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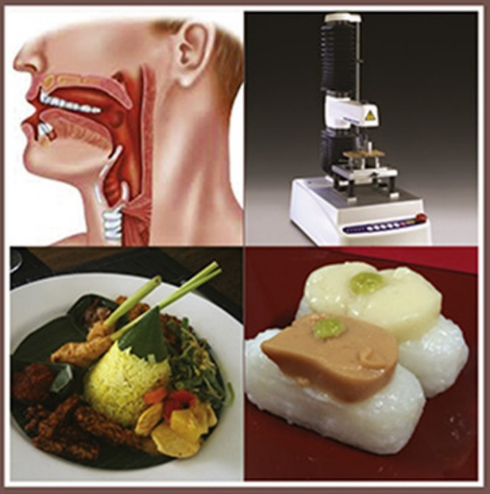

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Volume 1: Novel Ingredients and Processing Techniques

Edited by Jianshe Chen and Andrew Rosenthal

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Modifying Food Texture

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Preface

Food texture is a key sensory feature not only well appreciated by consumers but also used by consumers as a quality indicator of a food product. The study of food texture has become a very active scientific area of the food science and technology and has received growing attention from scientists of food science and related disciplines. In the past few decades, some significant progresses have been made in the fundamental understanding of the textural properties and practical applications of such knowledge in the development of healthy and tasty food products. The major developments in food texture study can be briefly summarized in three areas. The first achievement is a much better understanding of the underlying scientific principles of food texture and its sensation and perception. This is shown by the establishment of the physical, mechanical, and (micro)structural nature of food textural properties and the associated physiological and psychological factors that influence texture sensation and perception. Such developments provide a solid foundation for food texture study as a scientific discipline. Second, objective assessment of food textural properties has always been a research focus for its important implications to both fundamental understanding and industrial applications. A wide range of instrumental devices and experimental techniques have been developed, and many of them are now commercially available. These include various rheometers for the precise characterization of texture-related physical and mechanical properties, various empirical devices for fast characterization of some specific textural properties, and various instruments that mimic eating and oral processing and offer easy quantification of textural properties and prediction of consumers' perceptions. The third major development is the emergence of food texture modification in the past few decades for the purposes of either improved textural properties of a food product or altered texture of a food to suit the needs of consumers who have difficulty consuming normal food. This area has attracted much attention currently from both academic and industrial researchers due its close relevance to the fundamental understanding of food texture and microstructure and the growing needs in industrial applications. It is this latest development that has led to the production of this book.

The driving force behind the increased activities in food texture modification comes from two very different needs: the growing demands from consumers for healthy tasty food and the urgent needs of properly texturized food for safe food consumption by some specific consumer groups. For the former, food manufacturers have to find a balance between the health benefits and the sensory enjoyment of the food. With fast-changing lifestyles and the abundant availability of food, an oversupply of nutrients leads to negative consequences to human health and therefore becomes a concern to governments as well as consumers. Overweight and obesity are the two most obvious health-related problems as a result of food overconsumption. Food with

reduced contents of fat, sugar, and salt has become a preferred choice by many consumers. However, the major technical challenge to the food industry is not to reduce these components, but how to address the balance between the health benefit and sensory enjoyment. As a general principle, a food must be able to provide sensory enjoyment, a key function of the food that has profound influences on one's psychological and social well-being. A product that provides no sensory pleasing effect simply can hardly be categorized as a food, however healthy it claims to be.

Another very important reason for texture modification is the urgent need of properly texturized food for safe and easy consumption by some specific consumers who have difficulty in consuming normal food, including infants, elderly populations, and hospital patients. Proper modification of food texture is absolutely essential for the safety and well-being of these consumers. In recent years, provision of texture-modified food has been seen as a great business opportunity to the food industry. With a forecasted rapidly growing elderly population, a vast and fast-growing market is predicted for texture-modified food and also for functional ingredients and techniques needed for texture modification. Major food companies have put a lot of resources and efforts into exploring this opportunity, though many technical challenges remain to be solved.

The aforementioned two challenges imply the urgent need of the knowledge and techniques by the food industry on texture modification of food products. The focus of this book is exactly for this need, by addressing various aspects (both technical and practical) of food texture modification and specific needs of disadvantaged consumer groups for texture-modified foods. Unlike other textbooks where knowledge and applications are often the main focus, the primary concern of this book is on consumers' needs and well-being. Five essential aspects of food texture modification, including ingredients, methodologies, processes, products, and target populations, are arranged into two sequential volumes.

Volume 1 begins with our chapter to introduce the entire concept of texture modification. The chapter outlines the background knowledge of food texture and discusses progress in food texture study from its historical perspective. By setting food texture in its broad context, the chapter also explains the structural aspects of texture creation, texture oral breakdown, and oral appreciation. This is followed by chapters on the use of some novel food ingredients as effective functional components for texture formation and modification. This includes a chapter by Dr. Lin Chen on the food emulsifiers for microstructure creation of dispersed food systems; a chapter by Dr. Christos Ritzoulis and Dr. Panayotis Karayannakidis on food proteins, their structure-forming and stabilizing effects and various factors that influence such functionalities; and a chapter on another very important structural forming ingredient, the enzymes, authored jointly by Drs. Dilek Ercili-Cura, Thom Huppertz, and Professor Alan Kelly. The third part of volume 1 is on the methodologies for texture creation and modification, which include a chapter by Dr. Shekhar Kadama, Dr. Brijesh Tiwari, and Dr. Colm O'Donnell on the improved thermal processing techniques for much desirable texture; a chapter by Dr. Cheryl Chung and Prof. David J. McClements on the structure and texture of food emulsion products and the use of emulsification technique; a chapter by Dr. Hassan Firoozmand and Prof. Dérick Rousseau on

the phase behavior and controlled phase separation for texture modification; and a chapter by Drs. Morten Dille, Kurt Draget, and Magnus Hattrem on the emulsion gels and the use of filler particles for texture modification. The last part of the volume 1 deals with the texture of two major types of food products: a chapter on cereal breakfast and extruded products (by Dr. Frédéric Robina and Prof. Stefan Palzerb); and a chapter on soy-based products, a type of Oriental food growing popular in Western countries (by Dr. Jian Guo and Prof. Xiao-Quan Yang).

Volume 2 has two main focuses: the characterization of food textural properties and the needs and requirements of texture modification of target consumers. The volume begins by a chapter by Dr. Fumiyo Hayakawa on the vocabularies and terminologies of food texture and texture appreciation. This is followed by three chapters on the characterization of the textural properties of food, by taste panel analysis and by quantitative measurements using various instruments. Statistical analysis of taste panel data is discussed in detail by Dr. Peter Ho, whereas instrumental characterization of food texture are dealt with separately by Dr. Andrew Rosenthal on solid and semisolid (or soft solid) foods and by Drs. Guido Sala and Elke Scholten on fluid food. The second part of volume 2 contains topics on the need of texture modification for target populations. This include a chapter on the "free from" food by Drs. Maria Papageorgiou and Adriana Skendi, in which practices and techniques applied for the design and production of gluten-free food are discussed as a typical example. The need of texture modification for elderly populations are addressed in a chapter by Prof. Lisa Duizer and Ms. Katy Field on the weakened sensory capability of elderly and a chapter by Dr. Elisabeth Rothenberg and Prof. Karin Wendin on the practices of texture modification for elderly consumers who have developed difficulties eating and swallowing due to natural aging. Food provisions to hospital patients, in particular those who are diagnosed with dysphagia, are given in the chapter by Dr. Julie Cichero. On the other end of the spectrum, infants and babies also have specific requirements of food texture due to incomplete development of their eating capability. Texture modification of infant food is discussed in detail in a chapter authored jointly by Drs. Sophie Nicklaus, Lauriane Demonteil, and Carole Tournier. Finally, the book concludes with a chapter by Mr. Derek Johnson on the legislation and practices for texture-modified food for institutional food, with a particular focus on the cases of hospital food provision in the United Kingdom.

In addition to a brief abstract and a list of keywords for convenience of literature search, each chapter also has a final section that provides further relevant information so that readers can expand their reading when it is needed. We hope that, by addressing some key aspects of food texture modification, this book provides the knowledge and guidance urgently needed by R&D researchers in the food industry and those who seek to explore new business opportunities in the growing competitive global market. Many chapter topics of the book are also closely relevant to some food science textbooks, and therefore this book could also be used as a useful reference for undergraduate and postgraduate students studying food science and technology. However, we must also express our slight regret that the topic coverage of the book has not been as wide as we had initially planned. During the editing process, we had to drop a few chapters, largely due to a lack of literature on those chapter topics.

We would like to take this opportunity to thank all the contributors; their expert knowledge, enthusiasm, and hard work enabled us to put together a unique food texture book from very different scientific perspectives. We would also like to thank editorial staff at Woodhead (now Elsevier) for their support and advice through this process. And finally, we would like to thank our families for bearing with us through the long nights and weekend hours.

Jianshe Chen (Hangzhou, China)
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Part One

Food texture: an overview

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Food texture and structure

1

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1.1 Introduction

Texture used to be a term for the sensory description of the structure, feel and appearance of fabric materials. It was not until the middle of last century, when food production turned into industrial scale and a food science and technology degree was introduced for the first time, that texture became a sensory term for food description. The earliest literature record of food texture was made in 1949 by Carlson and Hoelzel (1949) in the journal *Science*. In the 1960s, food texture was commonly accepted as a key quality factor and was recognised as an important research area of the food science and technology discipline. The textbook *Food Texture*, published in 1962 by Matz (1962) was probably the most representative work of the early stage food texture studies. Since then, research activities in food texture have grown so fast that *Journal of Texture Studies* was launched in 1969 to disseminate research findings in this area. A quick search on Scoups shows a fast-growing number of published articles on food texture and related topics. In the entire decade of the 1960s, there were only 18 publications. This number increased to 107 in the 1970s, 217 in the 1980s, 741 in the 1990s and 3711 in the 2000s. In the past 5 years, over 700 food texture-related research articles were published each year.

The continuously growing activities in food texture research have been first driven by the need for fundamental understanding of human sensory perception towards food, in which food physics (in particular food rheology) and sensory psychology have been the most active areas. Another major factor in the study of food texture came from the food industry, driven by the need for functional ingredients and innovative technologies for improved textural quality of food and the need for reliable instrumental characterisation of textural properties for quality assurance and consumer preference prediction. In recent years the food industry is under a growing pressure to produce healthy food for the needs of some specific consumer groups: the fast-growing population of obese and overweight and the fast-growing population of elderly people. For the former, healthy food with significantly reduced energy density is required. However, for elderly and other vulnerable consumers, safety and convenience of food consumption is the top priority. Texture modification plays a key role in the design and manufacturing of quality food for both consumer groups.

Food texture study in the last half century has led to some major achievements that can be summarised in three areas. The first major achievement is on the fundamental understanding of the physical (mechanical) nature of food texture (van Vliet, 2002).

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Structure and structural deformation have been recognised as the core of texture sensation, and how a food material resists deformation is the key determining factor. Based on this recognition, food rheology was once seen as a base of texture study, but limitations of the approach were later pointed out by Bourne (1974). Fundamental physical principles associated with food fracture and deformation have now been superbly explained by van Vliet in his recent book (van Vliet, 2013). The second major achievement is the quantitative and semiquantitative methods for the characterisation of texture properties for a wide range of food materials, from thin liquid beverage to very cohesive paste, from a wobbly gel to a hard solid candy. The latest progresses in this area have been nicely summarised by Kilcast in his latest textbook, in which various instrumental approaches are discussed for texture characterisation for a wide range of food categories (Kilcast, 2013). The third major development has been on texture modification, a hot topic that emerged in the past decade driven by consumers' needs. The use of alternative ingredients and the application of novel processing techniques are probably the two main approaches adopted by the food industry for both texture creation and texture modification.

With this background in mind, this book aims to address the concerns of the food industry in terms of the use of novel food ingredients and new processing techniques for food texture modification. The emphasis of the book is on the needs of specific consumer groups, in particular those disadvantaged users such as elderly, hospital patients and infants. As an introduction, this chapter will discuss concepts of food texture, what it means and how it is defined. Food structure and its important implications to texture sensation and texture characterisation will also be discussed in this chapter.

1.1.1 Food texture, definition and classification

The real sensory meaning of food texture varies hugely among consumers as well as among food researchers. There has been a great effort from food researchers to agree on the definitions of texture terms. Based on the general consensus that texture is about the constitution, structure or substance of *anything* with regard to its constituents and formative elements, a number of definitions have been produced. A few of the most commonly referenced ones are given here:

- "All the mechanical, geometrical and surface attributes of a product perceptible by means of mechanical, tactile and, where appropriate, visual and auditory". International Organisation for Standardization (1992)
- "Primarily the response of the tactile senses to physical stimuli that result from contact between some part of the body and the food. The tactile sense (touch) is the primary method for sensing texture but kinesthetics (sense of movement and position) and sometimes sight (degree of slump, rate of flow), and sound (associated with crisp, crunchy and crackly textures) are also used to evaluate texture". Bourne (2002)
- "Texture is the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinaesthetic". Szczesniak (2002)

All these descriptions indicate that food texture is a sensory experience of multiple sensory stimuli working in combination and synchronisation. The texture of a food originates from its structure or microstructure (from the molecular level to the microstructure and macroscopic level). The sensation of food texture depends highly on how the structure deforms and breaks when handled and eaten. Therefore, any factor (e.g., ingredient interactions, processing conditions, storage and packaging, etc.) that influences the structural properties of the food will affect its texture.

According to the preceding definitions, textural properties of food can be categorised into three groups depending on the sensation mechanisms: the visual, the acoustic and the tactile. For visual texture, properties such as smoothness, glossiness, thin and viscous are the most common examples. The appearance and light reflection of the food determine visual texture (Chen, 2007). Texture properties associated with hearing are typically represented by the crispness and crunchiness, the two most beloved texture features linked to the noise produced during the fracture of a solid food. Such texture features are closely associated with other sensory stimuli detected by mechanoreceptors. Internal scull vibration has also been shown to be important to the sensation of these texture features (van der Bilt et al., 2010). Of all texture features, tactile texture is probably the most common and often the core focus of texture study. Tactile texture features are sensed by the direct contact between the food and human skin (hand or oral surfaces).

It has recently been proposed that sensation of food texture could be made through two very different physical principles: oral rheology and oral tribology (Chen and Stokes, 2012). Based on this theory, texture properties could also be categorised as texture associated with bulk deformation (rheology-originated; e.g., firmness, springiness, cohesiveness), texture associated with relative surface movement (tribology-originated; e.g., smoothness, roughness) and those sensed through combined rheology and tribology mechanisms (e.g., slipperiness, creaminess) (Chen and Stokes, 2012).

A big difficulty of texture analysis is the very wide range of texture terminologies and the very different use of textural vocabularies among different cultures and consumer groups. Hayakawa et al. (2013) conducted a comprehensive study of texture terms among Japanese consumers and noted as many as nine major categories, each consisting of a long list of textural terms and descriptions. More detailed analysis of texture terminologies and classification can be seen in Chapter 1, volume 2 of this work.

