor for the dry appearance, the creamy mouth feeling, the melting behaviour as well as the ice cream shrinkage. Electron micrographs (Goff *et al.*, 1999) show how the air bubble boundaries are occupied by fat globules and fat agglomerates.

Homogenizing is essential for ice cream production. Without good homogenizing, the fat globules would coalesce uncontrollably in the freezer. The ice cream would exhibit a crumbly texture with large air bubbles. In addition, there is a risk of the milk fat turning to butter. When homogenizing, milk proteins are built into a newly formed fat globule membrane. These proteins lead to an increased stability of the fat globules. However, a partial coalescence of the fat globules is necessary for developing a stable foam. Goff and Jordan's tests (1989) show that neither ice crystallization nor shear stresses are able to cause such a desired partial fat destabilization by themselves. In order to lower the stability of the fat globules in the freezer, the emulsifiers are of special importance (Berger, 1997; Bolliger *et al.*, 2000a,b). The emulsifiers, which are added to the mix before homogenization, deposit themselves beside the proteins into the newly developing fat globule membrane and partly weaken them. Thus, the emulsifying agents promote the emergence of fat globule aggregates during the whipping procedure.

The results from Rohenkohl and Kohlus (1999) show that variations of shear stress in the freezer lead to different destabilized fat globules. Thus they affect the distribution of the sizes of the air bubbles. In the conventional ice cream process, lower drawing temperatures cause smaller air bubbles.

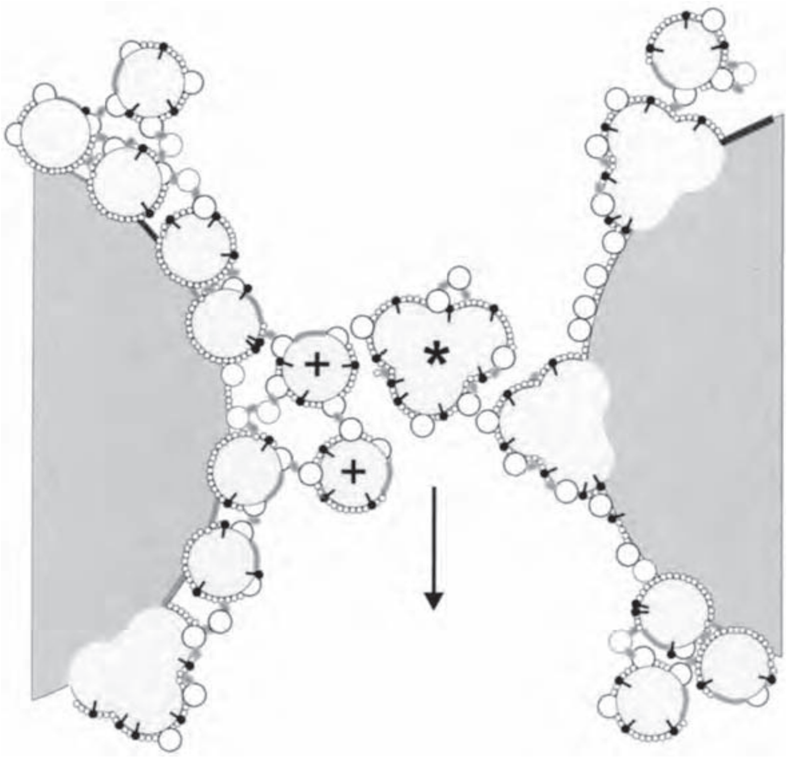

Fig. 15.5 Model showing foam stabilization during melting of ice cream (* fat globules; + further aggregated fat globules) (Koxholt, 2000).

Koxholt (2000) developed a model showing foam stabilization of the ice cream structure with fat globules and fat aggregates (see Fig. 15.5). Depending on the extent of fat destabilization, the boundary surfaces of the air bubbles in the ice cream are more or less stabilized through partially destabilized agglomerated fat globules (FA). There are regions nearby which are covered by intact fat globules and proteins. Further fat globules can be aggregated with calcium bridges between the caseins. This process also takes place in destabilized fat globule agglomerates through the membranes of the proteins in the serum. This coat of fat globules covered with proteins stabilizes the air bubbles.

The stabilization of the foam structure is dependent on the sizes of the fat globules and fat agglomerates. Koxholt's (2000) experiments show that above a critical diameter of fat globules (aggregates) ($D_{50,3}$ = 0.85 μm) the melting rate slows down the drainage rate. Figure 15.6 clarifies this. The maximum melting rate in % per minute corresponds to the maximal gradient of the ascending melting curve. Fat globules and fat agglomerates clog the foam lamellas and, therefore, slow down the drainage of the serum. Moreover, these globules and agglomerates

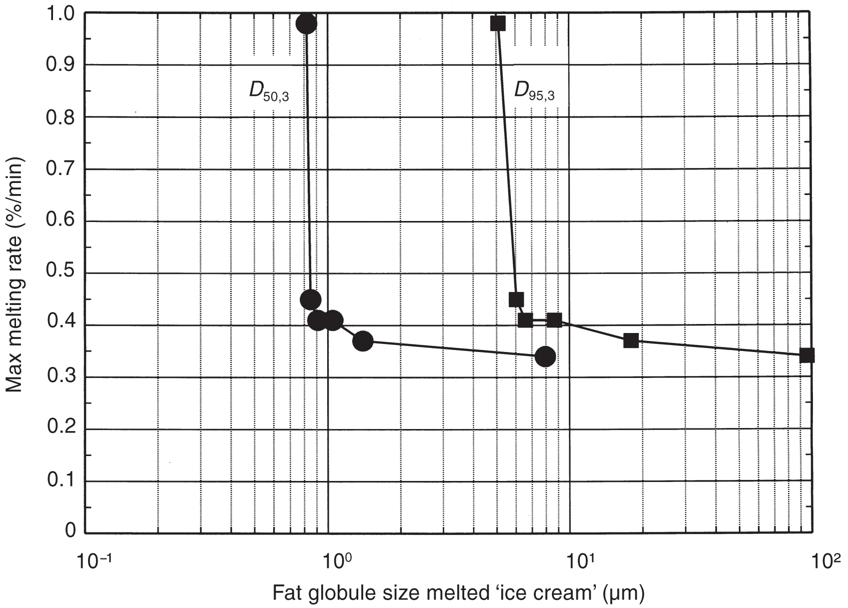

Fig. 15.6 Maximum melting rate of ice cream dependent on fat globule size (Koxholt, 2000).

form bridges between the air bubbles. They support one another and become a stable melting residue. Fat globules and fat agglomerates, which are smaller than the width of the lamella, flow off in the serum in the test. If the portion of small fat globules and small fat aggregates is too large, the ice cream is unstable and melts off quickly.

According to Koxholt's experiments (2000), stabilizing foam structure with particulated whey proteins happens in the same way. They block the foam lamellas with a size that corresponds to the width of the lamella. The effective diameter of the particle lies in the range of the critical diameter of the fat globule.

15.3.3 Stabilizers

Blends of different stabilizers find use mostly in ice cream. The specific characteristics of each component and their interaction are used. The components are usually polysaccharides, originating from plants or substances from them such as carrageen, alginate, locust bean gum, pectin and guar. Since the stabilizers used are hydrophilic, they affect the fat destabilization to a smaller extent than the emulsifying agents do (Goff and Spagnuolo, 2001). The strongly water-binding characteristics of the hydrocolloids increase the viscosity of the outer phase, favour the melting resistance and thereby improve the stability of the foam structure. However, a too high content of stabilizers results in an undesirable mouthfeel. Such sensations are often described as gum-like.

15.4 Future trends

This chapter shows that the recrystallization of the ice crystals is the determining factor for the distribution of the sizes of the ice crystals found in the later product. In the usual production process (freezing in a freezer while whipping simultaneously) the ice cream manufacturer should choose the lowest possible outlet temperature and aim for quick hardening conditions. The choice of the so-called drawing temperature provides a definite possibility for the operator to control the crystal sizes. In conventional freezers, apart from scraping and radial mixing of the partly frozen product, the dasher has the task of whipping the air while dispersing it finely into the product. This fine dispersion of the air bubbles together with the stability of the foam structure represents an important quality factor for the ice cream. Therefore, during the whipping procedure, the mechanical load should be as high as possible because of the foaming process. However, the whipping brings heat into the freezer and thus works against the desired withdrawal of heat. Since it is not to be expected that a deep temperature range is ideal for developing a foam structure with favourable characteristics, it appears more sensible to separate the freezing process from the whipping one. On the basis of these physical connections Windhab *et al.* (1993) conceived a process with a so-called low-temperature ice cream extruder. In the first step the ice cream mix is prefoamed. In the second step the mix is frozen in a freezer, before the partly frozen mix freezes further to approximately –15 °C in an extruder. Windhab and Bolliger (1998) show the positive effects of this new process in their results.

An interesting aspect about the structure of retarding agents of ice recrystallization comes from biology. So-called antifreeze proteins, which occur, among other things, in Antarctic fish, have the characteristic of definitely delaying the recrystallization. Feeney and Yeh (1998) report in their studies the use of these proteins which resulted in almost no recrystallization in the ice cream. This phenomenon can be explained by the modified interface between crystal and solution caused by these substances. Thus, the incorporation of water molecules into the crystal lattice is disturbed.

The fat agglomeration in the freezer with the resulting aggregate sizes is substantial for the melting behaviour. Apart from partially destabilized fat globule agglomerates, the air bubble boundaries are stabilized by proteins and by fat globules and agglomerates with intact membranes. Fat globules and fat agglomerates stabilize the foam structure at a critical diameter by clogging lamella and thus obstructing the serum drainage. Huss and Spiegel (2000) have developed a process in which they produced particulated whey proteins with defined sizes. The theoretical background is described by Spiegel (1999). For the ice cream manufacturer these microparticulated whey proteins represent an ideal fat substitute.