It is also a common knowledge that texture sensation occurs simultaneously with the appreciation of the taste and aroma. However, which of these three contributes most to one's sensory experience and liking of a food product has caused disputes among food researchers. However, most food researchers now agree that all these sensory features play an indispensable role in influencing consumers' liking and preference, though through very different mechanisms. Taste and aroma are based on molecular mechanisms and are sensed by chemosensors in the oral and nasal cavities, respectively. Molecule release, diffusion, and detection are the dominating mechanisms of these sensory features. In contrast, texture sensation is largely a physical mechanism, where mechanoreceptors are the main devices for the detection and sensation of such features. Even though texture sensation has little dependence on

molecule release and diffusion, the textural nature of a food often has a direct influence on the release and diffusion of taste and aroma molecules. Because of the very different underlying principles, food texture has historically been perceived as an independent scientific discipline, as is taste and aroma.

1.2 Mapping food structure

The structure of modified foods depends to a large extent on the ingredients that make it up and on the processes involved in their manufacture. We identify two key parameters in the perception of the structure, being moistness–dryness and the degree of openness/porosity. Of course, moistness is not just a measure of water content, for many foods are made moist by the presence of fats and oils. Consequently, we took it upon ourselves to attempt to map the variety of food structure, expressed in terms of the water and the fat content as measures of moistness–dryness. A frequently used way to represent water content is on a dry weight basis, that is, the percentage of water present divided by the dry matter. Taking this concept a stage further, it is possible to express both fat and water on a fat-free, dry weight basis (FFDWB). Taking data from McCance and Widdowson's Composition of Foods Integrated Dataset (Institute for Food Research, 2002), we are able to select example foods with varying water and fat contents. The range of values on a FFDWB tend to range from about 1/1000 up to about 1000, and the data can be compressed on a log:log plot to yield a structure map (Figure 1.1).

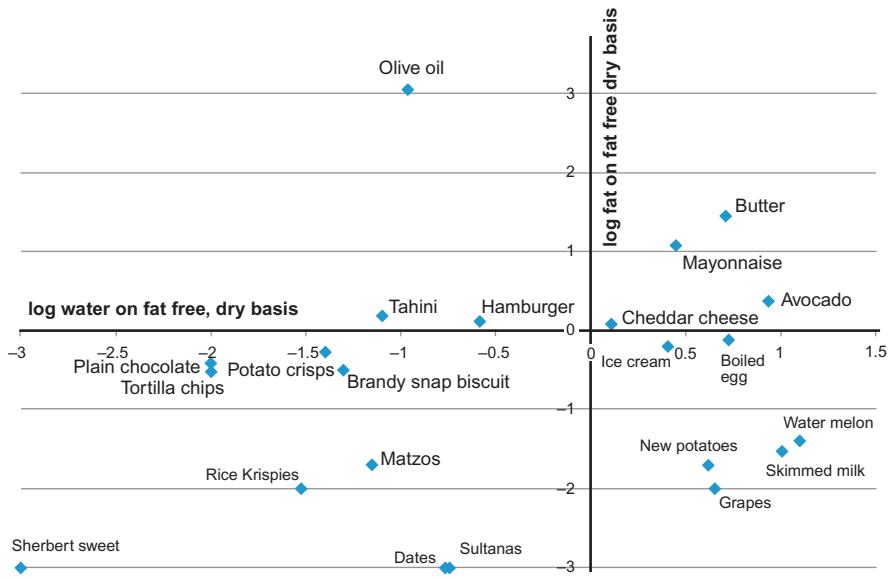

Figure 1.1 A log:log map of food composition and structure.

Of course, when the water has the same mass as the fat-free dry matter, the value on an FFDWB would be 1. When this is transformed to a logarithm, it becomes 0. Thus the intersection of the axes corresponds to equal amounts of water (or fat) to fat-free dry matter.

The foods on this two-dimensional map, start to cluster together:

1. Fresh fruit and vegetables are in the bottom right-hand corner—with high-water and low-fat content. An obvious exception among this group of foods is avocado, whose higher fat content shifts it over the fat on the FFDWB axis.
2. The top right quadrant with high-fat and high-water foods contain emulsions like butter and margarine.
3. Top left are the oils and fats—their extreme values of fat on FFDWB are due to their purity with virtually no fat-free dry matter.
4. The bottom left with low water and low fat are dry foods in the form of crackers, crisps, chocolate and sugar confectionary, as well as foods preserved by drying such as dried fruit. By extension of the concept of FFDWB, foods that are low in both water and fat must be rich in one or two of the other major food components (i.e., proteins or carbohydrates), and in the case of this quadrant, the food's fat-free dry matter is generally carbohydrate in the form of starch or concentrated sugars achieved through drying of fruits.

The third dimension of solidity—openness of structure—can be added, which helps us to separate products. For example, in the area of sugar confectionary, we can manufacture hard candy (hard boiled sweets) or cotton candy (candy floss). From an FFDWB perspective, both products have similar water and fat contents, yet they are very different in terms of openness of structure. Quantification of openness of structure is difficulty, and although there are related characteristics such as density or porosity or overrun or specific gravity for which limited data is available, for now it will be left as a notion/'guesstimate' based on anecdotal experience. Figure 1.2 is obtained by rotating Figure 1.1 and attempting to add the third dimension in perspective. Now we can add arrows to some of the data points such as Rice Krispies or ice cream to drag the point from the 2D plane to roughly where they should lie in 3D.

Generally, the structure of fresh foods is due to the biological organisation of the tissues in the raw materials, thus fruits and vegetables tend to depend on elements such as pectin cementing cells together, turgor pressure and botanical tissues such as sclerenchyma. Similarly, meat is derived from muscle, and its texture depends on the quantities of the different proteins present in the muscle, the conditions of slaughter and the postmortem changes. In contrast to these observations, the structure of manufactured foods depends far more on the chemical composition and interaction between ingredients and components during processing.

As this book is about modified texture, we will point the reader to existing reviews on the texture of fresh foods; however, we will, of course, consider how the structure and texture of fresh foods change during domestic food preparation. The rest of our attention will be directed to foods that have been constructed or reconstructed through a manufacturing process or those foods whose structure has been deliberately modified to meet the needs of a particular group of consumers (e.g., the elderly).

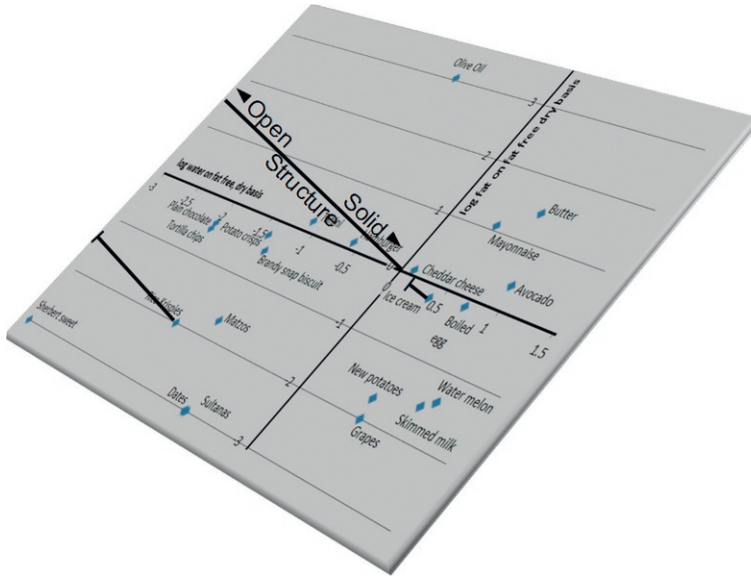

Figure 1.2 A 3D representation of food structure.

1.3 Textural changes during preparation

1.3.1 Domestic food preparation

1.3.1.1 Fruits and vegetables

The structure and texture of fresh vegetables and fruits has been comprehensively reviewed by Edwards (1999). There is no uniform basis for structure in vegetables and fruits, for example, pineapple owes its texture to bundles of fibres that fail along planes parallel to the bundles when compressed; in contrast, watermelon collapses in on itself when compressed (Peleg et al., 1976).

Domestic food preparation of fruits and vegetables often commences with cleaning and peeling operations to remove surface soil, surface defects and less-attractive skins and outer layers. Such operations will lead to a degree of softening by removal of inedible outer layers. Subsequent size reduction through cutting influences the bite size for later consumption, but more important, it limits the minimum distance from the surface to the centre as well as increasing the surface area to mass ratio—these factors are important if heat is to be applied, as they affect rates of heat penetration and 'cooking' as well as leaching of soluble plant material.

The types of operation need to be distinguished in terms of the heating medium.

Boiling and steaming

In domestic boiling and steaming at atmospheric pressure, the surface temperature does not exceed 100 °C. Importantly, the surface of the food does not dry out, and we may observe absorption of water as well as leaching of water-soluble components.

Although it is not a domestic process, canning is effectively wet cooking albeit at a higher temperature and will also be considered here.

The high-water environment is an ideal situation for starch pasting and gelatinisation, whereby starch granules swell, losing crystallinity through the absorption of water. The overall effect is a softening and the creation of a gel when cooled.

Pectin solubilisation is also favoured in such aqueous environments at elevated temperatures. Moreover, pectin solubilisation is also influenced by pH and dissolved minerals in the cooking water, whereby divalent cations (e.g., Ca^{2+}) will cross link, aiding pectin retention and leading to firmer materials after cooking. Conversely, the addition of bicarbonate will soften vegetables during cooking.

Turgor pressure of plant cells requires intact and biologically functioning cell membranes. Application of heat and the subsequent denaturation of proteins results in inactivation of bound enzymes that control the water and salt balance. Turgor is no longer established, and the tissues are irreparably softened.

Whatever the mechanism of loss of texture, the rates of softening of vegetables have been studied during wet cooking. Softening of vegetables during cooking is thought to be a first-order reaction, though over long periods of cooking such a model does not fit so well. Huang and Bourne (1983) identified different mechanisms and speculated that one is due to changes in pectin in the middle lamellar.

As a corollary to thermal sterilisation, the idea of *C* values (cook values) to suggest degrees of degradation of nutrients and sensory attributes (Holdsworth, 1985), sadly the idea has not been taken up as extensively as would have been useful to predict changes in texture or the underlying structure.

Dry cooking

During frying, roasting, grilling, baking and griddling, the surface temperature of the food frequently exceeds 100 °C. The surface can dry out and form a crust along with Maillard browning, which results in both characteristic flavours and colours. As with wet cooking operations, starch gelatinisation still occurs within the tissues in the bulk of the food as is a loss of turgor through protein denaturation of enzymes in the cell membranes. However, pectin solubilisation is more limited.

1.3.1.2 Flesh foods

Unlike fruits and vegetables, meat is derived from a living organism, though normally the animal has been dead for a while—this results in biochemical changes to proteins in muscle through postmortem glycolysis, resulting in a lowering of the pH and subsequent precipitation. Meat is predominantly derived from muscle, and the fibrous structure persists. In addition to the contractile proteins, the connective tissue—notably collagen—has a profound impact on texture. The structure and flesh foods have been comprehensively reviewed by Greaser and Pearson (1999); they outline some of the factors that contribute to the structure and the texture as well as discussing ways to modify them. These include enzymic treatment with proteases, marinating, prerigor cooking and curing.

1.3.2 Construction and reconstruction of foods from food ingredients

The previous section of domestic cooking dealt with the preparation methods commonly undertaken in the home. Although some of the ideas pursued in the rest of this chapter could be undertaken in a domestic kitchen, they are more akin to a manufacturing situation. Furthermore, whereas the previous sections dealt with fresh food, the rest of this chapter looks at products derived from food ingredients. This is to say that the original foods have been 'deconstructed' into constituent parts, for example, the food may have been milled or particular fractions of the original food may have been separated, for example, the starchy endosperm of cereal grains or a refined oil with a particular melting point fraction.

The purpose of this chapter is not to explain how ingredients are created or to consider technical details of unit operation that we discuss. This chapter is more an overview of principles and ideas. We have divided the operations/processes that are used to achieve a modified texture into two categories, which are shown in Table 1.1.

Before dealing with particular food groups, it is useful to consider some underlying principles involved in the techniques for structure modification.

1.3.2.1 Lapalace excess pressure

Colloidal systems (e.g., foams, emulsions, suspended crystals) are all influenced by the interfacial tension between the continuous phase (e.g., liquids, solids) and the dispersed phase (e.g., bubbles, droplets, particles, crystals). This interfacial tension exerts a stress on the dispersed phase such that there is a pressure difference inside the dispersed bubble (for the rest of this section, we will deal with foams, i.e., gas bubbles

Table 1.1 Techniques to achieve structure modification

Openness modifying procedures/operations	Firming/solidifying procedures/operations
• Aeration – Whisking – Gas evolution ○ Leavening agents ○ Microbial fermentation – Sparging • Flash evaporation—e.g., extrusion-cooking • Puffing • Cooking in oil, i.e., frying and roasting • Drying – Air drying – Freeze drying • Emulsification	• Hydrocolloids • Thermal setting, e.g., during baking – Starch gelatinisation – Protein denaturation • Crystallisation • Formation of glasses

suspended in a liquid continuous phase—though the principles are the same for other colloidal systems) and the bulk of the continuous phase. This excess pressure (ΔP) is proportional to the interfacial tension (γ) and the radius of the bubble (r).

$$\Delta P = \frac{2\gamma}{r}$$

Consider bubbles formed by whisking egg whites. The excess pressure in small bubbles is greater than that in large bubbles. This results in different concentrations of dissolved gas in the egg white adjacent to the small and the large bubbles. This concentration gradient gives rise to diffusion from the vicinity of the small bubbles toward the larger ones. As gas diffuses away from the small bubbles, the equilibrium is disturbed, causing more gas to dissolve from the bubble to the surrounding egg white, but in doing so, the bubble volume shrinks, leading to reduction in the radius and consequently a rise in the Laplace excess pressure. This is a self-perpetuating cycle, and the small bubbles shrink until they disappear while the larger bubbles continue to grow. This phenomenon is referred to as disproportionation (in the case of foams). Similar phenomena occur in emulsions, where it is referred to as Ostwald ripening, and in crystals, where it is referred to as crystal growth.

A further consequence of the Laplace excess pressure is that when the radius is almost zero, the excess pressure is virtually infinite, and this prevents the spontaneous creation of bubbles or droplets or crystals. Thus, to produce a bubble, we normally require mechanical motions, such as whisking of batters or mechanical mixing and folding of doughs. Gas production from chemical leavening agents or yeast fermentation is unable to produce a high enough pressure to generate *de novo* bubbles. Instead, the gas dissolves in the continuous phase and diffuses to existing bubbles, which consequently grow.

Just as bubble formation in foams is hindered by the Laplace excess pressure, so too is ice crystal formation during the freezing of foods. Generally ice crystal formation is considered a two-step process, being nucleation and crystal growth. The Laplace excess pressure prevents ice crystals from forming spontaneously, and homogeneous nucleation can only occur if the food is supercooled by rapid heat removal. More commonly ice crystals grow on inhomogeneities in the material such as cell organelles. Once nuclei exist, they continue to grow through further deposition of water on the existing crystals and due to the Laplace excess pressure causing the larger crystals to grow at the expense of the smaller ones.

1.3.2.2 Crystals and glasses

Crystals are key structural components in many foods, and their presence can influence textural properties in a number of ways, for example:

1. Ice crystals formed during freezing, especially large crystals formed by heterogeneous nucleation due to slow freezing and crystal growth, can lead to substantial cellular damage and softening of texture through mechanical disruption. Such crystals result in high levels of drip when the frozen foods are thawed.

2. Lactose crystallisation in ice cream can give rise to gritty textures.
3. Fat crystals in margarine maturation leads to the development of a crystal-based exoskeleton that immobilises the dispersed water droplets in place (Knoester et al., 1968).
4. Polymorphism in cocoa butter affects textural attributes like the crispness of the first bite as well as predicating the melting temperature of the chocolate in the mouth.
5. Amylopectin crystals in starch granules undergo gelatinisation when cooked with excess water (as in bread and cakes); under low-water conditions (e.g., cookies), they melt at elevated temperatures.
6. In starch retrogradation, amylose chains align and crystallise within starchy food products—losing their affinity for water and causing the products to dry out.

Due to their importance, it is worth examining how crystals are formed and dissolve. For the purpose of this discussion we will consider sucrose crystal. Figure 1.3 is the concentration–temperature state diagram for sucrose—to be more precise, it is two diagrams superimposed, the solid lines being common to both (whereas the faint broken line belongs to the equilibrium state diagram, and the bold broken lines are part of the nonequilibrium diagram). In the absence of water (i.e., 100% sucrose), T_m is the temperature at which sucrose melts.

The equilibrium state diagram includes the light broken line that corresponds to the eutectic temperature. Below this line (in the equilibrium state) the material consists of ice crystals and sucrose crystals, whereas the region labelled **'A'** consists of sucrose crystals in a saturated solution. If the mixture at point *X* were to be heated, the temperature rises through the saturation line and the crystals dissolve, such that at point *Y* we have a solution with a similar composition to point *X*, but with no solid crystals. The process is reversible; however, it is time dependant, and for crystals to form we

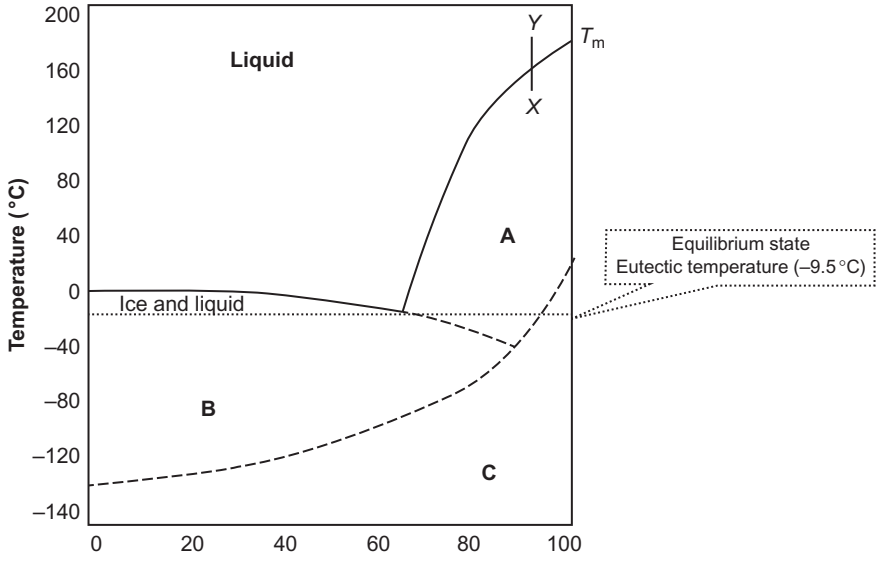

Figure 1.3 An illustration of the concentration–temperature state diagram for sucrose.

need time for the crystal growth. If inadequate time is allowed, then we force the system into nonequilibrium conditions.

Under nonequilibrium conditions there is no concept of eutectic temperature, for sucrose crystals do not form by rapid cooling from point *Y*. Instead, it is a supersaturated solution whose liquid sucrose content is higher than in the equilibrium state at the same temperature. The region labelled **'A'** (in the nonequilibrium state) is a high-viscosity supersaturated solution. As the temperature falls, the viscosity of the solution correspondingly increases to the extent that it starts to behave like a rubbery material. In the nonequilibrium state, there is no eutectic region; instead, the area labelled **'B'** consists of solid ice crystals suspended in a supersaturated sucrose solution. Only when the temperature falls below the lower broken line and enters the region labelled **'C'** does the material solidify in the form of an amorphous glass.

In the case of sucrose, rapid cooling of concentrated solutions (such as point *Y* in Figure 1.3) into the region labelled **'A'** yields hard candy (boiled sweets), which is an amorphous rubbery material—further cooling takes us into the glassy state.

There are many examples of glasses in foods. Although sucrose is a relatively small molecule, most of the food polymers such as starch also follow this behaviour. Thus when the amylopectin crystals in starch granules gelatinise, the resultant gel when cooled is actually a supersaturate solution of starch whose viscosity rises as the temperature falls. Although nonequilibrium cooling of a concentrated solution will result in a supersaturated viscous rubbery material, if it remains a supersaturated material it will with time start to crystallise. If, on the other hand, it is cooled to a glass, it will remain a stable amorphous material. So in the case of boiled sweets, which are glassy at room temperature—the product is stable and does not recrystallise, but in the case of starch, which at room temperature exists as a supercooled rubbery liquid—it will recrystallise on storage leading to retrogradation.

1.4 Structure of specific texture-modified food

1.4.1 Expanded, low-water, high-carbohydrate foods

Puffing techniques involve the expansion or release of gas within a material to create a structure. A number of techniques have been used such as extrusion-cooking in which water present in the ingredients is heated under pressure and when released through the die, it flashes off as steam, achieving vast expansion of the material and rapid cooling. The overall effect with starch rich cereals is the creation of a highly expanded, porous, glassy matrix. Such foods include some expanded snack products, some breakfast cereals and some crackers (see Chapter 10, this volume; Payne et al., 1989).

The open and porous cellular structure of the products, often consisting of thin walled glassy materials, gives rise to crisp, brittle materials when compressed. To an extent these properties may be predicted through mathematical models of force distribution in cellular matrices (Jeronimidis, 1991).

Steam explosion puffing is used for some fruits such as apples to produce expanded dried fruits. The raw material is heated under pressure with steam and then the

pressure suddenly released. A similar approach is used to create rice cakes; however, the expansion step is carried out in a confined mould to create the characteristic shapes yet achieving the expanded cellular structure.

Traditional baking techniques are generally less open in structure, due to the less explosive nature of the expansion step. Yet yeast fermentation or the generation of carbon dioxide from a leavening agent followed by thermally induced setting either from gelatinised or melted starch cooling to the rubbery or even glassy state does achieve varying degrees of expansion—the interaction of other ingredients plays a key role on the degree of expansion, and modified formulations can result in products becoming less successful (Pateras and Rosenthal, 1992).

1.4.2 Hydrocolloid thickened and gelled foods

Carbohydrate polymers are common ingredients in many naturally occurring foods and have been added to manufactured foods to achieve the development of solid structure when otherwise it may not exist. The choice of hydrocolloid has much to do with the intended mouthfeel, the chemical nature of the raw materials and the composition of the product. Achieving the balance of concentration is crucial—too little may not achieve the viscosity or firmness desired, whereas too much may make the product unpalatable.

Of particular interest to the authors are the medical applications of thickening liquid foods for dysphagic patients or creating restructured products for the elderly, who may find chewing of the conventional analogue a challenge (see Chapters 6 and 7, volume 2).

1.4.3 Mousses and whipped desserts

Generally mousses have spherical bubbles entrained in a liquid phase. The viscosity is elevated during early stages of whisking by Einstein thickening, though at high levels of overrun other mechanisms are involved.

Hydrocolloids mentioned in the last section may form the basis of the thickening of many dessert-type products; however, thickened structures are not always due to carbohydrate gums or gelatins, and some ingenious products exist where flavoured, sweetened powders containing phosphates are whisked by the consumer with milk, resulting in a calcium phosphate gel.

1.4.4 Chocolate and sugar confectionary

The earlier discussion on crystals and glasses is particularly relevant here, for in chocolate confectionery the creation of the β' cocoa butter polymorph is of importance in achieving the appropriate gloss, and although the variation in melting point between the different polymorphs is not great, it does have an impact on mouthfeel. However, in the case of sugar confectionary, we are dealing with glasses and rubbery materials resulting from rapid cooling of sucrose and sometimes other ingredients such as butter or cream.

Confectionery is luxurious and not intended as a staple. In this respect the processing is crucial to avoid grittiness, which would detract from quality. The inventiveness of the confectioner and the technologist in creating confectionery structures is inspirational. Although the basic materials are similar, the ways in which the structure can be compressed together or expanded into set bubbly or flaky products is wonderful. Often simple modifications such as increasing the thickness of a bite of chocolate has a profound impact on the mouthfeel, despite virtually no change in the structure or the raw materials.

1.5 Texture properties of different types of food

Texture properties vary greatly from one type of food to another, as do consumers' texture interests. For example, our texture concern for a beverage is more on its flow behaviour, but for a solid product such as biscuit, how it fractures becomes a main concern. Therefore, there is a need to identify key texture properties at the category level according to different physical forms of food: solid, soft solid and fluid. A solid food normally refers to those that have specific geometric features and shapes. This type of food can only stand for a small strain deformation before being catastrophically fractured. A fluid food means that the material has a little resistance against a stress, however small, and is unable to stand against gravity. A main feature of fluid food is that it has no geometric shape but takes the shape of whatever container it is in. A soft solid (also called semisolid) refers to a wide range of materials between fluid and solid. These foods are neither easily flowable nor solidly rigid.

1.5.1 Textural properties of solid food

Solid is one of the basic states of matter. A solid is characterised by its structural rigidity and resistance to shape and volume change. The molecules in a solid are tightly bound to each other, either in a regular geometric lattice (crystalline solid) or in an irregular form (amorphous solid). In the case of food materials, a solid can further be divided into two groups: dry solid and wet solid. The two types have very different packaging requirements for storage and handling. In relation to eating, moisture intake and moisture release are the two most distinctive features of the two respective subgroups. A dry solid food (e.g., biscuits, crisps) has a low-moisture content, which often requires packaging and storage conditions to minimise moisture intake. During eating, fractured particles of such a food will need a large quantity of saliva for surface coverage as well as in some cases for absorption and moistening. A wet solid food such as fruits and vegetables contains a high amount of moisture (juice). Moisture loss during storage is often a main concern of such foods. During eating, the rapid moisture release is often a major sensory pleasing factor for a wet solid food.

Mechanically, solid foods normally have a short strain deformation against fracture. Breaking test is the most effective way for texture characterisation of dry solid foods. A typical feature of the force–displacement curve is a short linear relationship before

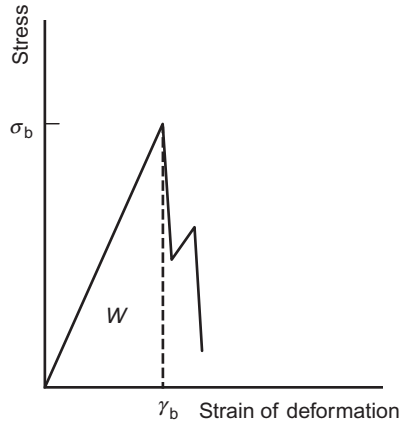

Figure 1.4 A typical force–displacement curve of solid food. The force (stress) increases quickly to reach a maximum, s_b, when the food breaks at a relative small strain, g_b, and in a catastrophic manner. The area underneath the force line is often referred as the work of fracture, *W*. Note two distinctive features of this type of food: a small fracture strain and a catastrophic fracture when it breaks.

a sudden and often catastrophic structural breakdown (see Figure 1.4). The breaking force and the breaking strain are two quantifiable parameters that have direct correlations to sensory experience of such a food. The ratio of the two factors gives Young's modulus, a derived physical parameter that is also commonly referred to in literature for texture characterisation. The area underneath the force line gives an estimation of the amount of work (energy) required for breaking. Hardness and brittleness are probably the most relevant texture terms for solid food. Crispness, crunchiness and crumbliness, as well as adhesiveness, are also commonly used for texture descriptions of this type of food.

Another very important feature of solid food breaking is the size distribution of the newly formed particles, a feature hugely important to oral experience and oral strategy of eating (van der Bilt, 2012). This feature can be quantified by a parameter called the *breakage function*, proposed by van der Glas et al. (1987). This function gives a quantitative analysis of the breaking and fracturing of a solid food. It is quantified by the weight fraction of newly formed particles of the size below the half of the parent particle. Most crisp, crunchy and crumbly dry solid foods have a high breakage function due to easy fragmentation, whereas a wet solid food usually breaks only along the stress line with little fragmentation and therefore has a low-breakage function. For this type of food, continuous chewing will be essential for further particle size reduction and bolus formation. This could be another major texture difference between the two solid subgroups.

1.5.2 Texture of soft solid food

There is no agreed definition about soft solid. In many cases, semisolid is also used to represent the same group of food materials. A general consensus is that a soft solid has similar structural features of a solid, such as its shape and geometry, but a soft solid

requires a much smaller stress for deformation. Also it can be deformed to a much larger strain with no major structural failure. Structure and texture formation of soft solid food are largely through bonding and networking of large structure-forming molecules such as proteins, polysaccharides and colloidal interactions. Soft solid probably covers the widest range of food products providing the richest possible texture experiments. The rich texture variation of this type of food originates from the very different responses of soft solid to an applied stress. The resistance against deformation can be summarised into a few major types as shown in Figure 1.5. Food type A has an initial elasticity followed by a major structure failure. Food type B also shows initial linear elasticity, but permanent structural damage starts to occur once the stress is higher than its yield point. Food type C has a J-shaped curve. It is initially easily deformable, but it becomes hard to deform at high strains, a case of strain hardening.

Gels are the most typical examples of soft solid food, available almost in every part of the world. Food gels can be roughly divided into two types according to their structural nature: polymer networking and particulate gels. The former is a result of the networking of long-chain biopolymers spanning across the whole volume, capable of holding a high proportion of liquid. Diverse mechanisms could be involved in gel formation, including cross-linking, double or triple helices, egg-boxed structures and bundled structures (Aguilera and Stanley, 1999). Gums, polysaccharides, gelatin and other hydrocolloids are the most common ingredients for gel making (see Chapter 9). Such gels mostly behave viscoelastically with a great sense of springiness. They are normally transparent and can be easily turned into all sorts of colours for an enhanced visually pleasing effect. Strain hardening often occurs to this type of gels (the J-shaped force–displacement curve in Figure 1.5).

Particulate gels are made of strands of more or less spherical aggregates ordered into a string-of-beads or cluster arrangement (Aguilera and Stanley, 1999). Gobular proteins (whey proteins, egg proteins, soy proteins and others) are common

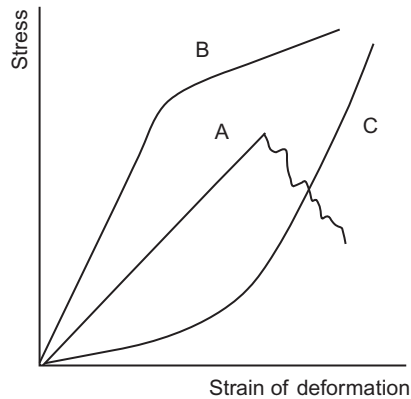

Figure 1.5 A schematic representation of the three different types of force–displacement curves for soft solid food. (a) A linear increase of force to the point of major structural breaking; (b) existence of a yield point where permanent internal structural damage begins; and (c) a J-shaped strain hardening material where the food becomes firmer before it is finally snapped.

ingredients for such gels. A particulate gel often appears translucent or white coloured due to light scattering of colloidal particles. Rheologically, a particulate gel often behaves with strain weakening, due to quickly weakened interactions between colloidal particles. Set yoghurt is a most typical example of particulate gel. Their deformation pattern is mostly represented by curve A in Figure 1.5.