Koxholt *et al.* (1999) successfully used these particles in ice cream and got a creamier product. The effective sizes of the particles lay in the range of the critical diameters of the fat globules.

15.5 Sources of further information and advice

The entire area of the ice cream industry, beginning with the chemical, physical, technical and biological principles of production up to the distribution, is treated in greatest detail in the fifth edition of *Ice Cream* by Marshall and Arbuckle (1996). For the user this standard work has meanwhile become an indispensable tool. The chapter 'Ice cream' by Berger (1997) in *Food Emulsions* represents a further classic in ice cream literature. In particular the characteristics and interaction of the individual components and their effects on the structure are intensively treated. Published by Buchheim (1998), the International Dairy Federation presents the Proceedings of the Symposium held in Athens 1997, which supply an overview of current research work, for example in emulsion and foam stability, functionality of ingredients, additives and process techniques.

The Department of Food Science at the University of Guelph, Canada has excelled with numerous research results on the ice cream structure. The publications by Goff, to which the preceding text referred several times, are to be emphasized in particular.

In the German-speaking countries in the 1990s, there were a number of institutes that contributed greatly to the present level of knowledge of ice cream texture. The Institute of Food Science at ETH Zurich, headed by Professor Windhab, stands out for its efforts especially on the on-line measurement of structure-relevant parameters within the process. Furthermore, great progress in structure research in the area of ice crystallization as well as foam structure was obtained by the Institute of Food Process Engineering and Dairy Technology at the Technical University of Munich as well as by the German Institute for Food Technology in Quakenbrueck.

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16

Controlling textures in soups, sauces and dressings

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16.1 Introduction

Soups, sauces and dressings are core to one of the largest market segments within both the general and specific food category of semi-solid foods. A consumer, for example, rarely eats a meal to which some type of sauce has not been applied, even if it is just tomato ketchup on a plate of chipped potatoes or a dressing to a salad (Deis, 2001).

This chapter will demonstrate why a careful understanding of raw materials and a consideration of how they interact is critical in developing and controlling the textures of soups, sauces and dressings. The main focus, in contrast with other texts, concerns the end use application rather than, for instance, the functionality of a particular raw material or class of raw materials such as emulsifiers or thickening agents (Barsby *et al.*, 2001; Dickinson and McClements, 1995; Dickinson and Rodriguez Patino, 1999; Frazier *et al.*, 1997; Friberg and Larsson, 1997; Hartel and Hasenhuettl, 1997; Imeson, 1997; Stephen, 1995; McClements, 1999; Phillips and Williams, 2000; Roller and Jones, 1996).

In order to complete this task, the whole system needs to be examined. The first section will detail how the optimum product needs to be defined and what factors affect this. Then the products have to be divided up to understand where the main ingredient classes lie and what role each type can play in controlling the final texture. After this division, the process continues by understanding some of the key texturising requirements that need to be fulfilled. At this point the nature of the

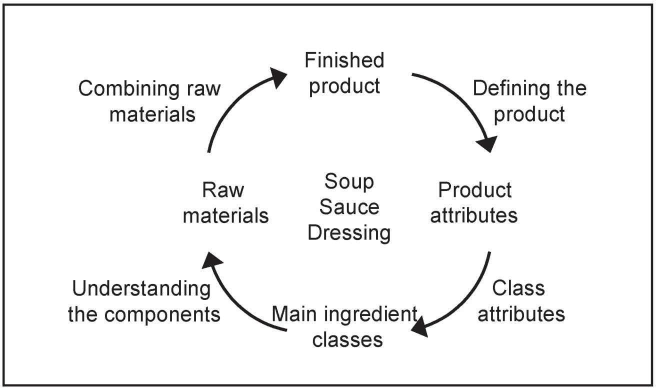

Fig. 16.1 Product evaluation cycle.

individual texturising raw materials are examined to show how they can contribute to the final macroscopic picture. Finally, the circle is completed by demonstrating how the raw materials can be combined to produce the appropriately textured soups, sauces and dressings (Fig. 16.1).

Throughout the chapter a series of tables will allow the reader to pick out the ingredients and routes that are most appropriate to their needs.

16.2 Defining the product

As the key first step in developing the texture, the product needs to be defined by taking into consideration the constraints of the main stakeholders for the soup, sauce or dressing. Although more time is taken up by defining the product, textures and processing route before samples are prepared, the method benefits from many longer-term advantages. The product developer will gain a greater understanding of the functionalities, contribution and interdependent interactions within the product and process and how they contribute to the whole (Earle *et al.*, 2001).

16.2.1 Product, process, price

Initially the main product attributes need to be defined by understanding the interrelations between the three main stakeholders: product selectors and marketing; processing and manufacturing; controlling and purchasing and the product development specialists (Fig. 16.2). The product development specialist's role, working with the stakeholders, is always geared towards the final goal.

Product selection and marketing

The product developer works intimately with marketing departments or product selectors to understand the consumer's interpretations, product feedback, sensory

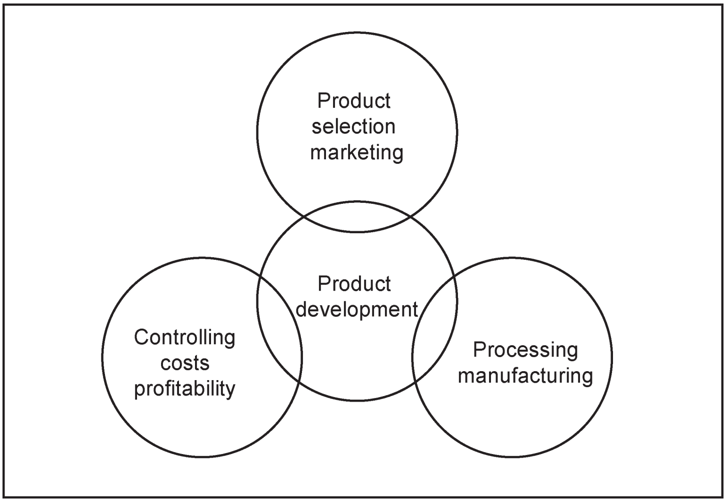

Fig. 16.2 Stakeholder interactions.

evaluations and market trends in order to produce new products that will delight. The total performance of the product, including the texture, is key to the success and the product developer must be able to interpret these textural attributes and convert them into actual products by using the right combination of raw materials (Borwankar, 1992; Kokini, 1985).

Processing and manufacturing

The product development specialist liaises closely with the processing and production departments because it is important to know how these textural properties can be generated during the manufacturing process. Ultimately the processing and production departments do not need to know why that textural effect has to be produced but they need to know how to make it that way with parameters, such as how much, how fast, how 'safe' and for how long?

Pricing and controlling

Textures can be created in many different ways through the use of different ingredients and processing routes, which in turn can lead to different costs and levels of profitabilities. Any resulting constraints over the choice of texturising materials that could be used due to cost issues has to be clearly managed with the financial controllers.

The complexity of the product development process means that the specialist needs many skills to create the right texture. Knowledge and expertise across a wide range of disciplines as well as food science and technology skills are needed in order to balance to needs of the stakeholders. This is why it is so important to spend this time 'up front' in order to establish the right parameters before starting the job of designing and texturising the right product.

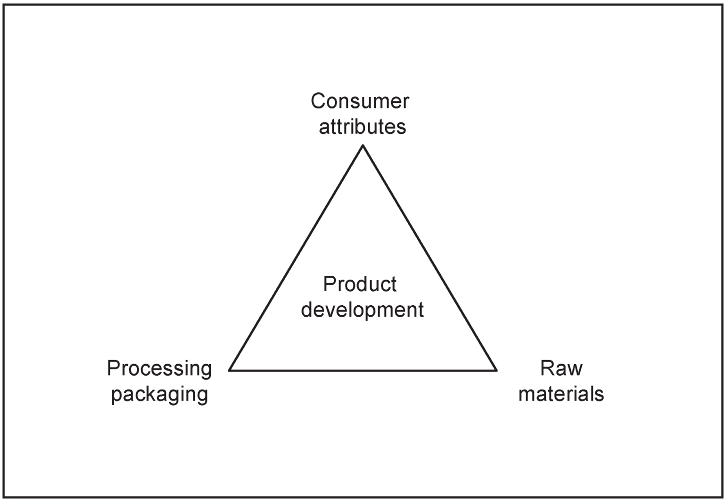

Fig. 16.3 Product attribute relationship.

16.2.2 Product attributes

Now the first steps towards developing the texturising solution can be taken by defining the general consumer, processing, packaging and raw material attributes that will be required. There is a strong triangular relationship between these attributes that the product development specialist must respect and use in the development process (Fig. 16.3). These attributes can then be evaluated to see which are the potential options for a particular solution.

Consumer attributes

There is an extensive range of consumer formats for products within the soups, sauces and dressings market. The selection of the appropriate option is a function of a number of factors that depend on how the product is to be stored and consumed. This selection is the first step in defining how a textural solution may be derived and the requirements for the consumer at the point of delivery. There are a number of categories that need to be considered for each product such as meal occasion, usage, flavours and textures (Table 16.1).

This summary allows the developer to determine the first parameters of the product and it is often done instinctively or is constrained by the market segment or the manufacturing equipment available. The table does not detail other important areas for texture generation such as the effect of meal location or degree of consumer involvement. These two areas can have a significant effect on texture as the consumer may need to process the product further to get the final desired texture. There are also legislative requirements for certain products in a number of countries that determine prerequisites for some of the products such as salad dressings.