Texture characterisation of soft solid is much more complicated than that of a solid one. Firmness is probably the most well-known texture term for this type of food, referring to the resistance of the food against deformation. This term can usually be satisfactorily estimated by either the Young's modulus or the breaking stress obtained from the force–displacement curve. Cohesiveness, springiness, adhesiveness, gumminess and so on are also commonly used for texture descriptions of such food systems. Texture discrimination is also the most delicate and challenging among foods of this type.

1.5.3 Texture of fluid food

Fluid food refers to those food products that are flowable, from very low viscosity beverages to highly cohesive materials that can almost be categorised as a soft solid. The most obvious feature of this type of food is its easy deformability. A fluid food cannot stand against the gravity and will flow even under a minimal stress. During eating, a fluid food requires no chewing or mastication, but deforms and flows between the tongue and the hard palate. Because of no involvement of chewing or mastication, a low-viscosity fluid food (e.g., beverage drinks) tends to have a much shorter oral stay (oral resident time). Once ingested, a fluid food almost goes straight to swallowing. For some highly cohesive or high-viscosity fluid food (e.g., honey, peanut butter), oral manipulation is needed by tongue pressing and squeezing to ensure appropriate saliva mixing and dilution for both flavour release and easy swallowing (Chen and Lolivret, 2011).

Viscosity and flowability are probably the most important physical properties influencing textural sensation of a fluid food. A large amount of food texture literature in the past few decades has been on viscosity measurement or fluid rheology study (Rao, 1999). Viscosity or consistency sensation can be most satisfactorily predicted by rheology approaches, at least for those near-Newtonian fluids. The use of different biopolymers or thickeners is an effective method in altering the viscosity, in particular in beverage products. Molecular weight, degree of branches, mixture of biopolymers, presence of suspended particles, as well as concentration, are the most important influencing factors. A major challenge in the study of oral viscosity sensation is the fact that most fluid foods are non-Newtonian, and their apparent viscosities are shear rate-dependent. With this in mind, Shama and Sherman (1973) conducted a well-known experiment to demonstrate the possible shear rate and shear stress applied to fluid foods during oral processing. Even though this work has been well accepted and extensively referred in literature, contradictions and disputes continue today (Akhtar et al., 2005; Koliandris et al., 2010).

Another main feature of fluid food is its immediate formation of a thin layer at oral surfaces after ingestion. The relative surface movement becomes critically important

as a result of tongue manipulation against the hard palate or other parts of oral surfaces (e.g., teeth, lips or cheeks). Sensations of smoothness, slipperiness, and for some food creaminess become most dominating. Astringency sometimes also plays a role in the sensation of a fluid food (such as wines, teas).

The thin layer formation and relative surface movement during the oral process of fluid food has the essential features of a tribometer setup. Based on this recognition, 'oral' tribology or 'oral' lubrication has been accepted as an important approach to texture study of fluid food. This work was started almost four decades ago by Kokini et al. (1977) and then resumed again in the last decade by a group of Unilever scientists (Malone et al., 2003). In the past few years, 'oral' tribology study has received great attention from food scientists (Dresselhuis et al., 2008; Goh et al., 2010). Rheology to tribology transition has been seen as the underlying physical principles of texture sensation during oral processing of various fluid foods (Chen and Stokes, 2012). Even though 'oral' tribology study has been seen as a very promising approach, there is still a lack of solid experimental evidence for a direct link between tribology results and texture sensation. The main difficulty is the lack of a feasible technique for an *in vivo* study. While conducting *in vitro* tests, the exact oral pressure, the viscosity change due to saliva mixing and due to oral shear, are still little known, making lubrication interpretation still less certain.

1.6 Sensation and appreciation of food texture

Even though it is certain that textural properties are closely linked to the physical and mechanical properties of the food, the determining mechanisms of food texture sensation and appreciation are far more complicated than what food physics can describe. Food texture sensation is largely a result of food–body interactions, where both oral physiology and sensory psychology play an equally important role. Based on this recognition, the integrated research of food physics, oral physiology and sensory psychology has established its momentum in recent years (Chen, 2009, 2014; Chen and Stokes, 2012; Lucas et al., 2002; van Vliet, 2002).

The perception of food texture can be seen as a perceived opinion, developed through contacts (visual, hear and touch) with the food material. The formation of such an opinion is not always straightforward. It is not a direct reflection of the properties of the food, but involves a complicated process of information transition, conversion, and interpretation. Food researchers are well aware of the variations of texture perception among different individuals and the gap between the objectively measured texture and human perceived texture, though to fully understand the causes behind these variations remains a challenge.

1.6.1 Physiological principles of texture sensation

Oral surfaces (including lips) are probably the most touch-sensitive parts of the human body. The main physiological determining factor of skin sensitivity to tactile touch is the number (or density) distribution of mechanoreceptors under the skin

surface. A higher number of receptors means a higher capability in detecting a tactile stimulus. So far four different types of mechanoreceptors are known to exist under human skin: the Pacinian corpuscles, Meissner's corpuscles, Merkel's discs and Ruffini endings. Each type of mechanoreceptor has its own unique properties and functions. For example, a Meissner's corpuscle consists of a stack of flattened cells located just below the epidermis. This type of receptor is most responsible to light touch and vibrations at a frequency of around 50 Hz. The Ruffini endings are highly branched fibres inside a roughly cylindrical capsule, detecting tension deep in the skin. The Merkel's discs detect sustained pressure. The deeply located Pacinian corpuscles are an onion-like capsule consisting of a body of fluid with a suspended nerve fibre, extremely sensitive to any rapid vibration of frequency range between 200 and 300 Hz. Based on the rate of adaption, mechanoreceptors can be categorised as slowly adapting (SA) and rapidly adapting (RA). Merkel's discs and Ruffini endings are the two SA receptors, which produce sustained responses to a static stimulation. Meissner's corpuscles and Pacinian corpuscles belong to RA receptors. They respond to sudden changes of an external stimulation and fire only at the onset and offset of the stimulation.

With the four different types of mechanoreceptors working together, any external tactile stimulus in the form of pressure, stretch, distortion or vibration will be detected, and signals will be sent to the brain for processing and interpretation. The ratio between the number of RA and SA receptors is generally 36–64 at most parts of body surfaces. However, it was found that on the tongue surface the number of RA mechanoreceptors was much more dominant (Bukowska et al., 2010), suggesting that the tongue is much more sensitive to on/off application of tactile stimuli. Considering the dynamic nature of food oral processing and constantly changing textural properties, one would think that denser RA receptors on the tongue surface is hugely advantageous for acute texture sensation.

1.6.2 Psychophysical principles of texture sensation and perception

Sensory perception is a most complicated process that converts external stimuli to a sensory opinion. This not only relies on the physiological capacities of an individual, but is also directly influenced by one's psychology. Many researchers have shown that texture perception and preference are highly related to one's previous experience. To some extent, one may claim that texture sensation is a learned (or trained) experience, rather than a born capability.

Observations on the eating behaviour of infants reveal that the introduction of different food texture could have an important influence on children's texture preference in later years as well as on the oral physiological developments and acceptance of new food at weaning (Nicklaus, 2011). This finding clearly shows the determining influences of experience and environment on one's preference of food and food texture. This has also been confirmed by Antmann et al. (2011), who observed that between different Spanish-language-speaking countries (Spanish speakers in Spain versus

those in Argentina and Uruguay), subtle differences were clearly noticeable due to cultural and geographical environment differences. By comparing texture terms among the different languages, Bourne (2002) observed that Japanese culture produces most comprehensive texture terms, due to probably the delicacy of their cuisine culture.

Applying physical instruments for objective texture characterisation and reliable prediction of consumer preference has always been a core concern of the food industry, driven mostly by cost saving and reliable quality control. However, the discrepancy between objectively measured texture and perceived texture is so common and sometimes so large that people suspect the usefulness of such approaches. Deviation of instrumental results from human perception could be caused by many different reasons. The most obvious is the unique oral conditions that are extremely difficult to mimic by an instrument. The oral shear rate, the food temperature, the deformation speed during biting and mastication, the food moisture content and so on are constantly changing from the first bite until after swallowing (Chen, 2009). How to satisfactorily incorporate these oral conditions into instrumental measurements remains a big challenge.

Another fundamentally important and practically very interesting problem is the human scaling of texture sensation, which is believed to be very different from that of a physical instrument. Modern equipment is designed as measurement tools that give a reading of the true value of a designated physical parameter. The instrumental reading has a linear scaling to the true value, with no deviation at least within the instrument applicable range. Examples include a balance for weighing, a thermometer for temperature reading, a load cell for force measuring, a pH meter for the logarithm of acidity, a reflectometer for sugar concentration determination, and many others. However, humans' response to a stimulus is almost certain not in linear scale and depends highly on the nature of the stimulus. Take an example of electric shock. If the intensity of the current is doubled, the perceived intensity by human could be 11.3 times higher according to Stevens' power law relationship (Stevens, 1960). For food sensory features, human beings are normally much less sensitive than an instrument. For example, the taste of saccharine could only be perceived 70% higher even when its physical intensity (the concentration) is doubled. Cutler et al. (1983) studied human perception of viscosity change against a series of constituted fluid samples and found that the perceived viscosity did not match the real viscosity change of the fluid sample measured by an instrument. Human perception in this case was again less sensitive than the instrument. Perceived viscosity increase was found to be much lower than the real viscosity increase as determined by an instrument.

Although the fundamental cause to humans' sensory deviation from its real physical intensity is still open for investigation, the most critical issue to food researchers would be the correlation between the perceived sensory intensity and the physical intensity of the sensory stimulus. The power law model proposed by Stevens more than a half-century ago still provides a valid theoretical tool to correlate human perceived sensory intensity with instrumental measured intensity (Stevens, 1960). However, possible applications of the theory to food sensory properties still require further experimental studies.

1.7 Conclusions

Food texture is a collective term of sensory experiences originated from visual, audio and tactile stimuli. The sensation of food texture plays a crucial role in influencing consumers' liking and preference of a food product. Consumer concern and interest of food texture vary from one type of food to another. For solid foods, sensory experience associated with fracture and breaking could be the most relevant textural features, whereas the sensation of flow behaviour could be the most critical texture-related feature for fluid foods. For semisolid or soft solid foods, different patterns of stress–strain deformation provide key information for the delicate texture variation among this type of food.

Food texture and food structure are the two internally linked properties. Although food structure influences textural properties of a food, it is regarded as material property of the food. The term *food texture* has a strong inclusion of sensory experience. Ingredient interactions and food processing and preparation are the most important industrial approaches for food texture (or food structure) creation or modification. Moisture content and fat content are the two key determining factors for texture creation. Content of air, as expressed as structure openness, also plays a critical role in texture creation. Using these parameters as three dimensions, foods can be conveniently grouped for their textural properties.

It is only when food physics interacts with oral physiology that the sensation and perception of food texture becomes possible. The underpinning principles of food texture sensation are very different from that of taste and aroma. Mechanoreceptors are the key for the detection of texture stimuli. However, the interpretation of these sensory stimuli is a very complicated internal process where the underlying psychophysical principles are still not fully understood.

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Part Two

Novel use of food ingredients for food texture modification

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Emulsifiers as food texture modifiers

2

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2.1 Introduction

Commercial food plays 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 substances that display many functions in relation to food texture. They can either increase colloidal stability or provide controlled destabilisation in emulsion or aerated food products. Interactions between emulsifiers, proteins and carbohydrates in bakery products or other starch-based foods improve both texture and shelf life.

The functionalities 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 reviews the major types of emulsifiers used in food products followed by interactions of emulsifiers with other food components, emphasising the interfacial interactions between emulsifiers and food components such as water, proteins, carbohydrates and oils in relation to the effects of emulsifiers on food texture and rheology. Finally, we describe the mouth feel characteristics of emulsifiers.

2.2 Types of emulsifiers

This section gives a brief description of the most common types of food emulsifiers, their chemistry and physical properties and discusses some of the factors that would influence food texture.

2.2.1 Small-molecule surfactants

Small-molecule surfactants refer to those relatively small surface-active molecules ($\leq 10^3$ Da) that consist of a hydrophilic 'head' group, which has a high affinity for water, attached to a lipophilic 'tail' group, which has a high affinity for oil. Surfactants are added to food product formulations to perform a range of functions, possibly but

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not necessarily related to emulsification. In food processing and food storage, the three main functions of surfactants are

(1) to improve emulsion/foam formation and stability by controlling the state of dispersion and agglomeration of oil droplets or fat globules;
(2) to modify shelf life, texture and rheological properties through interaction with carbohydrate and protein components; and
(3) to control of texture and morphology of fat-based products by influencing oil droplet size distribution or structure of fat crystals.

Though some food emulsifiers occur 'naturally' (such as lecithins or monoglycerides [MGs]), many are considered to be synthetic 'food additives'. At present, most permitted food emulsifiers are esters (or partial esters) formed from animal or vegetable fatty acids with polyvalent alcohols such as glycerol, propylene glycol or sorbitol. For further effectiveness, these molecules may be reacted with ethylene oxide or esterified with organic acids such as citric acid or lactic acid. However, due to toxicological, legal and marketing constraints, the acceptable daily intake of surfactants available for use in food is quite restricted and is becoming increasingly so. The surfactants can be represented by the formula RX, where X represents the hydrophilic head and R the lipophilic tail. The characteristics of a particular surfactant depend on the nature of its head and tail groups. The head group may be anionic, cationic, zwitterionic or nonionic, although most surfactants used in the food industry are mainly nonionic (e.g., monoglycerides, polysorbates, acetic acid esters of MG, lactic acid esters of MG), anionic (e.g., fatty acid salts, stearyl lactylate salts, diacetyl tartaric acid esters of monoglycerides, citric acid esters of MG) or zwitterionic (e.g., lecithin). The tail group usually consists of one or more hydrocarbon chains with between 10 and 20 carbon atoms per chain. Each type of surfactant has functional properties that are determined by its unique molecular structure and the physicochemical environment that it operates within.

Food emulsifiers are often classified according to the so-called *hydrophile–lipophile balance* (HLB). This is a semi-empirical concept based on the idea that, for a given oil and water system, there is an optimum balance between molecular hydrophilic and lipophilic character, which leads to maximum emulsification efficiency and emulsion stability. The HLB is described by a number that gives an indication of the relative affinity of a surfactant molecule for the oil and aqueous phases. The HLB values of some commonly used food surfactants are listed in Table 2.1.

The HLB number of a surfactant gives a useful indication of its solubility in either the oil and/or water phases and can be used to predict the type of emulsion that will be formed by a surfactant (Table 2.2). The predominantly hydrophilic emulsifiers (O/W; large HLB) stabilise oil-in-water emulsions, whereas predominantly lipophilic emulsifiers (W/O; small HLB) stabilise water-in-oil emulsions. According to the Griffin scale, surfactants with HLB values in the range of 3–6 are suitable for making water-in-oil emulsions, those with HLB values in the range of 10–18 are suitable for making oil-in-water emulsions and those with HLB 7–9 have no preference one way or the other. Molecules with HLB values below 3 (very hydrophobic) and above 18 (very hydrophilic) are often not particularly surface-active because they tend to

Table 2.1 HLB values of some commonly used food surfactants

Surfactant name	HLB value
Sodium lauryl sulphate	40.0
Sodium stearoyl lactylate	22.0
Potassium oleate	20.0
Sucrose monoester	20.0
Sodium oleate	18.0
Polysorbate 80	15.0
Polysorbate 60	15.0
Sucrose monolaurate	15.0
Polysorbate 65	14.9
Decaglycerol monooleate	14.0
Ethoxylated monoglyceride	13.0
Decaglycerol dioleate	12.0
Polysorbate 65	11.0
Hexaglycerol dioleate	9.0
Sorbitan monolaurate	8.6
DATEM	8.0
Soy lecithin	8.0
Decaglycerol hexaoleate	7.0
Triglycerol monostearate	7.0
Sorbitan monopalmitate	6.7
Glycerol monolaurate	5.2
Calcium stearoyl lactylate	5.1
Sorbitan monostearate	4.7
Propylene glycol monolaurate	4.5
Sorbitan monooleate	4.3
Glycerol monostearate	3.8
Glycerol monoleate	3.4
Sorbitan tristearate	2.1
Sorbitan trioleate	1.8
ACETEM	1.5
Oleic acid	1.0

accumulate preferentially in bulk oil or bulk water, rather than at an oil–water interface. Emulsion droplets are particularly prone to coalescence when they are stabilised by surfactants that have extreme or intermediate HLB values. At very high or low HLB values, a surfactant may have such a low surface activity that it does not accumulate appreciably at the droplet surface and therefore does not provide protection against coalescence. At intermediate HLB values (7–9), emulsions are unstable to coalescence because the interfacial tension is so low that very little free energy is required

Table 2.2 **Comparison of functional attributes of different types of surfactants (Krog and Sparso, 2003)**

Chemical name	Solubility	Emulsion type	Usage level (g/g oil)	pH stability	Salt stability	Temperature stability
Nonionic (low HLB)	Oil	W/O	~0.05	Good	Good	_
Nonionic (high HLB)	Water	O/W	~0.05	Good	Good	Poor at PIT
Ionic	Water	O/W	~0.05	Good	Poor at I > CFC	Poor at PIT

Note: PIT, phase inversion temperature and CFC, critical flocculation concentration.

to disrupt the membrane. Empirical observations suggest that maximum emulsion stability is obtained for O/W emulsions using surfactants with an HLB number around 10–12, and for W/O emulsions around 3–5. This is because the surfactants are surface-active, but do not lower the interfacial tension so much that the droplets are easily disrupted. Under certain circumstances, it is possible to adjust the 'effective' HLB value by using a combination of two or more surfactants with different HLB numbers. Surfactant blends are often used in the food industry to improve the overall functional properties of surfactant systems in commercial products.

The most widely used food emulsifiers are mono- and diglycerides. They are only sparingly soluble in water and are predominantly lipophilic. The HLB for glycerol monoleate is 3.4, and that for glycerol dioleate is 1.8. However, because of difficulties of separation, pure mono- or diglycerides are not used as food ingredients.

The properties of a number of food-grade surfactants commonly used in the food industry are briefly discussed next and summarised in Tables 2.1 and 2.2. Water-soluble surfactants with relatively high HLB values (10–18) are normally used to stabilise O/W emulsions, such as beverages, dressings, desserts and coffee creamers. Nevertheless, they are also used to displace proteins from the surfaces of protein-stabilised fat droplets during the production of ice creams, whipped creams and toppings (Goff, 1997a, 2000). Water-soluble surfactants may also bind to proteins or polysaccharides and modify their functional properties. Oil-soluble surfactants with relatively low HLB values (3–6) are used to stabilise W/O emulsions, such as margarines and spreads. They are also used to inhibit fat crystallisation in some O/W emulsions because this improves the stability of the food product to refrigeration conditions, for example, dressings. Oil-soluble surfactants can also be used in conjunction with water-soluble surfactants to facilitate protein displacement from fat droplets during the production of ice creams, whipped creams and toppings. Surfactants with intermediate HLB values (6–9) have a poor solubility in both oil and water phases and are not particularly good emulsifiers when used in isolation. Nevertheless, their emulsification properties can be improved by using them in combination with other surfactants.

Let us briefly review some of the types of chemical compounds that are permissible as food emulsifiers. In the first place, most are nonionic surfactants: monoglycerides, sorbitan ester, polysorbates, sucrose esters and so on. Amongst the few anionic food emulsifiers are sodium and calcium stearoyl-2-lactylates, succinylated monoglycerides and diacetyl tartaric esters of monoglycerides. Cationic surfactants are not used as food emulsifiers. An important amphoteric emulsifier is phosphatidylcholine (PC) (lecithin); this zwitterionic surfactant carries zero net charge under neutral pH conditions (Krog and Sparso, 2003).

2.2.1.1 *Monoglycerides*

Monoglycerides refers to a series of surfactants produced by interesterification of fats or oils with glycerol. The most widely used food emulsifiers are mono- and diglycerides. They are nonionic oil soluble surfactants and are only sparingly soluble in water. The HLB for glycerol monoleate is 3.4, and that for glycerol dioleate is 1.8. Monoglycerides are dispersible in water only in the presence of co-emulsifiers

(sodium salts of fatty acids). However, because of difficulties of separation, pure mono- or diglycerides are not used as a food ingredient. Also available are purer 'distilled monoglycerides' with a monoester content of 90–95%.

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 (Goff, 1997a, 2000).

Distilled monoglycerides are very effective aerating agents and commercial emulsifier gel products, which 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 to stabilise the α-crystalline structure of the lipid bilayers.

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. Large-deformation rheology is typical for a particle gel with a relatively small strain at fracture, both in shear deformation and compression.

Monoglyceride coagels are used as texturising agents in low-fat foods (e.g., spreads), due to their water-binding capacity. 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 2.3.

2.2.1.2 *Organic acid esters of monoglycerides*

Monoglycerides can be esterified with a variety of organic acids (e.g., acetic, citric, diacetyl tartaric and lactic acids) to form surfactants with different functional properties. The most common examples of this type of surfactant are acetylated monoglycerides (ACETEM), lactylated monoglycerides (LACTEM), diacetyl tartaric acid monoglycerides (DATEM) and citric acid esters of monoglycerides (CITREM).

Table 2.3 Application of liquid crystalline phases, α-gels and β-hydrates in foods (Krog, 2001)

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)	✓	✓	✓
Texturing of low-fat spreads	–	✓	✓

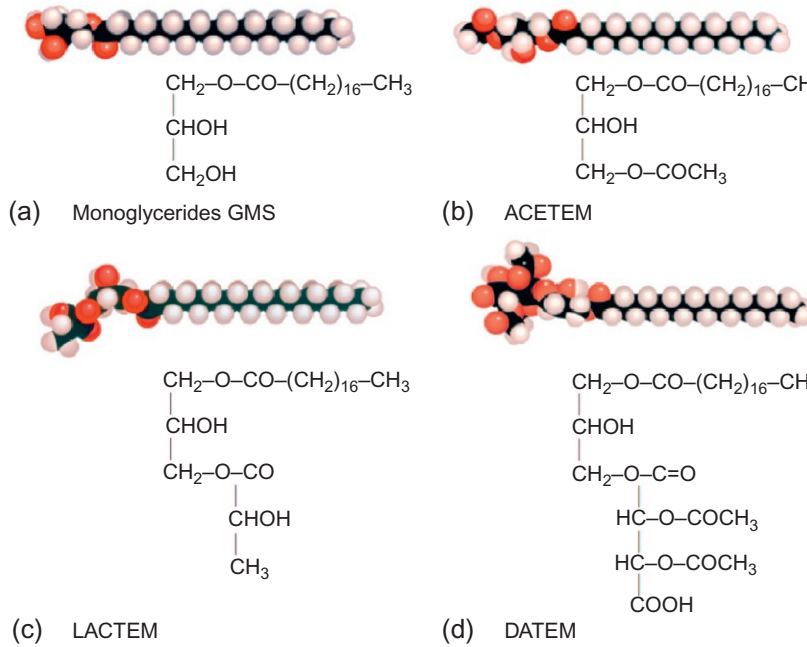

Figure 2.1 Chemical structure and molecular models of (a) glycerol monostearate (GMS) and the main component of organic acid derivatives of monoglycerides; (b) ACETEM, monoacetylated monoglycerides; (c) LACTEM, lactylated monoglycerides; (d) DATEM, diacetyl tartaric acid ester of monoglycerides (Krog and Sparso, 2003).

The chemical structure and molecular models of monoglycerides and their organic acid esters are shown in Figure 2.1. ACETEM and LACTEM are nonionic oil-soluble surfactants with low HLB values, whereas DATEM and CITREM are anionic water dispersible surfactants with intermediate or high HLB values.

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 solid state of such products. The melting points of organic acid esters of monoglycerides are, therefore, considerably lower than those of corresponding monoglycerides. The properties and typical application of organic acid esters in foods are summarised in Table 2.4.

The esterification of monoglycerides with organic acids considerably alters their polarity and hydration and swelling properties in water. Neither ACETEM nor LACTEM form liquid crystalline mesophases in water due 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

Table 2.4 Typical properties and applications of monoglyceride derivatives

Emulsifier	Properties	Typical applications
ACETEM	α-Crystalline Nonionic Oil soluble	Film forming, coating agent (fruit, frozen meat), whippable emulsions, cake shortening
LACTEM	α-Crystalline Nonionic Oil soluble	Whippable emulsions, powdered topping bases, cake shortenings, cake improves
DATEM	α-Crystalline Anionic Water dispersible	Dough strengthener, bread improver, emulsion stabiliser (coffee creamers, salad dressing, beverages)
CITREM	α-Crystalline Anionic Water dispersible	Emulsion stabiliser (W/O or O/W emulsions, meat products, beverages)

attraction between the lipid bilayers. The thickness of the water layers formed is relatively small, usually in the order of 10–20 Å. However, the interaction between water and ACETEM or LACTEM is important for their function in whippable emulsions (nondairy creams, toppings) and cake shortenings (Euston, 1997).

Esters of dicarboxylic organic acids and monoglycerides, such as diacetyl tartaric acid ester of monoglycerides (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, whereas 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 I 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.

2.2.1.3 Polyol esters of fatty acids

Surfactants with different functional characteristics can be produced by esterification of polyols with fatty acids. The type of polyol and fatty acids used to prepare the surfactant determine its functional characteristics. The polyols that are most commonly esterified with fatty acids are polyglycerol, propylene glycol, sorbitan, polyoxyethylene sorbitan and sucrose. The fatty acids used to prepare these types of surfactants may vary in chain length (typically 12–18 carbon atoms) and degree of unsaturation. The chemical structure and molecular models of such polyol esters are shown in Figure 2.2. The solubility and functional properties of polyol esters of fatty acids

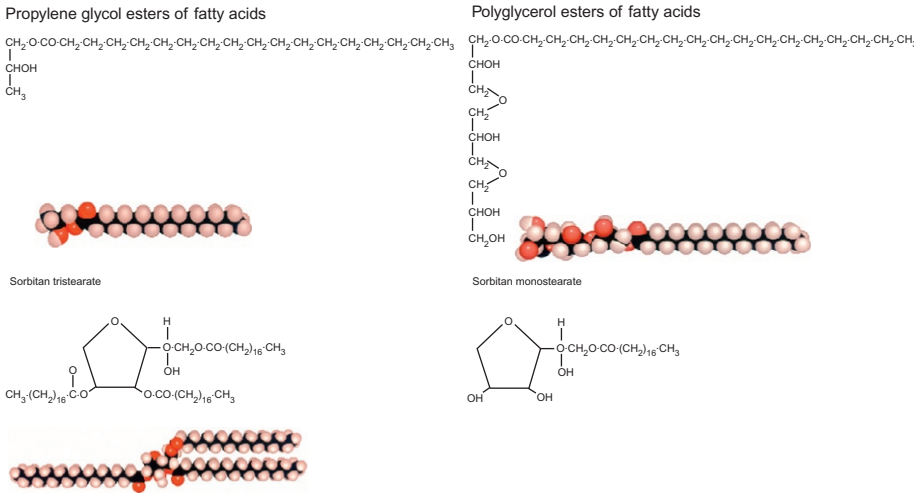

Figure 2.2 Chemical structure and molecular models of polyol esters of fatty acids (Krog and Sparso, 2003).

depend on the relative sizes of the hydrophilic and lipophilic parts of the molecules. Surfactants with large polyol head groups tend to be water dispersible and have high HLB values (e.g., polyglycerol and polyoxyethylene sorbitan esters), whereas those with small polyol head groups tend to be oil soluble and have low HLB values (e.g., propylene glycol esters). The ratio of hydrophilic to lipophilic groups can be varied appreciably within some series of polyol esters of fatty acids by changing the size of the polyol group, which leads to both oil-soluble and water-dispersible surfactants being present in the same series, for example, sucrose esters. Sorbitan esters (so-called Spans) are formed from the reaction of sorbitol with fatty acid. These low HLB oil-soluble emulsifiers are often used in combination with high HLB water-soluble polyoxyethylene sorbitan esters (so-called Tweens). These polysorbates are formed from the reaction of sorbitol esters with ethylene oxide. These oil- and water-soluble surfactants are often used in combination to improve the overall stability of emulsions.

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.

2.2.1.4 *Sodium stearoyl lactylate and calcium stearoyl lactylate*

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). It should be noted that SSL (HLB = 21.0) is one of the most hydrophilic food emulsifiers. Combinations of SSL and distilled, saturated monoglycerides are used as aqueous dispersions to improve the aeration of bakery products (cakes) or low-fat, nondairy whipping creams (Faergemand and Krog, 2003).

2.2.1.5 Lecithin

In contrast to these synthetic emulsifiers, lecithin is a natural food emulsifier that can be extracted from a variety of sources, including soybeans, rapeseed and egg. Soy lecithin is the most widely used surfactant ingredient in the food industry because it can be economically extracted during the processing of crude soybean oil. Natural lecithins contain a complex mixture of different types of phospholipids and other lipids, although they can be fractionated to form purer ingredients that are enriched with particular fractions. The most common phospholipids in lecithin are PC, phosphatidylethanolamine (PE) and phosphatidylinositol (PI). The hydrophilic head groups of these molecules are either anionic (PI) or zwitterionic (PC and PE), whereas the lipophilic tail groups consist of two fatty acids. Natural lecithin has intermediate solubility characteristics and HLB numbers (~8), which means that it is not particularly suitable for stabilising either O/W or W/O emulsions when used in isolation, but it may be effective when used in combination with other surfactants (van Nieuwenhuyzen and Szuhaj, 1998). In addition, lecithin can be chemically or enzymatically hydrolysed to break off one of the hydrocarbon tails to produce more hydrophilic surfactants that are capable of stabilising O/W emulsions.