Table 16.1 Consumer attribute options

	Soup	Sauce	Dressing	Comments
Packaging				
Tinned	Y	Y	–	Traditional for long shelf-life.
Pouch	Y	Y	Y	Product may be cooked in pouch.
Sachet	–	Y	Y	For smaller/individual portions.
Glass jar	Y	Y	Y	Product can be viewed in container.
Carton	Y	Y	–	Often for fresh/short-life products.
PET/foil lid	Y	Y	–	For long shelf-life individual portions.
Aseptic brick	Y	Y	–	For long shelf-life products.
Usage				
Home	Y	Y	Y	Most products and formats used.
Office	Y	Y	Y	Speed and convenience of use.
Alfresco	–	Y	Y	Anywhere with no preparation.
Restaurant				
in house	–	Y	Y	Consumer can choose/serve.
back of house	Y	Y	Y	Chef can use for on plate presentation.
Styles				
Regular	Y	Y	Y	Non-fatty sauces, soups, dressings.
Cream style	Y	Y	Y	Emulsified fat sauces, soups, dressings.
Concentrated	Y	Y	–	Requirement to dilute before use.
Condiments	–	Y	Y	For application by the consumer.
Reduced fat	Y	Y	Y	Non-fat or reduced fat products.
Two parts	–	Y	Y	Two parts to combine or prepare in stages.
Viscosities				
Drinkable	Y	–	–	For soups that can be drunk in a cup.
Pourable	–	Y	Y	For pouring over another part of the meal.
Spoonable	Y	Y	Y	For spooning out individual portions.
Cooking instruction				
Cold usage	Y	Y	Y	No further preparation required to use.
Hob top	Y	Y	–	Heated – with gentle stirring in a saucepan.
Oven baked	Y	Y	–	Longer, hotter environment but no stirring.
Microwaved	Y	Y	–	Rapid – aggressive heating.
Stir frying	–	Y	–	Very hot with free oil.

From a development and manufacturing point of view this summary gives an overview of all the products. Much can be learnt by transferring the experiences and technology from one segment to another or allowing a manufacturer to expand its range into another segment.

Process and packaging

Processing and packaging methods form the second side of the triangular relationship and they are clearly interlinked to the end user. The texture of the

Table 16.2 Processing route options

	Soup	Sauce	Dressing	Comments
Process				
Cold	Y	Y	Y	No heat used – only mechanical energy.
Ambient	–	Y	Y	No heat used – only mechanical energy.
Pasteurised	Y	Y	Y	85–95 °C for 15–120 min.
UHT	Y	Y	–	110–140 °C for 0–6 min.
Retorted	Y	Y	–	110–140 °C for 6–60 min.
Packaging				
Tinned	Y	Y	–	Hot fill and sterilised.
Pouch	Y	Y	Y	Can be sterilised or pasteurised.
Sachet	–	Y	Y	Usually cold filled.
Glass jar	Y	Y	Y	Hot fill and pasteurised or sterilised.
Carton	Y	Y	–	Hot filling.
PET/foil lid	Y	Y	–	Sterile (hot/cold) filling.
Aseptic brick	Y	Y	–	Sterile (hot/cold) filling.
Storage conditions				
Deep frozen	–	Y	–	Freeze–thaw stable product required.
Chilled	Y	Y	Y	Often for short shelf-life 'fresh' products.
Ambient	Y	Y	Y	Long shelf-life, pasteurised or sterilised.
Storage times				
Days	Y	Y	Y	Fresh chilled/products often sauces.
Weeks	Y	Y	Y	Not many products are in this category.
Months	Y	Y	Y	Pasteurised or pH-controlled products.
Years	Y	Y	Y	Fully sterilised or pH controlled.
Process factors				
Time	Y	Y	Y	These factors may limit the choice of raw material due to incompatibilities of raw material functionality and stability under certain conditions.
Temperature	Y	Y	Y	
pH	Y	Y	Y	

product is a function of the manufacturing process and how it is stored. Under some circumstances the processing route and raw materials are incompatible and an alternative combination has to be found. Linking the consumer requirements to the purely technical attributes of the processes in terms of machinery layout, equipment installed and exact processing and packaging conditions will determine how the texture could be generated (Table 16.2).

Raw materials

The third side of the product attribute triangle takes the developer to the heart of controlling textures of soups, sauces and dressings. The raw materials and their interactions are the principal methods to develop and control the texture of a product. As part of this process it will become clear that the same end product can be attained by different raw material routes and this is where the constraints of the

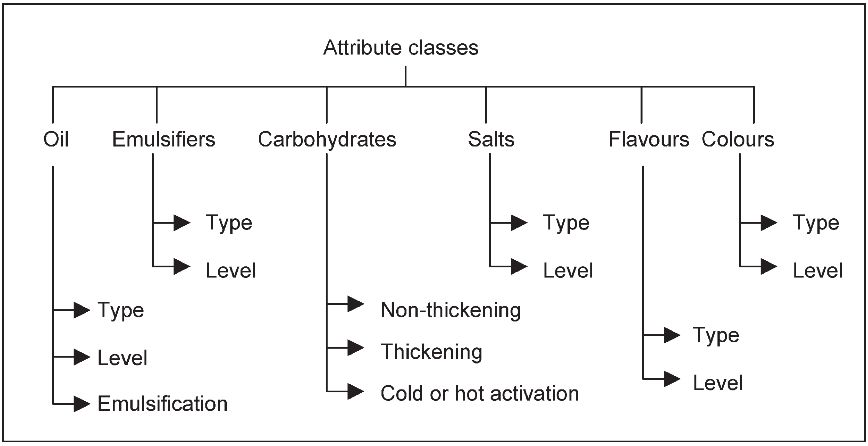

Fig. 16.4 Product attribute classification.

stakeholders can play a significant role in the route to the end texture. At this point the route, the textural requirements and cost structures should have been defined for the end product.

16.3 Ingredient and product classification

The first level of understanding of the textural effects starts with considering the principal classes of ingredients that are within the soup, sauce or dressing (Fig. 16.4). It is important to determine to what extent each of these different classes contributes to the final texture (Heertje, 1993). This evaluation process is done on both existing and new products with no need to relate it directly to the intended end use.

16.3.1 Oils and fats

As the first class, oils and fats immediately divide the product into the two main areas of hydrophobic and hydrophilic regions and separate the ingredients into those that are either oil- or water-soluble. Soups, sauces and dressings are generally characterised by the continuous water phase and thus how the oil is distributed and stabilised has a significant effect on the texture (Franco *et al.*, 1997).

Most of the oils and fats within the system are oils rather than fats, the only exceptions being butter and animal fat which are solid when the sauce or gravy is at, or below, room temperature. The most common oils are sunflower, rapeseed and soya, with others used for specialist reasons. At low levels the oil can often be incorporated as a coarse mixture without the need for it to be emulsified. The oil is mixed and broken into small enough droplets so that once dispersed they are not likely to coalesce (Deis, 2002).

As the oil level increases, two effects are observed that have to be considered. With more oil, the ratio of the discrete and continuous phases changes resulting in less of the hydrophilic region and thus less water is available to dissolve or suspend the hydrophilic ingredients. This alone can affect the hydration of certain ingredients, which then affects their texture, as well as flavour impact. Secondly, in order for the oil to be held, it needs to be emulsified to create an oil-in-water emulsion. This, in turn, requires the introduction of an emulsifier. The preparation of an oil-in-water emulsion often requires a step change to the processing route by requiring homogenisation equipment to be introduced.

16.3.2 Emulsifiers

The type and level of any emulsifier will dramatically affect the quality, stability, texture and flavour of an emulsion and thus the final product. The effects are very interdependent and thus the emulsifier needs to be chosen with care. The emulsifier is also the one ingredient that is the key interface between the hydrophobic and hydrophilic parts and has a major influence on the texture of the end product. There are a range of texts that go through the detail and aspects of each type and this is an area of extensive research, development and understanding of how to get the best from emulsified systems (Dickinson and McClements, 1995; Dickinson and Rodriguez Patino, 1999; Flick, 1990; Franco *et al.*, 1995; Friberg and Larsson, 1997; Garti, 1999; Hartel and Hasenhuettl, 1997; McClements, 1999; Moore *et al.*, 1998; Robins, 2000; Wendin and Hall, 2001; Wendin *et al.*, 1999).

Table 16.3 summarises some of the main classes and attributes associated with them.

The type and level of the emulsifier chosen versus the level of oil significantly affects the product. The relative ratio has an effect on the stability of the emulsion and also the size of the oil droplets that are generated. Some emulsifiers, weight for weight, have a better emulsifying capacity and can give more surface contact. This can reduce the average size of the oil size droplets, which affects the creamy texture of the sauce. The size and distribution of the droplets also affect the viscosity and rheology of the sauce. Different emulsifiers also have different stability towards heat or pH – something that must be considered in hot or acidified prepared sauces (Anton and Gandemer, 1999; Deis, 2002; Franco *et al.*, 2000; Hunt and Dalgleish, 1995). The nature and stability of the emulsion through the inter-oil droplet interactions, either steric or ionic repulsion, are affected by the other ingredients in the product and also how it is prepared. The type of emulsification equipment also has an effect on the particle size distribution.

It may appear that proteins have been missed out of the classification system but this has been deliberate. The main role of proteins in soups, sauces and dressings is within the class of emulsifiers where even in the addition of cheese for a flavour purpose, the dairy protein is the emulsifier for the fat that is added with it. Proteins also contribute to the overall effects of the products as they can bind and form networks if there are sufficient quantities present.

Table 16.3 Emulsifier options

Emulsifier	Example	Comments
Dairy proteins	Whey protein Caseinates Cheeses	Can be pH and heat sensitive but add good dairy, creamy flavours.
Vegetable proteins	Pea Soya	Vegetable derived products with a good range of protein functionality with non dairy flavours.
Phospholipids	Egg yolk Lecithins	More pH tolerant, natural emulsifiers.
Fat derived	Mono- and diglycerides Esters of mono- and digylcerides	Ability to vary the hydrophilic lipophilic balance (HLB) values to suit. 'E' number or equivalent is often required on label.
Carbohydrate derived	Starch esters Sucrose esters Polysorbates	Ability to vary the HLB values to suit. 'E' number or equivalent is often required on label.
Hydrocolloids	Gum arabic	Natural emulsifying capacity of a gum.