2.2.2 Protein emulsifiers

The interfacial membranes formed by proteins are usually relatively thin and electrically charged, hence the major mechanism preventing droplet flocculation in protein-stabilised emulsions is electrostatic repulsion (McClements, 2004). Consequently, protein-stabilised emulsions are particularly sensitive to pH and ionic strength effects and will tend to flocculate at pH values close to the isoelectric point (IEP) of the adsorbed proteins and when the ionic strength exceeds a certain level (Dickinson, 2010). Emulsions stabilised by globular proteins are also particularly sensitive to thermal treatments because these proteins unfold when the temperature exceeds a critical value, exposing reactive nonpolar and sulphydryl groups (Galazka et al., 1999). These reactive groups increase the attractive interactions between droplets, which may lead to droplet flocculation. It should be noted that a number of methods have been developed to attempt to improve the emulsifying properties of protein ingredients, including limited hydrolysis to form peptides, modification of protein structure by chemical, physical, enzymatic, or genetic means, and blending of the proteins with other ingredients, although not all of these processes are currently legally allowed.

2.2.2.1 Milk proteins

Protein ingredients isolated from bovine milk are used as emulsifiers in a wide variety of emulsion-based food products, including beverages, frozen desserts, ice creams, sports supplements, infant formula, and salad dressings. Milk proteins can be conveniently divided into two major categories: casein (~80 wt%) and whey proteins (~20 wt%). Casein precipitation can be achieved by adjusting the pH close to the IEP (~4.6) of caseins or by adding an enzyme called rennet that cleaves the hydrophilic fraction of casein that is normally responsible for stabilising casein micelles. Casein and whey protein fractions can then be separated from each other by causing the casein to precipitate from solution (the curd) and leaving the whey proteins in solution (the whey). If isoelectric precipitation is used, the separated fractions are called 'acid casein' and 'acid whey', whereas if enzyme precipitation is used, the separated fractions are called 'rennet casein' and 'sweet whey'. The fractions separated using these two processes have different compositions, and therefore ingredients produced from them may have different functional properties. Curd formation is a critical step in the creation of cheese, and the large quantities of whey remaining from this process can be used to make functional whey protein ingredients. A variety of milk protein ingredients are available for usage as emulsifiers in foods, including whole milk, whey proteins and caseins. These ingredients are usually sold in a powdered form, which is light cream to white in appearance and has a bland flavour. These powders are normally available in the form of protein concentrates (25–80% protein) or protein isolates (>90% protein). There are four main protein fractions in casein: α_{S1} (~44%), α_{S2} (~11%), β (~32%) and κ (~11%). In general, these molecules have relatively random and flexible structures in solution, although they do have a limited amount of secondary and tertiary structure. The caseins also have some regions that are highly nonpolar and others that are highly charged, which plays a major role in determining their molecular and functional properties in food (Dickinson, 2001). In their natural state, the caseins tend to exist as complex molecular clusters called 'micelles' that are typically between 50 and 250 nm in diameter and are partly held together by mineral ions (such as calcium phosphate). In commercial ingredients, caseins may also be present in a number of other sorts of molecular clusters depending on the way the proteins were isolated, for example, sodium caseinate, calcium caseinate, acid casein and rennet casein.

Caseinate-stabilised emulsions have been shown to be unstable to droplet flocculation at pH values (3.5–5.3) close to the IEP of casein and at relatively high ionic strengths (Dickinson et al., 1997). Caseinate-stabilised emulsions tend to be more stable to heating than whey protein–stabilised emulsions, presumably because the relatively flexible casein molecules do not undergo appreciable heat-induced conformational changes like globular proteins do. It should be noted that sufficiently high concentrations of nonadsorbed caseinate can promote emulsion instability through a depletion flocculation mechanism (Dickinson, 1999).

Whey protein is also a complex mixture of different individual proteins, with the most common being β-lactoglobulin (~55%), α-lactalbumin (~24%), serum albumin (~5%) and immunoglobulins (~15%). Normally, β-lactoglobulin dominates the functional characteristics of whey proteins because of its relatively high concentration and

unique physicochemical properties. Whey protein-stabilised emulsions tend to flocculate at pH values (~4–5.5) close to their IEP (~5.0), at high salt concentrations and on heating above the thermal denaturation temperature of the adsorbed proteins in the presence of salt (Kulmyrzaev et al., 2000; Demetriades et al., 1997).

2.2.2.2 Meat and fish proteins

Meat and fish contain a number of proteins that are surface-active and capable of stabilising emulsions, for example, gelatine, myosin, actomyosin, sarcoplasmic proteins and actin. Many of these proteins play an important role in stabilising meat emulsion, that is, products formed by blending or homogenising fat, meat and other ingredients. Emulsion stabilisation is partly due to their ability to adsorb to the oil–water interface and partly due to their ability to increase the aqueous phase viscosity or to form a gel in the aqueous phase. Gelatine is one of the few proteins that have been isolated from meat and fish and sold commercially as a functional emulsifier ingredient. Gelatine is a relatively high-molecular-weight protein derived from animal collagen, for example, pig, cow or fish. Gelatine is prepared by hydrolysing collagen by boiling in the presence of acid (Type A gelatine) or alkaline (Type B gelatine). The IEP of type A gelatine (~7–9) tends to be higher than that of type B gelatine (~5). Gelatine exists as a random coil molecule at relatively high temperatures, but undergoes a coil–helix transition on cooling below a critical temperature, which is about 10–25 °C for pig and cow gelatine and about 0–5 °C for fish gelatine. Gelatine has been shown to be surface-active and capable of acting as an emulsifier in O/W emulsions (Surh et al., 2006). Nevertheless, when used on its own, gelatine often produces relatively large droplet sizes during homogenisation, so that it has to be hydrophobically modified by attachment of nonpolar side groups or used in conjunction with anionic surfactants to improve its effectiveness as an emulsifier (Djagny et al., 2007; Kristinsson and Rasco, 2000). Research has been carried out to establish the ability of various other protein fractions of fish and meat muscle to act as emulsifiers (Kristinsson and Rasco, 2000). The ultimate objective of this work is to be able to convert waste products from fish and meat production into value-added functional ingredients for use as emulsifiers in foods (Huidobro et al., 1998). Nevertheless, there are currently few examples of functional ingredients derived from fish or meat products (other than gelatine) designed especially as emulsifiers.

2.2.2.3 Egg proteins

Both egg yolk and egg white contain a mixture of protein and nonprotein components that are surface-active. Egg ingredients can be purchased in a variety of different forms for use in food emulsions, including fresh egg yolks, frozen egg yolks, dried egg yolks, fresh whole eggs, frozen whole eggs and dried whole eggs. Different egg ingredients are usually prepared using different processing treatments, which often influence their effectiveness at stabilising emulsions. In the food industry, egg white is more commonly used for stabilising foams, whereas egg yolk is more commonly used for stabilising emulsions. Egg yolk is widely used as an emulsifier in the production of mayonnaise, salad dressings, sauces and cake batters. The mean

particle diameter of emulsions stabilised by egg yolk decreased from pH 3 to 9, suggesting that egg yolk was more efficient at forming emulsions at higher pH values (Chang and Chen, 2000). Like other globular proteins, the proteins in eggs will unfold and aggregate on heating above their thermal denaturation temperature, which influences the stability and rheological properties of emulsions (Guilmineau and Kulozik, 2006). Emulsions stabilised by egg yolk have been shown to have poor stability to freeze–thaw cycling (Castellani et al., 2006).

2.2.2.4 Plant proteins

Surface-active proteins can be extracted from a variety of plant sources, including legumes and cereals. A considerable amount of research has been carried out to establish the ability of these proteins to stabilise emulsions and whether they could be made into commercially viable value-added ingredients for use as emulsifiers in foods. One of the most widely studied proteins extracted from a plant source is soy protein, which is commercially available as a protein concentrate or isolate. Soy protein ingredients are a complex mixture of many individual protein fractions with different molecular and functional characteristics, for example, 2S, 7S, 11S and 15S fractions. In addition, each of these fractions contains a mixture of different protein subunits that also have different molecular and functional characteristics.

Previous studies have shown that soy proteins can decrease the interfacial tension between oil and water and therefore facilitate emulsion formation (Molina et al., 2001). Researchers have shown that it is possible to form stable O/W emulsions using soy proteins or their fractions as emulsifiers (Roesch and Corredig, 2003). Nevertheless, compared to the other sources of proteins mentioned earlier, there have been far fewer systematic studies on the influence of environmental conditions (pH, ionic strength and temperature) on the stability of soy protein-stabilised emulsions. Emulsions prepared using soy protein concentrates or isolates tend to be highly flocculated, possibly because of bridging of the relatively large soy protein aggregates between droplets (Chen et al., 2011a,b). Consequently, soy proteins could be used to stabilise emulsions where droplet creaming is not usually a problem, for example, food products with relatively high droplet concentrations or high continuous phase viscosities. On the other hand, soy protein ingredients may be unsuitable for stabilising relatively dilute emulsions where creaming would be accelerated by droplet flocculation (Nishinari et al., 2014). Nevertheless, researchers are examining methods of improving the emulsifying properties of soy proteins by fractionating them, by physically, chemically, enzymatically, or genetically modifying them, or by using them in combination with other ingredients.

2.2.3 Polysaccharide emulsifiers

2.2.3.1 Gum arabic

Gum arabic is widely used as an emulsifier in the beverage industry to stabilise cloud and flavour emulsions. It is derived from the natural exudate of *Acacia senegal* and consists of at least three high-molecular-weight biopolymer fractions. The surface-active fraction is believed to consist of branched arabinogalactan blocks attached

to a polypeptide backbone. The hydrophobic polypeptide chain is believed to anchor the molecules to the droplet surface, whereas the hydrophilic arabinogalactan blocks extend into solution (Dror et al., 2006). The interfacial membrane formed by gum arabic is believed to provide stability against droplet aggregation mainly through steric repulsion, but with some contribution from electrostatic repulsion also (McNamee et al., 1998). The influence of a variety of processing conditions on gum arabic functionality has been examined. For example, it has been shown that gum arabic-stabilised emulsions remain stable to droplet flocculation when exposed to a wide range of conditions, for example, pH (3–9), ionic strength (0–25 mM $CaCl_2$) and thermal treatment (30–90 °C) (Nakauma et al., 2008; Chanamai and McClements, 2002). Nevertheless, gum arabic has a relatively low affinity for oil–water interfaces compared to most other surface-active biopolymers, which means that it has to be used at relatively high concentrations to form stable emulsions. For example, as much as 20% gum arabic may be required to produce a stable 12 wt% O/W emulsion. For this reason, its application as an emulsifier is restricted to products that have relatively low droplet concentrations (Dickinson et al., 1991; Buffo et al., 2001). Gum arabic has a high water solubility and a relatively low-solution viscosity compared to other gums, which facilitates its application as an emulsifier.

2.2.3.2 *Modified starches*

Natural starches are hydrophilic molecules that have poor surface activity. Nevertheless, they can be made into effective emulsifiers by chemically attaching hydrophobic moieties along their backbones (Nilsson and Bergenstahl, 2006). These modified starches are widely used as emulsifiers in the beverage industry. One of the most commonly used modified starches is an octenyl succinate derivative of waxy-maize. It consists primarily of amylopectin that has been chemically modified to contain a side group that is nonpolar. These side groups anchor the molecule to the oil droplet surface, whereas the hydrophilic starch chains protrude into the aqueous phase and protect droplets against aggregation through steric repulsion (Nilsson and Bergenstahl, 2007). Because the dominant stabilising mechanism is steric repulsion, emulsions stabilised by modified starch are resistant to changes in pH (3–9), ionic strength (0–25 mM $CaCl_2$) and temperature (30–90 °C) (Chanamai and McClements, 2002). Like gum arabic, modified starch has a relatively low interfacial activity (compared to proteins or surfactant), and so a large excess must be added to ensure that all the droplet surfaces are adequately coated. For example, it is recommended that about 12% modified starch is required to produce a stable 12 wt% O/W emulsion (Chanamai and McClements, 2001). Modified starches usually come in powdered or granular forms that are easily dispersible in cold water.

2.2.4 *Protein–polysaccharide complexes as emulsifiers*

Proteins tend to be better at producing small-emulsion droplets when used at low concentrations than polysaccharides, whereas polysaccharides tend to be better at producing emulsions that are stable to a wider range of environmental conditions than proteins, for example, pH, ionic strength, temperature, freeze–thaw cycling. It may

therefore be advantageous to combine the beneficial attributes of these two kinds of biopolymers to produce small-emulsion droplets with good environmental stability. A number of researchers have shown that protein–polysaccharide complexes may have better emulsifying properties than either of the biopolymers used in isolation (Akhtar and Dickinson, 2007; Chevalier et al., 2001). These complexes may be held together either by physical or covalent interactions and may be formed either before or after homogenisation. Ingredients based on protein–polysaccharide interactions will have to be legally acceptable, be economically viable and show benefits over existing ingredients before they find widespread usage in the food industry. It should be noted that gum arabic is a naturally occurring protein–polysaccharide complex that is already widely used in the food industry as an emulsifier (Dickinson et al., 1991).

2.3 Interfacial properties of emulsifiers

2.3.1 Interfacial properties of surfactants

Surfactant molecules adsorb to oil–water interfaces because they can adopt an orientation in which the hydrophilic part of the molecule is located in the water, whereas the hydrophobic part is located in the oil. This minimises the contact area between hydrophilic and hydrophobic regions and therefore reduces the interfacial tension. This reduction in interfacial tension is important during homogenisation because it facilitates the further disruption of emulsion droplets, that is, less energy is needed to break up a droplet when the interfacial tension is lowered. Once adsorbed to the surface of a droplet, the surfactants must provide a repulsive force that is strong enough to prevent the droplet from aggregating with its neighbours. Ionic surfactants primarily provide stability by causing all the emulsion droplets to have the same electric charge and therefore electrostatically repelling each other. Nonionic surfactants primarily provide stability by generating a number of short-range repulsive forces that prevent the droplets from coming too close together, such as steric, hydration, and thermal fluctuation interactions (Baret et al., 2009). In addition, even oil droplets stabilised by nonionic surfactants often have an electrical charge, and therefore electrostatic repulsion may also contribute to their stability. Some surfactants form multilayers (rather than monolayers) at the surface of an emulsion droplet, which has been found to greatly enhance the stability of the droplets against aggregation (Sanatkaran et al., 2014; Yaari et al., 2014). In summary, surfactants must have three characteristics to be effective at enhancing the formation and stability of emulsions. First, they must rapidly adsorb to the surface of the freshly formed emulsion droplets during homogenisation. Second, they must reduce the interfacial tension by a significant amount. Third, they must form an interfacial layer that prevents the droplets from aggregating under the solution and environmental conditions pertaining to emulsion.

The interfacial membranes formed by some surfactants (especially those containing saturated hydrocarbon chains) are capable of undergoing liquid–solid phase transitions on changes in temperature. Above a critical temperature (T_c), the hydrocarbon chains have a relatively high-molecular mobility and can be considered to be ‘fluid-like’,

but below T_c the chains lose their molecular mobility, pack closely together and can be considered to be more 'solid-like'. The transition of the chain packing from fluid-like to solid-like usually causes an appreciable decrease in the interfacial tension and may have important consequences for the functional properties of some emulsions (Goff, 2000).

2.3.2 Interfacial properties of amphiphilic biopolymer

Usually, amphiphilic biopolymers must be fully dispersed and dissolved in an aqueous solution before they are capable of exhibiting their desirable emulsifying properties. Solvation of biopolymer ingredients prior to homogenisation is therefore an important step in the formation of many food emulsions. After a biopolymer ingredient has been adequately dissolved in the aqueous phase, it is important to ensure that the solution and environmental conditions (e.g., pH, ionic strength, temperature and solvent composition) will not promote droplet aggregation during homogenisation or after the emulsion is formed. For example, it is difficult to produce protein-stabilised emulsions at pH values close to the IEP of the proteins or at high salt concentrations because the electrostatic repulsion between the droplets is insufficient to prevent droplet aggregation once the emulsions are formed.

The interfacial activity of many biopolymers is due to the fact that they have both hydrophilic and lipophilic regions distributed along their backbones. For example, most proteins have significant numbers of exposed nonpolar amino acid side groups, whereas some polysaccharides have nonpolar side chains attached to their polar backbones. The major driving force for adsorption of these amphiphilic biopolymers to oil–water interfaces is therefore the hydrophobic effect. When the biopolymer is dispersed in an aqueous phase, some of the nonpolar groups are in contact with water, which is thermodynamically unfavourable because of hydrophobic interactions. When a biopolymer adsorbs to an interface it can adopt a conformation where the nonpolar groups are located in the oil phase (away from the water), and the hydrophilic groups are located in the aqueous phase (in contact with the water). Adsorption also reduces the contact area between the oil and water molecules at the oil–water interface, which lowers the interfacial tension. Both of these factors favour the adsorption of amphiphilic biopolymers to oil–water interfaces. The conformation that a biopolymer adopts at an interface, and the physicochemical properties of the membrane formed, depend on its molecular structure and interactions. Flexible random-coil biopolymers adopt an arrangement where the predominantly nonpolar segments protrude into the oil phase, the predominantly polar segments protrude into the aqueous phase, and the neutral regions lie flat against the interface (see Figure 2.3). The membranes formed by these types of molecules tend to be relatively open, thick and of low viscoelasticity. Globular biopolymers (usually proteins) adsorb to an interface so that the predominantly nonpolar regions on the surface of the molecule face the oil phase, whereas the predominantly polar regions face the aqueous phase, and so they tend to have a particular orientation at an interface (see Figure 2.3). Once they have adsorbed to an interface, biopolymers often undergo structural rearrangements so that they can maximise the number of contacts between nonpolar groups and oil. Random-coil biopolymers are relatively flexible molecules and can therefore rearrange their

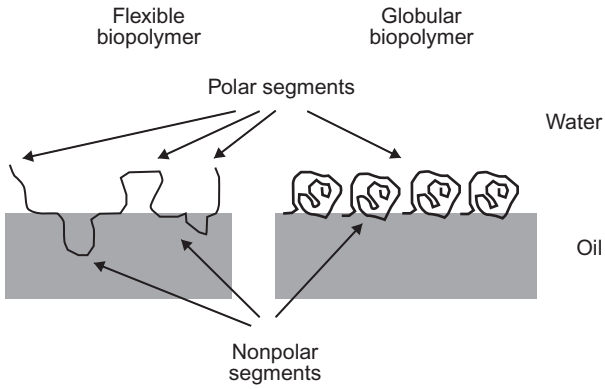

Figure 2.3 The structure of the interfacial membrane depends on the molecular structure and interactions of the surface-active molecules (McClements, 2005).

structures fairly rapidly, whereas globular biopolymers are more rigid molecules and therefore rearrange more slowly. The unfolding of a globular protein at an interface often exposes amino acids that were originally located in the hydrophobic interior of the molecule, which can lead to enhanced interactions with neighbouring protein molecules through hydrophobic attraction or disulphide bond formation. Consequently, globular proteins tend to form relatively thin and compact membranes that have high viscoelasticities. This may account for the fact that membranes formed by globular proteins are more resistant to rupture than those formed by more random-coil proteins.

To be effective emulsifiers, biopolymers must rapidly adsorb to the surface of the emulsion droplets created during homogenisation and then form an interfacial membrane that prevents the droplets from aggregating with one another. The interfacial membranes formed by biopolymers can stabilise emulsion droplets against aggregation by a variety of different mechanisms, for example, steric, electrostatic and hydration repulsion. The stabilising mechanism that dominates in a particular system is largely determined by the characteristics of the interfacial membrane formed, for example, thickness, electrical charge, internal packing and exposed reactive groups. The dominant stabilising mechanism operating in a particular emulsion determines the sensitivity of the system to droplet aggregation under different solution and environmental conditions, for example, pH, ionic strength, temperature and solvent quality.

2.4 Interaction between surfactants and biopolymers

Under certain circumstances, surfactant molecules bind to proteins and polysaccharides, and the resulting surfactant–biopolymer complexes may have very different functional characteristics than either of the individual components. Interactions between surfactants and proteins are used in many types of food processes to improve food properties. These interactions may be either direct or indirect. Direct interactions involve binding of surfactants to proteins and can cause substantial changes in the conformation, stability, or interactions of protein molecules. Depending on the nature of

the interaction, these changes may have either a beneficial or detrimental influence on the functional properties of proteins, for example, surface activity, foaming capacity, gelation and solubility. Surfactants may also interact indirectly with proteins by either competing with them or displacing them from interfaces (Chen and Dickinson, 1998). For example, small-molecule surfactants are added to some emulsified food products to displace proteins from the surface of oil droplets, thereby facilitating the coalescence of droplets during subsequent chilling and shearing operations, for example, ice cream and whipped cream (Goff, 1997a,b).

Interactions between surfactants and polysaccharides are also commonly used to improve processing operations or product properties. For example, surfactants (e.g., monoglycerides and stearoyl lactylates) are often incorporated into starch-based products, such as breakfast cereals, pasta and potato products, to improve their quality. The surfactants form inclusion complexes with starch by inserting their hydrocarbon tails into helical coils formed by amylose or linear regions of amylopectin. These lipid–starch complexes are believed to improve the quality of starch-based products such as bread by increasing loaf volume, reducing crumb firmness, and delaying staling, mainly through their ability to retard the retrogradation of starch (Kim, 1992). The ability of surfactants to bind to starch depends on the molecular characteristics of the starch (e.g., chain length), as well as of the surfactants (e.g., head-group polarity, tail group length and degree of unsaturation). Starch tends to bind more ionic than nonionic surfactant and binds more saturated than unsaturated surfactants. Surfactants may also interact with a wide variety of other types of polysaccharides (e.g., cellulose, pectin, chitosan, carrageenan), thereby altering their conformation, association and/or stability, which in turn leads to alterations in their functional properties, such rheology, appearance, stability and phase separation. Judicious usage of these interactions can create food products with novel properties or develop encapsulation or delivery systems.

Certain types of surfactants have been shown to be capable of modifying the nucleation and crystallisation of lipids, which is used to control crystal formation in some food products. Surfactants have been shown to be capable of preventing clouding in salad oils by retarding the growth of fat crystals. The surfactants are believed to adsorb to the surface of any nuclei or small fat crystals formed in the oil, thereby inhibiting their further growth by preventing adsorption of additional lipid molecules (Diftis et al., 2005). Surfactants have also been shown to inhibit undesirable polymorphic transitions of lipid crystals in chocolates, shortenings and margarines.

2.5 Mouthfeel characteristics of emulsifiers

The mouthfeel of food emulsions has been shown to be strongly influenced by the type, concentration and interactions of the colloidal particles and macromolecules present. The perceived ‘fattiness’, ‘creaminess’ and ‘thickness’ of oil-in-water emulsions has been found to increase as the droplet concentration increases. The creaminess of oil-in-water emulsions was also found to depend on droplet size, which was partly

attributed to the associated change in emulsion viscosity. Creaminess has also been found to depend on the type of emulsifier used to stabilise the droplets, possibly due to differences in their impact on droplet flocculation and emulsion viscosity. Previous studies indicate that there is a strong correlation between perceived creaminess and emulsion viscosity; however, these studies also suggest that other factors that depend on droplet characteristics are important (Daget et al., 1987). Products with reduced fat contents may contain 'fat-replacers' that are designed to provide a mouthfeel similar to that of the conventional full fat product. These fat replacers are often designed to have characteristics that are similar to the emulsion droplets in conventional products. For example, spherical particles (0.1−20 μm) have been formed using biopolymer aggregates made from proteins and/or polysaccharides to mimic emulsions droplets. Another contribution to mouthfeel that may be important during consumption of some food emulsions is the cooling sensation associated with melting of emulsified fat in the mouth due to the endothermic enthalpy change associated with fat crystal melting. It has been shown that the breakdown of fat droplets within the mouth and the ability of the released fat to coat the tongue may also play an important role in determining the mouthfeel of emulsions (Akhtar et al., 2005).

Biopolymers, such as polysaccharides or proteins, are often added to food as emulsifiers, emulsion stabilisers or texture modifiers. The type and concentration of biopolymers present, as well as their interactions with each other, influences the microstructure, thin-film rheology and bulk rheology of emulsions. The perceived mouthfeel of food emulsions is normally changed by the presence of biopolymers that influence their microstructure and texture. The relationship between the rheological properties of aqueous biopolymer solutions and their perceived mouthfeel has been reviewed (Stanley and Taylor, 1993). It has been shown that there is a strong correlation between the perceived 'thickness', 'stickiness' and 'sliminess' of polysaccharide solutions and their shear viscosity or modulus measured under shear conditions representative of those in the mouth. In general, the shear rates experienced by foods within the mouth depend on the foods' unique rheological characteristics, and it may vary from 5 to 50 s^{-1}. As a simple rule of thumb, it has been suggested that shear rates of 10 s^{-1} for large-deformation shear viscosity measurements or 50 rad s^{-1} for small-deformation dynamic shear modulus measurements give a reasonable representation of mouth conditions. It has been suggested that the small-deformation measurements often give a better correlation to initial perceived mouthfeel because they do not cause appreciable breakdown of food microstructure (Stanley and Taylor, 1993). Polysaccharides have also been shown to reduce the perceived 'flavour intensity' of flavoured aqueous solutions, which has been attributed to their ability to suppress the mixing of the food within the mouth.

The ability of biopolymers to alter food rheological characteristics may also influence the way that foods coat the surface of the mouth during mastication. Biopolymers may also influence the force required to deform and disrupt gelled emulsions into smaller fragments within the mouth.

At present our understanding of the physicochemical basis of the mouthfeel of emulsifier-contained foods is still rather limited. It is clear that more systematic research is needed to establish the influence of characteristics of emulsifier-contained

foods, such as particle/droplet size, particle/droplet concentration, emulsifier–biopolymer interactions, oil type, and aqueous phase composition, on the mouthfeel of emulsifier-contained foods. Such studies will require a combination of sensory analysis and instrumental measurements of the properties of tested food samples with various compositions and microstructures. Some of the major factors that need to be studied in more detail have recently been identified: (1) the initial rheology and mechanical properties of the food being consumed, (2) the changes in food properties during mastication, (3) the influence of food microstructure and composition on flavour partitioning and release, (4) the physiology of the mouth and (5) food–mouth interactions. The rational design of food with desirable mouthfeel profiles depends on an understanding of the influence of emulsifiers on food texture. Nevertheless, a quantitative understanding of this influence is difficult because of the complexity of the physicochemical, physiological and psychological processes involved. However, it should be noted that the oral processing technique, recently reported by Dr. Jianshe Chen, may be a feasible way to evaluate the mouthfeel characteristics of emulsifiers. The study of 'food oral processing' covers areas of food physics, oral physiology and sensory psychology (Chen, 2009, 2014). The core is the interplay of these disciplines (see Figure 2.4). Study of food physics aims to reveal the determining physical, mechanical and microstructural principles involved in the deformation and fracturing of food and their implications to eating and sensory perception. Oral physiology study focuses on the oral behaviour and physiological responses to changing food properties during an eating process, including saliva secretion, the extraction and relaxation of facial muscles, jaw movements, tongue activities, oral receptors stimulations and so on. On the other hand, sensory psychology has the main focus on the techniques of sensory analysis and the psychophysical principles of sensation and perception. Therefore, to establish a reliable correlation between instrumental sensory characterisation and human perception, proper understanding is greatly needed on the psychophysical principles of sensory perception (Prakash et al., 2013).

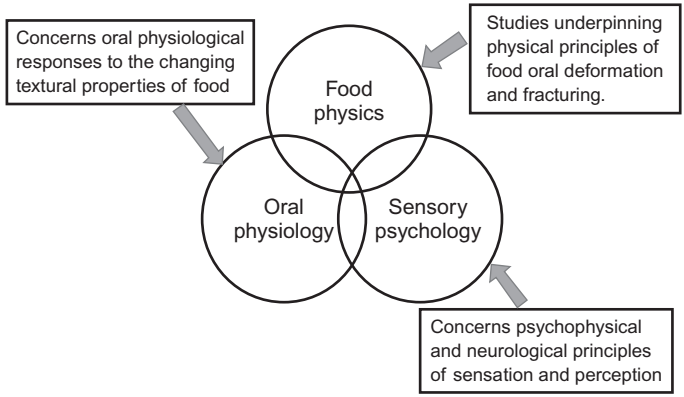

Figure 2.4 Core disciplines of food oral processing studies (Chen, 2014).

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Proteins as texture modifiers

3

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3.1 Proteins as modifiers of the mechanical properties of foods

It has been discussed extensively in other chapters of this book that no single or universal correlation exists so far between (i) the mechanical properties of a food (viscosity, viscoelasticity, fracture/yield stress, compliance/relaxation), (ii) its structural parameters and (iii) its perceived texture. However, as a connection among the three should obviously exist, one must first understand the effect of the food's components and structure to its mechanical properties before moving on to the effect of the food's components to the complex ensemble of processes and stimuli that make up its perceived texture.

Proteins, as major food constituents, are among the obvious materials that can be utilized to manipulate food properties. From a physicochemical perspective, proteins can provide a vast pool of food-grade polymers of varying size and hydrophobicity, with structural and functional properties that are tunable with temperature, ionic strength and pH, to choose from and use to manipulate the structure and rheology of the semiliquid matrices that form most foods. One should not forget, though, that food materials based on proteins should not be considered *de facto* safe: Many proteins are allergenic and may conditionally be toxic or harmful. The physical state of the proteins can also be of importance; potential hazards associated with protein nanostructures in food are reviewed by Raynes et al. (2014).

Figure 3.1 gives a broad layout of the main mechanisms by which proteins can influence or control the mechanical properties of foods. The simplest scenario involves one or more populations of proteins being dispersed into a continuous aqueous phase. At low mass fractions and as a first approximation, proteins behave as typical polymers, altering the viscosity of their dispersion medium; at higher concentrations, the binding of water that results from protein hydration can become of importance. A typical scenario is that of sodium caseinate being added as a water binder into foods, or of meat proteins being tumbled as to alter the mechanical properties of the final product.