16.3.3 Carbohydrates and fibres

Within the hydrophilic part carbohydrates and fibres constitute the largest part by weight. These have a number of different texturising roles and can be split into two groups simply defined as thickening or non-thickening. Within the non-thickening group there are products such as short chain carbohydrates or sugars which are good water binders but not thickeners in their own right. For instance, a 5% gelatinised starch solution is thick, while 5% sugar solution is very thin (Deis, 2001). The main contributions to the texturising effects are: water binding, thus reducing free water available for other ingredients; sweetening; bulking; increasing body and mouthfeel; affecting freezing and boiling points and overall specific gravity.

The second group is the thickeners, where a small amount of these materials not only binds water but also restricts the movement of the systems and thus increases the viscosity. These can be further divided into those that do this by bulk, such as starches, and those by networking, such as pectins. All these agents thicken the system and produce a change in the structural and textural properties.

Within this class there are also fibrous products that can act in a similar way to the carbohydrates and are differentiated only by their resistance to enzymatic breakdown within the gut. Some are purely water binders, such as potato fibres, which hold on to water and thus reduce the free water available to give the viscosity. Others form networks such as xanthan gum, which reduce the movement around the system and thus give viscosity and texture in that way.

Table 16.4 Carbohydrate options

Carbohydrates	Examples	Comments
Sugars	Extracted sugars, e.g. sucrose Mono glucose units, e.g. dextrose Starch polysaccharides (high dextrose equivalent, DE)	Sugars hydrate very rapidly and bind water preferentially over other ingredients which can affect other ingredient functionality.
Maltodextrins	Starch polysaccharides (low DE)	The low DE polysaccharides are good non-sweetening bulking agents that give body and mouthfeel to products.
Dextrins	Heat, acid treated starches	These are more film forming although some have been produced as fat mimetics.
Hydrocolloid gums (low viscosity)	Water binding agents, e.g. gum arabic	There are a range of gums that will bind water but do not rapidly increase viscosity. These can be used to complement high viscosity gums.
Celluloses	Microcrystalline cellulose Carboxy methylcellulose Methyl cellulose Hydroxypropyl cellulose Hydroxy propyl methyl cellulose	The chain length of the cellulose will determine how much viscosity can be generated. The shorter the chain the less viscosity. All hydrate and hold water.
Starches	Rice Tapioca Corn Waxy maize Wheat Potato	The range of starch base raw materials is wide and the number of resultant products is extensive. Starch is the most commonly used thickening agent in soups, sauces and dressings.
Hydrocolloid gums (medium viscosity)	Guar gum Locust bean gum Propylene glycol alginate Carargeenan Pectins	These gums are extracts and have a range of viscosities. They bind water and will act as viscosifying agents. Some, under certain conditions, will also gel.
Fibres	Potato Oat Wheat	These fibres will take up water and retain it. Through this action they can reduce the flow characteristics by their steric bulking effects.
Hydrocolloid gums (high viscosity)	Xanthan (microbial exopolysaccharride)	Xanthan gum is produced by fermentation. It gives highly viscous solutions but is also thixotropic.

Any thickening agents that form networks and increase the viscosity will contribute enormously to the texture and this is one of the most significant classes in terms of general texturising effects (Table 16.4). In most cases the networks that are formed are described as weak gels and are characterised by a low rigidity modula, very low yield stress, shear reversibility, homogeneous flow properties, shear thinning and viscoelastic properties at rest.

16.3.4 Salts and ions

Charged molecules and ions within soups, sauces and dressings can significantly influence the final texture, structure and flavour of a product. Salts include those with cations such as calcium, sodium, potassium and anions of chloride, carbonate or phosphates. There are also chelating agents such as ethylenediamine tetraacetic acid (EDTA) that are used to help reduce rancidity in products or sorbates and benzoates for preservation effects. These ions have strong interactions with the other raw materials: in particular proteins and carbohydrates. The ions can act to change the solubilities of proteins or by increasing the interaction between different molecules such that a strong network can be formed (Depree and Savage, 2001; Ramaswamy *et al.*, 2001). The salts also affect the freezing and boiling points of the product as well as the overall taste. The order of addition of the salts with respect to other ingredients can be used to change dramatically the functionalities if this is required.

Acidity has also been placed in this category as this is a function of the hydrogen ion. This key ‘salt’ affects the pH and this has major textural and stability effects on soups, sauces and dressings (Anton and Gandemer, 1999; Franco *et al.*, 2000; Hunt and Dalgleish, 1995). The interactions with other buffering salts and other ingredients can have a significant effect on the availability and solubility of the hydrogen ion and other salts which is critical for certain texturising effects as well as food safety and flavour.

16.3.5 Flavours and colours

Although the flavours and colours of the system do not significantly contribute to the physical attributes of the texture, they can still play a major role in the perceived texturising effects. In both cases the solubility between hydrophobic and hydrophilic media affects how and when these are tasted or seen and thus the perception by the end user (Wendin and Hall, 2001; Wendin *et al.*, 1999). The flavours and colours can be masked or enhanced by certain ingredients that affect the texture and thus an appreciation of how they interact is significant.

16.3.6 Integral approach

It is clear that since all the main categories have an effect on the end product, in terms of structure, texture and flavour, they are integral to the end success of the product. Within the main area for semi-solid soups, sauces or dressings a triangular

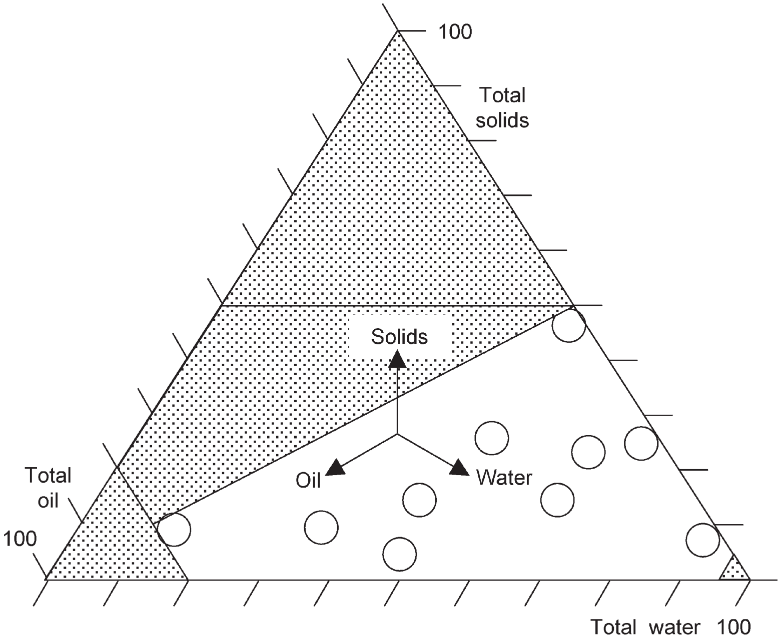

Fig. 16.5 Ingredient combination map.

relationship between oil, water and the solids can be mapped (Fig. 16.5). This immediately demonstrates to the end user that, even with the many possible combinations, not all the potential mapped space can be used, particularly very high oil, very high solids, high oil–solids combinations or pure water. The circles represent some of the products within the segment.

16.4 Texturising requirements

At this point the analysis is half way around the cycle, having examined the type of product and the main classes of ingredients that have an effect on the final texture. In advance of examining the texturising effects and uses of the raw materials, the general texturising functions need to be clarified.

Texture is the umbrella name associated with the physical attributes of the product and can be further divided into areas such as visual appearance, mouthfeel and viscosity. This is different from the sensorial characteristics of taste and aroma, although clearly it is in combination that the final eating performance is evaluated. In this chapter the primary aim is to focus around the texture and structure but the interrelated factors must always be taken into consideration (Bourne, 2002; Rosenthal, 1999; Yackinous *et al.*, 1999).

The texture of the product is a facet of a number of different attributes and these

Table 16.5 Texture requirement range

Facet	Characteristic	Comments
Visual	Visual appearance	Surface texture, colour, opacity, uniformity, and particulates
Rheology	'At rest' physical properties	Viscosity, elasticity, gelling
	Flow characteristics	Short, long texture Spoonable, pourable, gelled
	Yield flow characteristics	Newtonian, Bingham, thixotropic
	Coating ability	Cling, hot holding, water binding
	Oral factors	Creamy, slimy, full bodied, melt in mouth
Suspension	Particulate suspension	Uniformity in container Heat transferring Early versus late particulate suspension
Processing	Manufacturing effects and tolerance	Early, late texture generation Texture stability Time, temperature, pH effects

can be sub-divided in order to give a clearer framework (Table 16.5). These factors should be considered for all products, although they will have different levels of importance in every case.

As a brief introduction the following sections highlight some of the principal considerations within certain of the texturising elements. These are the areas that are perceived as the most important and critical attributes.

16.4.1 Requirements: visual/physical

The visual appearance of the product is one of the first points at which the consumer will interact with the product and build up an expectation of the eating experience and thus is a primary factor in acceptability. There are a whole range of attributes that can be examined and controlled such as visual texture from smooth, pulpy, colour or inclusion of particulates.

The origins of the general texture of many products are derived from the natural materials from which the 'authentic' dish was once produced. These natural sources include tomatoes or other vegetables in either their whole or puréed form and are still one of the key ways of producing major visual texturising effects. The natural fibrous part of the vegetable enhances the product texture and viscosity and can be used directly or, as in the case of puréed tomatoes, more often in a concentrated form.

In the modern food processing era it has become more acceptable to use other value-adding materials to replace some, or all, of this texturising effect for specific reasons. This is where the next category starts to contribute to the visual texture and can include the previously extracted and dried fibrous material. Examples include pea, potato and cereal fibres. These products, when extracted and dried, can be

Table 16.6 Visual texturising agents

Raw material types	Ingredients
Natural raw materials	Tomatoes, crushed, pureed, sieved Vegetables whole, diced, pureed Fruits whole, diced, pureed Herbs whole, rubbed Spices whole, ground
Dried extracted raw materials	Tomato fibres Vegetable fibres: pea, potato Cereal fibres: wheat, oat
Non-fibres	Starches, as extracted Celluloses, as extracted
Manufactured products	Granular products derived from starches Agglomerated products derived from starches
Dairy products	Cheeses

used in other food areas where the original product is not wholly suitable and can thus diversify their applicability. The products can also be further processed to give a range of grades which, again, can be used to improve product structure and textures.

In a further category are products that are extracted from one source and can be used as an alternative to other texturising agents. One such example is potato starch, which, because of its particle size distribution, gives a pulpy texture on use and can enhance the pulpiness of tomato-based products. There are many hydrocolloids that can be used in this way to give different desired textures and visual effects.

Finally, there are products that start from a base material that is modified through a manufacturing process to give it a different textural effect. Agglomerated starches are included in this category as these are based on smooth starches, but by processing can be altered to give pulpy textures. Visibility of the particles is a major factor in the success of this type of application.

Table 16.6 shows the flow of visual texturising agents. This example concerns those raw materials that add pulpy texture, although this principle can be carried out for a wide range of other visual appearances such as the use of dairy products and cheeses. By understanding the nature of the system these different ranges can be chosen in order to obtain the desired effect.

16.4.2 Requirements: rheology

The rheological properties and requirements of the system is the next area that needs to be considered (Morrison, 2001; Rao, 1999). Here materials that will thicken a product are considered as these tend to affect the rheological properties the most. There are a wide range of characteristics available, including thixotropic, and from watery through to gelled products. The main classes of gelled products have been discussed elsewhere in this volume and generally, in soups, sauces and dressings are usually seen as undesirable.

From a rheological point of view, the bulk of the products either show a Newtonian behaviour or act like a Bingham plastic where a yield stress is required. The variation in viscosity from very thick to thin is a function of the end use and is usually controlled by the amount of a particular thickening agent. Changing the type of thickening agent will change the rheological properties: for example products made with xanthan gum tend to have what is described as a 'long' and sometimes slimy texture, while those made from a starch can be what is often described as 'short'. In all cases these are the weak gels where there are strong enough interactions to hold the products together but not to the point of gelling so hard that once broken they will not re-form.

16.4.3 Requirements: suspension

Another part of the textural effects of soups, sauces or dressings is often one of the visual – uniform mix. It is commonly accepted that in most semi-solid foods in the marketplace the product is in a uniform mix with any particulates being evenly distributed throughout the product. The exceptions are some of the vinaigrette dressings, which have to be shaken before being used. In fact the need for suspension is often a more critical step for the manufacturer for even production distribution rather than an acceptance factor in the end product. Choice of the suspension agent or processing route can have a dramatic effect on the texture for the end use and thus care has to be taken to consider the effects all the way through the system.

At one extreme the suspension agent works throughout the whole process: xanthan gum, for instance, retains its viscosity through a wide range of processing conditions and throughout the manufacturing process. In contrast, other suspension agents are used only in one part. A good example is the use of a native starch in combination with a modified starch. The native starch is used to gain initial viscosity in a pre-processing step before packing. Once in separated portions, the product is heated, causing the native starch to break down, allowing better heat transfer before the modified starch takes over and gives the final suspension viscosity. Here the design is also to allow the thermal (food safety) process to take place in an efficient way and yet reduce the overall processing time.

The suspension is important for the overall appearance and consumer appeal as well as for the manufacturers' benefit. For other systems, such as vegetables, it can also be carried out by controlling the specific gravities to make them equal so that they can be more balanced in the end product. For emulsions the controlling of the oil size droplet to reduce the creaming rate is also very applicable.

16.4.4 Requirements: processing

All forms of food preparation can be classed as a type of processing, even in the simplest forms within a chef's kitchen. From an industrial point of view the key difference is generally scale, both in terms of physical volumes and lengths of time.

Semi-solid soups, sauces and dressings are processed in a variety of different ways to achieve the desired end product. Quality and safety are always key parameters for the consumer and thus the texturising agent must be able to cope with the processing conditions and go through to the final packaging format and ensure that the texture remains intact for the whole of the shelf-life. The raw materials can often be adjusted to cope with a large variety of processing conditions such as chemically modifying a starch to ensure that it can cope with a cooking and perhaps freezing process.

16.4.5 Measurement of texture

In the categories that have been highlighted above it is clear that the texture is a function of all these factors and, as such, cannot be simply written down or defined but needs to be evaluated for each case. In order to define the texturising effect a number of different, and complementary, methods are required. These fall into the two main categories: qualitative and quantitative analysis. Both have limitations and thus it is only when they are used in conjunction that a complete picture can be given (Bourne, 2002; Rosenthal, 1999).

From a qualitative point of view, product descriptions and sensory studies can be used. There is often a limitation: in product evaluation or sensory studies, a set of key words and descriptors are developed during the first stages of an evaluation and can only be used for that test. These cannot easily be interpreted outside the set as they are not general enough to give broader definitions, although within the set they help to define quantitative directions (Koeferli *et al.*, 1998).

There are a wide range of quantitative methods that are available to measure attributes of the end product. These physical methods can allow aspects of the texturising effect to be measured and result in numerical values to be supplied under certain conditions. This allows standards to be measured for reproducibility, which is often seen as an important quality critical control point (QCCP). In all cases the measuring technique provides some information about the product but it cannot be used solely to define the texture of the product as the different techniques have different limiting factors. Table 16.7 gives the main classes of texture or structure measurements that are generally used in soups, sauces and dressings. This is detailed more extensively elsewhere within this volume and in other texts (Bourne, 2002).

The limitations with all techniques, both quantitative and qualitative means that they need to be used in conjunction with each other and, where possible correlated. In so doing the complete picture can be gained and thus confidence in the texture of the system is fully understood and can then be designed to meet the consumers' desires.

Table 16.7 Texture measurement methods

Technique	Examples
Flow characteristic	Bostwick
Shear resistance	Brookfield, Haake viscosities
Oil droplet size	Malvern, Coulter counter
Oral assessment	Informal tasting
Sensory panel	Trained panel testing
Texture analyser	For thicker, near gel-like products
Salt analysis	Sodium
pH	
Microscopy	For starch structure evaluation
Viscograph	Brabender, rapid visco analyser

16.5 Texturising ingredients

In analysing soups, sauces and dressings so far, the complete system and main classes have been examined to understand the product texture from a holistic point of view. This has meant looking at the macroscopic picture and effects. The macroscopic effects are a function of the microscopic effects of the raw materials that compose the product. Now the raw materials need to be evaluated so that the development specialist can choose the ingredients to provide the texturising effects for the end product.

In order to continue to focus around the end use rather than the more traditional review by material type, a number of the different and main textural descriptors have been taken and the raw materials that can contribute to these effects have been identified. These groups have been simplified for practical purposes and are in line with the findings of various studies and reviews (Borwanker, 1992; Bourne, 2002; Kokini, 1985; Kokini and Cussler, 1983; Kokini *et al.*, 1984). It must be remembered that the ingredients can contribute to other effects and thus each part should not be used in isolation but with the others in a multidimensional matrix of interactions. These categories are for general guidance rather than for a specific product or application.

The series of lines in the figures below highlight the general texturising effects with a range of raw materials that can provide properties being shown. The ranges show a qualitative assessment of what they can cover within that effect, although the function of a specific type, level of incorporation or other factors such as specific processing conditions have not been described.

16.5.1 Surface texture

The surface of the product can take on a range of different appearances, depending on what is required for the end product. It can vary from being very smooth and glossy to being pulpy or particulate in nature (Fig. 16.6). For smooth textured

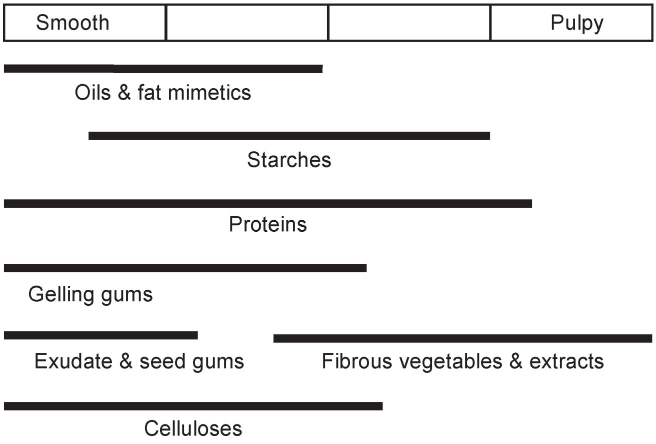

Fig. 16.6 Surface texture range options.

products, the ingredients must not interfere visually with the surface and thus tend to need to be fully solublised or have particulates so small that the eye cannot discern them. Most of the hydrocolloid gums, pectin and gelatin fall into this category as they are fully dispersed in the water phase.

Products such as cooked starches fall into a second category as they are more distinct particles with a granular structure. The size of the swollen granules affects the degree of visibility from the smallest, such as rice, through to the most pulpy potato starches. Emulsified oils also fit into this category since they have oil droplets that are small enough to appear indiscrete. In the case of the oil droplets and the starch granules they can be viewed as discrete entities under a microscope, unlike the fully solubilised raw materials. The degree of pulpiness can be increased by the introduction of natural raw materials, such as puréed vegetables, through to the use of whole vegetables, where the full natural fibrous part can be seen.

Within the fibrous products category are other materials ranging from microcrystalline cellulose to dried vegetable fibres. These materials do not solubilise to any degree, although they may hydrate and bind water and thus add a particulate dimension to the products. The raw material extracts can often be manipulated to offer a range of particle sizes.

Proteins can also introduce a range of visible textures depending on the state they are in. Dairy proteins, for example, range from being denatured and flocculated or discrete cheese particles through to fully solubilised with the use of emulsifying salts. There are also a wide range of vegetable proteins that can be used to texturise the soup, sauce or dressing.

The key goal is to choose materials that can have an effect on the surface that is required for the end product. The very nature of how they affect the surface means that they are interacting within the bulk of the product and thus affecting the total thickening characteristic of the product.

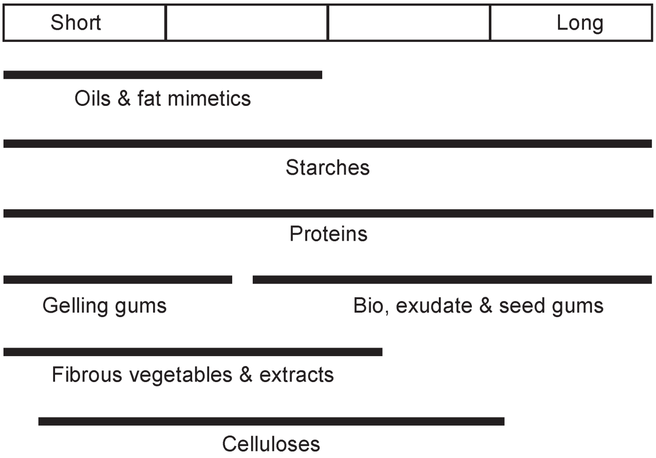

Fig. 16.7 Thickened characteristic range options.

16.5.2 Thickened characteristics

The two extremes of the thickened characteristics are gelled products and essentially pure water. The core of the soups, sauces and dressings products lies in between these extremes, forming weak gel networks (Fig. 16.7). Within the weak gel networks there are again two extremes in the nature of the thickened characteristic: short and long texture. These descriptors relate to the distance over which the thickening interactions are working.

In soups, sauces and dressings, the short nature comes from short distance interactions between discrete entities that form the weak gel network. This includes the interactions between discrete starch granules in a thickened sauce or low-fat dressings, the short distance interactions of microcrystalline cellulose particles or bulk-packing interactions of oil droplets in high-oil mayonnaises and dressings. The difference with gelled products is also clear because after applying mechanical energy to disrupt the system, the weak network is able to reform and reproduce the original short texture.

If raw materials are chosen where the interactions become more chaotic or over longer distances, then the thickened characteristic tends to become longer as the molecules need to unravel further before the split can occur. Fully solubilised molecules, such as xanthan gum or over-cooked starches, are good examples of the ability to create extensive networks.

Raw materials that are usually used to prepare gelled products can sometimes be chosen under certain circumstances. They are used at a levels where there is insufficient material to gel or other raw materials are adjusted, such as removing co-gelling ingredients like calcium, changing the pH or adjusting the total solids. The gelling materials can also be modified to change their interactions and reduce the tendency to gel, such as the production of propylene glycol alginate from sodium alginate.

It is important to stress that the thickened characteristic is not necessarily a reflection of the flow characteristic of the system. There are a number of ingredients that are thixotropic and, thus, under greater shear stress, thin more rapidly than others that do not have this pseudoplastic characteristic. In soups, sauces and dressings, most products have a degree of yield stress before they will flow. The nature of the structure, i.e. short versus long, is not always an indication of how much yield stress is required and the effects the shear will have on the product. All these factors need to be taken into consideration before the appropriate mix of raw materials can be made.

16.5.3 Coating ability

The coating ability is the other main characteristic of the at-rest viscosity and can be viewed in two ways. First, there is the physical effect of holding on to a piece of meat or coating of vegetables or pasta (Fig. 16.8). Secondly, there is the coating ability within the mouth, which is often associated with the body or the creaminess of a sauce (Fig. 16.9).

For the physical coating effect, the application and ability to maintain the coating through further processing and cooking need to be examined. The sauce needs to have a carefully managed consistency so that, when applied, it will flow over a product but not pour off. It cannot be too short otherwise a good coat cannot be applied, or too long since it can become too slimy and ‘slip’ off. On certain porous materials the sauce can be very easily absorbed into the material and this results in a poor visible appearance. The viscosity and water-holding capacity need to be controlled such that the ingredients are able to hold the water in the sauce, yet the sauce cannot be so thick that it will not coat in the first place. Finally, there are a wide range of sauces that need to be heated in the final stage of production and, thus, the sauce needs a degree of resistance to thinning on heating.

For a good coating ability, the water binding and retention can be created in a number of ways. In products that do not need reheating, low-viscosity water-binding agents, such as guar gum or other hydrocolloids, can be used because they preferentially bind water and prevent the sauce from being ‘diluted’ and thinned by the other materials. In products that need to undergo hot preparations, there are a number of materials that can maintain much of their viscosity while being heated, such as xanthan gum or the unique, thermally setting methylated celluloses. The celluloses thicken on heating and can prevent water migration and evaporation within a food by trapping it within a matrix in the sauce.

The second coating aspect is within the mouth and is particularly significant in cream-style products. The consumer feels a sensation of creaminess or body through the coating and lingering effects of the product within the mouth. There is a balance between products that are thin and pass easily through the mouth, those that last long enough to give a good mouthfeel and those that are retained for too long such that they feel too slimy. Studies have been carried out to determine the links between the physical characteristics of a product and its ability to give good coating and mouthfeel (Kokini, 1985).

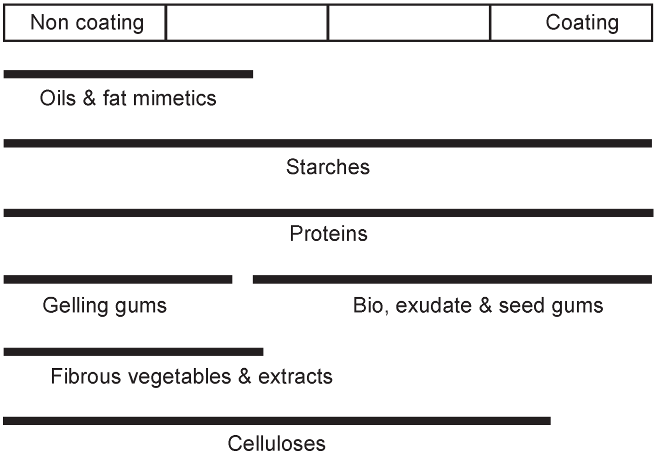

Fig. 16.8 Coating ability range options.

In terms of creaminess and coating ability, the emulsified fat or equivalents predominate. The consumers' desire to reduce their fat intake but still retain the sensation of creaminess has resulted in the extensive development of fat replacers that mimic the fat globules by creating small particulates or by coating in the mouth (Roller and Jones, 1996). Increasing the body of a sauce can also improve the coating ability because it takes more time for the tongue to thin down the product before it can be swallowed. 'Body' tends to mean increasing the solids or increasing the viscosity of the soup or sauce.

Another aspect of mouth coating that also has to be taken into consideration is the effect that the oral cavity has on the texture of the product. The ability of the mouth to warm products has an effect on the heat sensitivities of the various ingredients. For example, low melting point fats will soften within the mouth and can give a different textural effect than higher melting point fats. Gelatin-based products rely on this principle to give their melt-in-the-mouth characteristic.

The enzymatic activity within the oral cavity is the first part of the digestive tract and certain texturising agents, such as starches, are broken down rapidly. Hence starchy foods and sauces can start off with a thick, full body but can melt away relatively quickly in the mouth. This contrasts with other thickening agents that are not broken down and pass through the mouth with no change except a degree of dilution and mechanical shear. Some of the hydrocolloids that are held in the mouth for longer result in a slimy sensation.

The in-mouth attributes take soups, sauces and dressings on from evaluating their static nature to their flowing characteristics both orally but also importantly in the manufacturing processes.

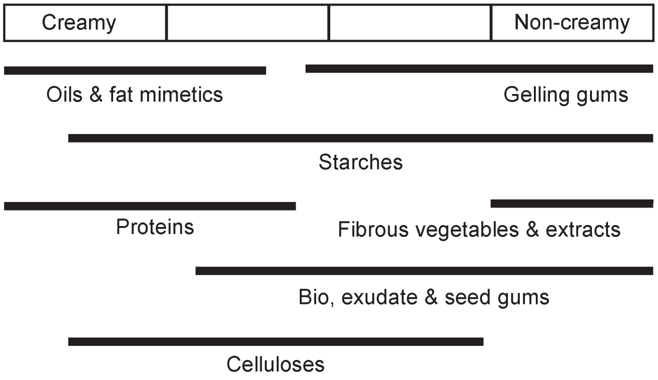

Fig. 16.9 Creaminess range options.

16.5.4 Flow characteristics

The flow characteristics and changes that can occur to the products have to be related and controlled in order to permit any mechanical energy that is required to move them through pipes to be deposited onto products and packed and stored (Fig. 16.10). Shear energy can break some of the products down, while others will flow in either a Bingham or pseudoplastic way.

Thermal energy can also result in changing the flow characteristics of the product and is important during any further processing stage, such as home preparation or in the mouth (Fig. 16.11). The range extends from those that thin and then rethicken on cooling to those that break down and the unusual ones that actually thicken on heating and thin on cooling. Here the choice is down to the textural requirement throughout the processes.

16.5.5 Raw materials matrix

In moving from the visible, through the static and on to the flowing properties of the products, the choice of raw materials to control these textural effects can be made. In all groups a range of raw material options are available that have some or all of the desirable characteristics and the ability to cope with the variable manufacturing and storage time, temperature and pH issues.

Within each raw material type, for instance starch, there are a number of variants which will give the full range of characteristics that the product development specialist can use to develop and control the textures. Alternatives include variations in the source of the raw material base, natural variations within the cultivars of the base and any modifications that are carried out later.

The scope of this chapter can highlight only (a) the main product categories that have the widest coverage and developed usage and (b) the principal effects that can be controlled. For a more in-depth understanding of these raw materials, including

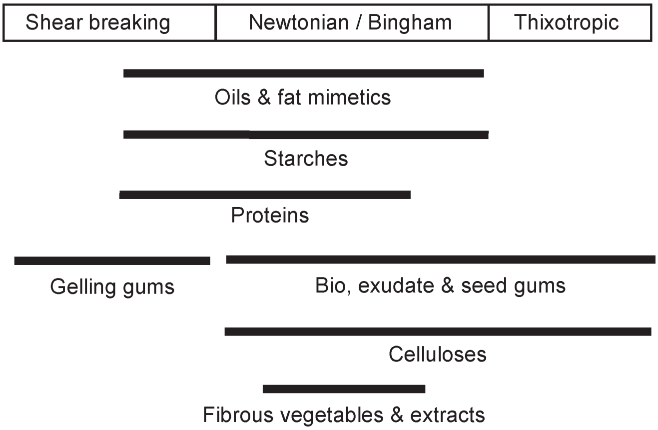

Fig. 16.10 Mechanical energy effect range options.

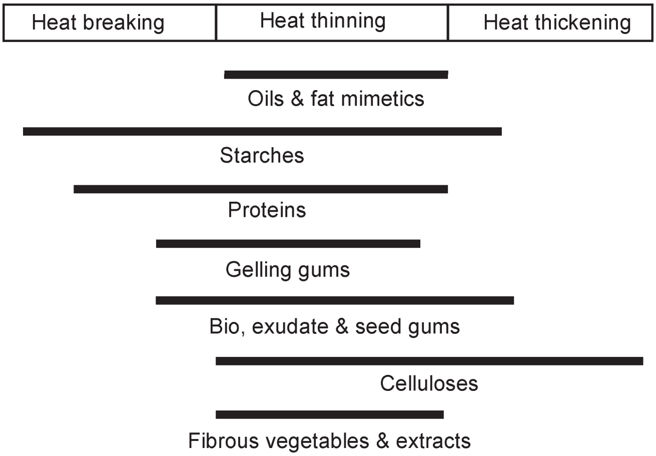

Fig. 16.11 Thermal energy effect range options.

the interactions at the molecular level, reference needs to be made to the relevant chapters on the raw material functionality. This will enable the product specialist to gain a deeper understanding of a particular raw material and how best to get the maximum texturising properties from it.

Tables 16.8–16.10 summarise and link the effects in a more traditional way: by raw material type.

Table 16.8 Selected thickening agents

Thickeners	'At rest' characteristics	Flow characteristics	Notes
Agars	Gelling	Gelled	Not used in soups, sauces, dressings
Alginate:sodium	Gels with calcium ions	Gelled, heat stable, thixotropic	Unstable in acidic conditions
Alginate:propylene glycol	Thickening and suspension agent	Thixotropic	Stable in acid conditions
Carrageenan: kappa	Gels with potassium ions	Low hot viscosities	Not used in soups, sauces, dressings
Carrageenan: iota	Gels with calcium ions	Low hot viscosities	Not used in soups, sauces, dressings
Carrageenan: lambda	Thickening and suspension agent		Suitable for thickening soups, sauces and dressings
Cellulose: methyl hydroxypropyl	Thickening and suspension agent	Heat thickening	Unique heat thickening properties
Cellulose: microcrystalline	Hydrates and gives viscosity to system	Thixotropic	Microcrystalline particles give opacity
Cellulose: carboxymethyl	Hydrates and gives viscosity to system	Thixotropic at low degree of substitution (DS) Bingham at high DS	Also known as cellulose gum
Exudate gums: Arabic, Tragancanth, Karaya	Low–high viscosity suspension agents		Gum arabic can act as an emulsifier Not generally used in soups, sauces, dressings
Gelatin	Gel forming	Gel will melt on heating	Only protein gelling agent Some use in dressings
Pectins: high methoxy low methoxy amidated	Gels under low pH or high solids Will act as thickening and suspension agents	Heat thinning	Total solids or pH controlled for gelling
Seed gums: guar tara locust bean	Thickening agents Good water binding agents	Generally heat thinning but heating helps initial hydration	Synergies with xanthan gum Low pH systems hydrolyse them
Starch: corn, maize, potato, rice, tapioca, waxy maize, wheat. Modified: cross-linked, substituted, oxidised.	Thickening and suspension agents Can be modified to gel if required Can be cook-up or pregelatinised	Generally heat thinning but heat is required at some point to activate and give viscosity	Need to be modified to reduce pH, heat and shear sensitivity Wide range of textures from smooth to pulpy textured Most commonly used thickening agents in soups, sauces and dressings
Xanthan gum: microbial exo-polysaccharride	Thickening and suspension agent	Will thin on heating but maintains a weak network	Thixotropic, shear stable, pH and heat stable

Table 16.9 Selected proteins

Proteins	Type	Properties	Comments
Milk derivatives	Whey proteins	Emulsifier/ stabiliser	Less heat stable but pH stable. A range of concentrates are available.
	Caseinates	Emulsifier/ stabiliser	Heat stable but not pH stable. Can have a range of counter ions including Na and Ca.
	Milk powders	For dairy notes	Contain all proteins, fat and carbohydrates.
	Cheeses	For cheese/dairy flavours	For cheese flavours and can add particulates to products.
Vegetable derivatives	Soya	Emulsifier	
	Pea	Emulsifier	
	Textured vegetable proteins	Meat substitutes	To add particulates to products.
Meats		Meat products	To add meat particulates to products.

16.6 Achieving the right texture

When a new development is commenced in a particular field, the first point of reference is often made back to any current texturising systems that either the manufacturer or a particular development specialist knows. Consumers are also used to the characteristics of the products that they have eaten previously and this can have a predetermined expectation for the texture that is delivered.

The key to controlling the textures of soups, sauces and dressings is to build up the knowledge of how the ingredients interact within the particular systems and then have the ability to change them by understanding what role they could play in a new product development. To complete the cycle, the different product types need to be built up with an understanding of the different types of textures and the routes to the final product. Ideally, this has to be carried out on an individual product basis but the nature of this chapter precludes discussion of every single example. The products can be reviewed in a number of broad product classes.

16.6.1 Fruity, low pH, clear sauces

Fruity, low pH, clear products are mainly found as sauces such as sweet and sour style products and, to a limited extent, in condiments and dressings. These sauces are typically non-emulsified products with little or no oil, emulsifiers or dairy products. By the very nature of the fruit they contain high levels of fruit sugars and other sweeteners and give the products quite high soluble solids. The acidic taste

Table 16.10 Selected fat mimetics and replacers

Starch derived including maltodextrins, and dextrins	Rice Potato Waxy maize Maize High amylose corn Tapioca Oats	Modified or hydrolysed starches or enzymatically treated starches to produce low DE maltodextrins
Sugars	Soy hemicelluloses Almond hemicelluloses Sugar beet hemicelluloses Polydextrose Inulin	
Cellulose	Microcrystalline cellulose Carboxymethyl cellulose Methyl cellulose Hydroxypropyl methyl cellulose Bacterial cellulose	Can be used in combination with guar, xanthan, carrageenan or maltodextrins
Fibres	Pea Sugarbeet Inulin Oats Wheat Soyabean Rice Corn Yeast	
Hydrocolloids	Carrageenan Gellan Xanthan/guar blends Konjac flour gel Citrus pectin gel	
Protein-based	Complete milk protein Milk, egg white protein, pectin Whey protein Pea proteins Soya proteins	Processed to give micro-particulates, be partially denatured, or make gels

is a result of the high levels of acids, which, in turn, have a low pH. From a visual point of view, these sauces have a high sheen and smooth finish and, in many cases, they have a high degree of clarity. They tend to be pourable with a good coating ability and thus have an intermediate texture length. The main bulk of the ingredients are fully solubilised to ensure there is good clarity, the only exception being the addition of fruit purees or vegetable pieces, such as chillies.

The high solids level gives the product a good body and mouthfeel with some viscosity, which is augmented by a thickening agent which will give a good, medium-length structure with suspension and clarity and yet be stable at the very low pH of the system. Tapioca starches show good clarity and structure in this application and can be modified to tolerate the low pH. Xanthan gum or pectins will fully solubilise and are stable to the low pH although care has to be taken with the pectin to ensure that the sauce will not gel under the high solids or low pH of the system. Combinations of starches and hydrocolloids can be used to balance the coating and thickening characteristics and reduce any long or slimy texture if hydrocolloids are used on their own.

16.6.2 Clear bouillon-types: soups, dressings

Clear bouillons and dressings are two extremes which share many common factors. Clarity and smooth texture are two of the important visual characteristics, often with suspended particles and they are differentiated from sauces by lower overall solids and viscosity. These products still have little or no oil, emulsifiers or dairy ingredients. As the clarity and smooth texture of the products are important, ingredients such as clarified hydrocolloids or potato and tapioca starches, which have very low residual protein or fat levels, can be used. The ingredients need to carry out two other functions: the first is to give suspension to particulates, especially for dressings; the second is to give a good body but with much lower solids.

In bouillon soups the pH is more neutral and the products are either pasteurised, short shelf-life products or fully sterilised. These products have a short texture but require a good body to give a full mouthfeel. Starches are the most appropriate thickening and suspension agents and can be complemented with low-viscosifying mouthfeel agents, such as oxidised starches, low-viscosity hydrocolloids such as guar gums or intermediate, 10–20 DE maltodextrins. These contribute to the mouthfeel but do not dramatically increase the solid and ensure good clarity.

For clear dressings, the pH is much lower (3.5–4.0) for longer shelf-life, ambient-stored products. Clarity is still important but these products have a longer structure so that they can cling and have a good coating ability on salad leaves or other products. Hydrocolloids such as xanthan, guar, locust bean gum or synergistic combinations predominate, although there are limited applications for starches, and carrageenans and pectins in the more gelled dressings such as mint sauce/jelly. As these products can be made cold, there is less of a problem from the combination of heat and low pH breaking down the hydrocolloids or starches. The products do need to be resistant to breakdown by the high shear in the process.

16.6.3 Gravies, dark-coloured products

The last main class of smooth glossy products, where there is little or no fat, is of the highly flavoured and coloured products, which include gravies and barbecue-type sauces. This category follows many of the same attributes as other clear products but has the added advantage that clarity is not an issue. This permits the use of thickening agents, such as the widely used waxy maize starches, where clarity is not such a major issue.

The only exceptions within this category are gravies where the stock has been produced from the meat itself. These can contain the extracted animal fat, which is not emulsified and remains as a layer on top of the gravy. Generally, the consumer mixes the fat layer in at the point of home preparation. These products sometimes contain gelling agents, such as gelatin or alginates, which thin on the reheating stage to give the smooth liquid product with a melt-in-the-mouth characteristic.

16.6.4 Spicy dishes

The main types of spicy products are Mexican and Indian and certain Asian dishes, although this category extends to any product which is extensively flavoured with spices. From a visual point of view, the very nature of the spices means that the products have a non-glossy textured surface. They are high in other particulates which naturally bind water together and this tends to give the products quite short texture. A certain number of the products contain oil but, in many of these cases, the oil is not emulsified. The appropriate thickening agents are starches or water-absorbing fibres, which support this short, non-glossy appearance and are able to hold the oil distributed within the matrix.

16.6.5 Tomato-based chunky or ketchups

A natural extension of the spicy-based products leads into a whole class of products based around one ingredient. Tomato-based products are extensive and products range from a smooth, puréed nature through to coarse and chunky. These products do not contain significant amounts of oil or emulsifying agents. The range of solids varies dramatically from low to over 40%, constituting tomato solids, sugars and salts. The pH is typically in the range of 3.5–4.0, as many of these products are pasteurised, ambient stable, long shelf-life products. Soups which may have been sterilised are the only exception.

The widest range of structures, visible textures and flow properties are in this class and thus the greatest diversity of thickening and suspension agents are used. In the chunky or pulpy, short-textured products, fibres or pulpy potato starches enhance the product characteristics. In smoother, short-textured products, smoother starches based on waxy maize or tapioca can be used. A longer texture with improved coating can be gained by using hydrocolloids. This is more common in condiment sauces, where xanthan gum, guar locust bean gum or pectins have been used. Synergistic combinations of starches and hydrocolloids allow a full range of textures and properties to be attained.

16.6.6 'Cream of . . .' style

'Cream of . . .' style products are the first types where emulsifiers and emulsified fats are introduced. The choice of emulsifiers depends on the nature of the texture, flavour, processing conditions, pH and other ingredients in the system. The emulsion gives the products a short to medium structure with a smooth visual texture. Particulates can be added after a homogenisation stage to change the visual appearance if required. From a thickening and textural point of view, the small granular starches with their short, smooth texture are the first choice. Xanthan gum can also be used to give a slightly longer texture, which is useful for cling and coating abilities. Synergistic starch and xanthan combinations can be used to improve the overall body and mouthfeel of the product.

In the health-conscious consumer's modern climate, reduction in fat intake always features highly on the product developer's agenda. As a result, an extensive array of fat replacers, mimetics and other functional raw materials have been developed (Roller and Jones, 1996). These work by giving the same mouthfeel and body as the fat droplets they are replacing. The interactions between the fat replacers and the rest of the system are critical to ensure that the right structure, texture and stability can be achieved.

16.6.7 Salad dressings

The salad dressing category is, in many ways, similar to the 'cream of . . .' style sauces, except for the fact that the emulsified fat tends not to be thermally processed and the system is pH-controlled. Salad dressings range from short to long texture, like spreadable products, to those suitable for coating other meal constituent, such as salad leaves or pasta. There is an overlap and a grey area between where a salad dressing ends and a mayonnaise begins, although dressings tend to have a lower oil content and are more highly flavoured.

The choice of emulsifier is more flexible when no heating is required after emulsification. Egg, dairy or vegetable origin, such as egg yolk, whey or pea proteins can be used. In dressings, the soluble solids are high enough to give the product a good body and, as in sauces, fat replacers or mimetics as well sugars and salts can contribute to this. The nature of dressings means that there is a preference for good coating, pH stable and shear resistant thickening agents, such as pregelatinised starches, hydrocolloid gums like xanthan, propylene glycol alginate, guar and locust bean gums, or their synergistic combinations.

16.7 Improving texture and the use of new texturising agents

The desire of the product development specialists to create new textural solutions is due to the need to constantly stimulate consumers and the marketplace. There are two principal areas that are being pursued. The first is to find new uses for current texturising agents by applying the general functionality of the ingredient in a different way. In these cases modifying how the raw materials are processed is the simplest route, such as the use of alternative equipment or changing the order of addition of ingredients to change the way they interact in the system.

The second is where the ingredient manufacturers are trying to improve the functionality of the raw materials. This includes improving the flexibilty and tolerance of the raw material to previously incompatible conditions, for instance different shear, heat or pH levels. The manufacturers also work with the base material and make changes by controlling the biochemical pathways of the raw materials. The functionality of the base raw material can be changed to give it other, unique textural properties. An example of this is a new product that can give long structures but is rapidly digested orally so that they melt away in the mouth and are not slimy.

The desire in all cases is to provide unique and differentiated solutions to give the suppliers and manufacturers an advantage against their competitors in the marketplace. Details of particular textural solutions are best gained from the relevant suppliers since these are constantly being updated as new knowledge and insight is gained.

16.8 Future trends and conclusions

The market for soups, sauces and dressings is under rapid change as a result of a number of trends in the marketplace. These trends are directly related to the changing lifestyles of consumers and the degree of interaction and skill that is needed to prepare the products. There are some principal drivers for the whole food market, not purely the textural part: ease of preparation, wholesome, good quality and authentic.

Ease of preparation is key because of the demand for speed, with a minimum level of involvement or skill and yet still resulting in a good quality, textured product. Skills in the kitchen for preparing meals from scratch is diminishing and thus ease of preparation is important. The changing pattern of meals is towards more single-serve portions that can be consumed while people are on the go with no chance of any further textural generation by the consumer.

Authenticity of flavours and textures is an on-going factor, especially with people travelling more extensively and tasting a wider variety of authentic dishes in their home market. This always challenges the developers as they have to be able to make the dishes in a manufacturing environment but with the authentic touch, unique to a chef style preparation.

Heathly and wholesome food consumption is another key area. Healthy involves low-fat, balanced products which contrast with and yet complement wholesome foods where the consumer wants to feel that they are eating products that are clean, pure, natural and with no additives.

Closer integration between suppliers and end product manufacturers is one of the product developers' main working environment changes. This approach, to the extension to their own development teams, allows new materials and texturising solutions to be be evaluated in real products and improve their speed of delivery to market.

The outlook for the segment is very positive as there are many new meal occasions for the developers to cater for and thus generate texture. These new combinations have to be managed through the development process so that the needs of the consumers are linked to processing, packaging and business successes for the manufacturer.

Controlling the textures of soups, sauces and dressings is a complex process where many different factors are involved. Throughout this chapter it has been important to consider the end use of the product and constantly to refer back to it. This is why the process started with the whole product and broke it down, via various ingredient classes through to the individual components. Once it was clear what each raw material could provide, the soup, sauce or dressing could be reconstructed to produce the desired outcome, with an understanding of the functionalities of the various ingredients.Then the product development specialist has a basis for developing new products and understands the key components that need to be added to make this up.

By reviewing the relevant chapters on the individual raw materials, the whole product can be put into context and the specialist will be able to make the best choice in order to create the desired, functional and appropriately textured product to delight the consumer.

16.9 Sources of further information and advice

There are a wide range of sources of further advice which the product development specialist can consult in order to obtain more information about texturising soups, sauces and dressings. First there are the technical representatives and scientists from the many raw material suppliers, who work to gain a detailed understanding of the functionalities of the raw materials. This can be further enhanced by application specialists, who combine the raw materials to give customers the end products. These people either work for a raw material supplier, an intermediary, such as a product development consultancy, or can be based in-house within the larger food manufacturing organisations. The next channel is through the various food research organisations or university groups, which can offer consultancy advice, training courses or access to resources that are not easily available to all manufacturers.

There is an extensive range of literature, ranging from periodicals, reviews, meeting proceedings or texts that can be digested to yield an in-depth understanding of particular raw materials, classes of raw materials or particular processes. A great deal can be found by searching on the Internet. Care has to be taken when carrying out searches as a great deal of time can be spent trying to locate particular references. It is also worth remembering that a lot of the information is often promotional and thus not always impartial. However, there is a good deal that is invaluable since it is possible to search various food science databases, obtain new information about particular ingredients through supplier sites or read a range of food science courses that are published. As the search addresses on the Internet can change rapidly I have not added them as this could lead to potential confusion but it will be possible to find many of these by using an appropriate search engine.

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