The preceding cases do not normally involve strong, direct interactions between the protein molecules and usually result simply in an increase in viscosity. Gelling, on the other hand, can occur when proteins bind adequate amounts of water and their concentration is sufficiently high to form a structure-spanning network, whereas at the same time they do interact with each other. That way, on the deformation of the proteins, momentum can be transferred to their immediate neighbours, the entire structure storing it as an elastic component. Interactions leading to the formation of gels can

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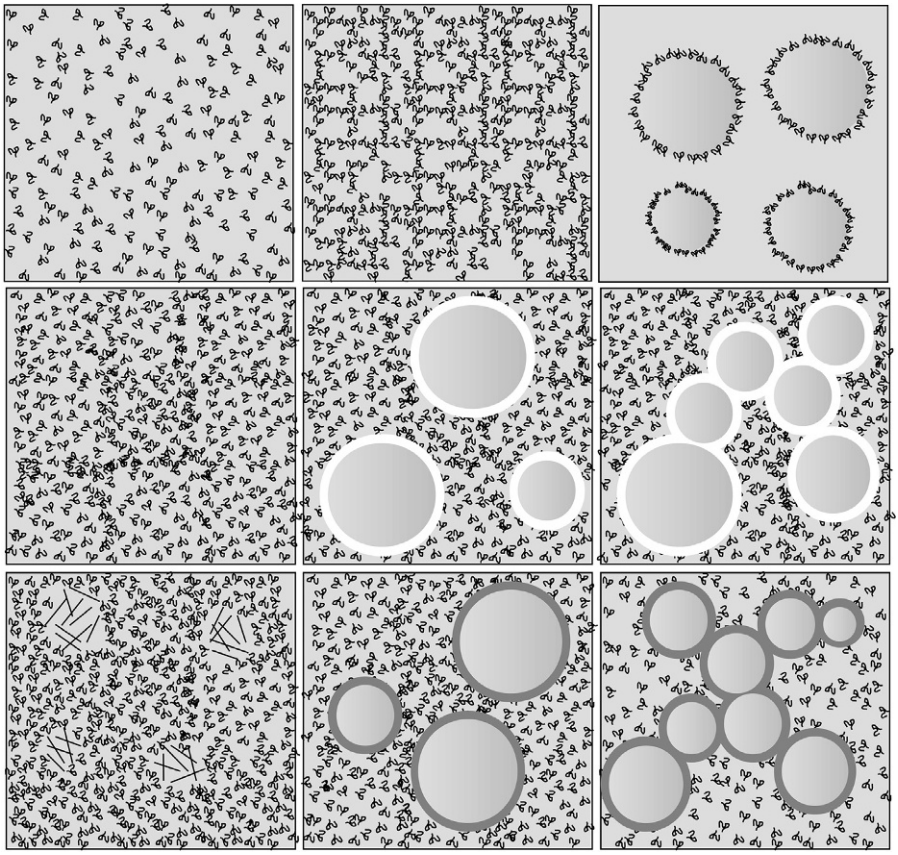

Figure 3.1 Schematic depiction of structures formed by proteins in foods. Left to right, top row: dilute dispersion; concentrated dispersion; adsorbed layers; middle row: concentrated dispersion; inactive filler particles in protein dispersion; aggregated inactive filler particles in protein dispersion; bottom row: water-in-water emulsion; active filler particles in protein dispersion; aggregated active filler particles in protein dispersion.

involve dispersion and hydrophobic forces, whereas stronger bonds such as electrostatic interactions, hydrogen and covalent bonds can be dominant if they form in adequate numbers. Yoghurt is a typical example of gels formed without covalent bonds forming between the gelled proteins to a great extent. Such products owe their particular texture to networks formed by proteins aggregating and eventually forming a gel due to acidification down to pH values close to the proteins' isoelectric points (i.e. Ritzoulis, 2013).

Another broad category of protein networks is the result of their denaturation, typically due to thermal treatment, application of high pressure, alterations in pH or in ionic strength or combinations of these. In that case, networks are formed by a wide range of interactions, mostly related to the exposure of parts of the proteins that were inaccessible before the denaturation event. Some of the most widely cited examples of

denaturation-induced networks involve the formation of the solid bodies of boiled eggs, whereas culinary experimentations involving solid white and liquid yolk should be correlated to differences in the denaturation temperatures (and maybe thermal history) between the various egg white and yolk proteins.

Chemical, rather than physical, changes in the protein structure can also have a profound effect on the structure, and indirectly to the texture of foods. In that sense, formation of most cheeses is due to the calcium-induced aggregation of caseins following the rennin-mediated cleavage of interfacial κ-casein. In bread making, covalent bonding between gluten molecules makes up for what is much of the end product's texture: Disulphide bridges and other direct covalent bonds between amino acids belonging to adjacent proteins are the principal interactions responsible for the transformation of viscous dough into elastic bread.

Proteins are well-known surface-active materials. Their surface activity is mainly related to the existence of non-polar amino acids in their primary structure. This is one of the principal reasons why proteins tend to adsorb onto the oil–water interfaces of oil-in-water and water-in-oil emulsions, and onto the air–water interfaces of foams. There they influence the stability of both emulsions and foams by means of presenting a barrier against droplet or bubble coalescence (at least in oil-in-water emulsions). If the proteins are able to cross-link to form a mechanically strong (partly elastic) layer at the interface, they may even protect against Ostwald ripening. Milk, egg and meat proteins have been traditionally utilized to emulsify or to foam, and thus to control food texture. Crucial in this aspect is the capacity of proteins to stabilize oil droplets, which, partially coalesced or not, adsorb in turn onto water–air interfaces and provide Pickering stabilization against coalescence and perhaps Ostwald ripening/disproportionation. Today this mechanism is well recognized as a significant contributor to the stability of whipped cream and of ice cream. The role of meat proteins and added non-meat proteins to the stabilization of fat-in-meat gel emulsions (e.g., sausages) remains unclear, the fat–meat gel interface in cured and processed meat products being yet largely unexplored; its visualization, study and understanding could help manipulate, up to a point, the stability, mechanical properties and, eventually, the texture of these intriguingly complex products.

Protein dispersions can become more complex when non-interacting colloidal bodies are integrated into the food structure; although a strictly non-interacting particle (inactive filler) is relatively rare, one can consider in this category composite sauces based on mixing cream (protein or phospholipid-covered milk fat droplets) and broths (dispersions of proteins and polysaccharides), especially at neutral pH and relatively low ionic strength. In non-flocculated systems, the rheology is strongly dependent on the rheological properties of the continuous phase and of the volume fraction and deformability of the dispersed particles. In very broad terms and with many exceptions, on the dispersion of particles (e.g., oil droplets, air bubbles or solid crystals) of a relatively low-volume fraction into a low-viscosity liquid, the resulting dispersion is reasonably expected to be liquid. Similarly, dispersion of such particles into a viscoelastic medium is typically expected to result into a viscoelastic character of the entire system. The low-volume fraction of the dispersed phase in the preceding cases limits its relative effect to the system's mechanical behaviour. Again, in broad terms

as earlier, (i) on the dispersion of a very high-volume fraction of particles into a liquid system, or (ii) on formation of a structure-spanning flocculated network of dispersed particles, the dispersed phase may dominate the mechanical response of the entire system. A typical example is the solidification of liquid cream on incorporation of very large amounts of air during the formation of whipping cream, or the formation of yoghurt from a relatively low-volume fraction of structure-spanning aggregated protein particles.

It is not unusual in such cases for the particles to become flocculated due to depletion forces arising from the high concentrations of bulk proteins or polysaccharides. A large body of literature is dedicated to these interactions and their effect on the rheology of emulsions, but its review is out of the scope of the present chapter. Of particular importance, however, is the flocculation of emulsions under the influence of the mucin glycoproteins of the oral cavity, an event that should be directly related to the perceived texture of emulsion-based foods and is to be discussed in more detail later. Mucin-mediated flocculation should serve as yet another reminder that the perceived texture is a complex stimulus that is not related to the rheology of the food alone, but also of its dispersion into saliva and its subsequent processing at the oral cavity.

An emerging research field on the protein-induced manipulation of emulsion rheology, and indirectly on emulsion texture, pertains to the heteroaggregation of two droplet populations covered by oppositely charged interfacial protein layers, for example, one above and one below their isoelectric point (i.e., Mao and McClements, 2013). In that way, flocculated networks with controlled mechanical properties can be formed, providing an additional tool for the manipulation of textural properties of foods.

It is not unusual in real foods for the interfacial layers of droplets, bubbles and other particles to interact with the proteins of the continuous phase (active fillers). Their mechanical behaviour is markedly different from that related to inactive fillers (i.e., van Vliet, 1988). These systems tend to be more common in real foods, whereas the contribution of the droplet (particle) gel and of the continuous phase can be dependent on a very large number of factors. For a comprehensive introduction to this intriguing issue, the reader is referred to recent reviews such as Dickinson (2012).

A scenario typical in foods is the coexistence of more than one macromolecular component in the bulk phase. Such cases have been studied in detail in model aqueous systems containing two biopolymers since the late seventies. In many cases, especially when the polarities are similar and at relatively low concentrations, a condition resembling complete mixing of the two macromolecular populations can be feasible, but phase separations are usual at higher concentrations. The term ‘water-in-water emulsion’ is usually reserved for such aqueous dispersions of phase-separated polymers. Proteins have been demonstrated that, along with polysaccharides, can be used in phase-separated mixtures to texturize water (Vis et al., 2014). Experimental reports suggest that, contrary to expectations, some water-in-water emulsions can be comprised of spherical-like droplets of one phase dispersed into the other. The sphericity of the ‘droplets’ implies the presence of interfacial tension between the two aqueous phases; values of such interfacial tensions have been recently reported

(Balakrishnan et al., 2012). As finite values of interfacial tension suggest thermodynamically unstable systems, the latter's stability could benefit from interfacially adsorbing proteins. An interesting property of proteins appears to be their capacity to stabilize such systems by adsorbing at the water–water interface (Ngyen et al., 2013). The rheology of the two separated phases has recently become an active research field. The effect of the microrheology of the individual phase-separated regimens to the mouthfeel and texture of foods has not yet been studied in detail.

3.2 Mechanistic aspects of textural modification by proteins

3.2.1 Influence of temperature, pH, ionic strength to the structure of proteins aggregates and gels

Gelation is one of the most important properties of food proteins that occurs at two stages: (i) partial denaturation or conformational changes of protein molecules and (ii) gradual association or aggregation of the individual denatured proteins (Matsumura and Mori, 1996). A protein gel is formed when attractive and repulsive forces are balanced. This has been clearly demonstrated in many studies regarding the effects of pH, temperature and ionic strength on gel formation of various proteins, such as pigeon pea protein concentrate, β-lactoglobulin (β-lg) and locust bean protein isolate (Renard and Lefebvre, 1992; Akintayo et al., 1999; Lawal, 2004). Because the preceding factors affect the gelation properties of food proteins, it is expected that they will have a significant impact on the textural properties of food as well. Barbut (1995) showed that NaCl level on whey protein isolate gels had a significant effect on texture-related mechanical properties. A progressive increase in the size of the protein strands was observed, using scanning and transmission electron microscopy, as the amount of NaCl in the gel increased, which led to protein aggregation and concomitant decrease in gel strength. Chung et al. (1993) studied the effects of pH and NaCl on gel strength (shear stress and strain) of Pacific whiting (*Merluccius productus*) surimi gels. It was found that at pH 5, which is very close to the isoelectric point of myosin (~5.5), no gel was formed, indicating that the gelling forces were too weak. Both shear stress and strain significantly increased as the pH of the gel increased, especially in the alkaline pH range. However, shear stress values of surimi gels with an alkaline pH were higher when salt was added at concentrations up to 0.9% (w/w) than those containing greater amounts of NaCl. Besides the aforementioned factors, high pressure treatment has also been reported to lead to protein gelation. Van Camp et al. (1996) showed that high pressure treatment (4 kPa for 30 min) led to the formation of whey protein concentrate gels, which exhibited similar gel strength with the respective heat-set (80 °C for 30 min) gels.

3.2.2 Maillard reaction-induced textural modification

Food processing is, perhaps, the oldest form of human-used chemistry, certainly outdating metallurgy, pottery and dyeing, and possibly even tannery. At the heart of the chemical modifications during which raw materials react to produce cooked/processed food, lies

the broad family of Maillard reactions. During the latter, amino acids react with sugars to yield a wide array of products, which give processed food its characteristic colour, odour and, of course, texture. The reader is referred to the rich existing bibliography on general food chemistry and on Maillard reactions for a discussion of the organic chemistry of these reactions. Of interest to the present chapter is the specific range of products that give cooked/processed food its characteristic crust. This composes extensive polymer networks that are the result of a very large number of case-specific reactions. Such phenomena have been utilized by cooks throughout human history, although their application in large-scale food processing remains largely an empirical task.

A point of interest with a potential impact on the texture of foods is the significant progress that has been made in the preparation of novel emulsifiers from the Maillard reactions between hydrocolloid polysaccharides and proteins. The broad idea between this volume of work (e.g., Dickinson and Galazka, 1991; Dickinson and Semenova, 1992; Mishra et al., 2001; Diftis and Kiosseoglou, 2003; Dunlap and Côté, 2005; Einhorn-Stoll et al., 2005) is the production of molecules that would ideally possess both the emulsifying properties of protein and the thickening properties of polysaccharide. Indeed, Maillard reactions are reported to produce efficient emulsifiers (e.g., Akhtar and Dickinson, 2007; Zhang et al., 2007) and impart interesting rheological behaviour (e.g., Spotti et al., 2014). Although the possible negative health implications of certain products of the Maillard reactions should not be overlooked, the production of novel materials using this route could provide alternatives to artificial emulsifiers and thickeners, allow for their easy formulation at the local level and, of course, provide useful insight to what reactions may be happening already as we cook at home.

3.2.3 Enzymes in texture modification

The enzymes used to manipulate the texture of protein-based foods can be broadly classified into two categories, namely, proteolytic and cross-linking enzymes. Proteolytic enzymes have been the subject of many research studies on improving the texture of various proteinaceous foods. For instance, tenderness is the primary textural characteristic of meat, and therefore, it is of paramount importance for the meat industry to produce high-quality meat of consistent tenderness, thus conforming to the requirements of consumers. Numerous studies in this field have shown that several tenderizing solutions containing various plant proteases can accelerate meat tenderization though injection, infusion or marinating. The plant proteases that have been studied thus far on different types of meat (beef, pork and lamb) include actinidin (Lewis and Luh, 1988; Han et al., 2009; Christensen et al., 2009), papain (Ashie et al., 2002), bromelain (Sullivan and Calkins, 2010; Ha et al., 2012), ficin (Kang and Rice, 1970) and zingibain (Ha et al., 2012). Furthermore, microbial collagenase has been studied in the tenderization of meat and was found to be potent in the presence of NaCl and $CaCl_2$ (Foegeding and Larick, 1985).

Regarding the applicability of cross-linking enzymes in food systems, the food industry has largely focused on the use of transglutaminase. Transglutaminase (E.C. 2.3.2.13) catalyzes the acyl-transfer reactions between γ-carboxamide groups

of protein-bound glutamine residues with primary amines or the ε-amino groups of lysine residues in proteins, leading to the formation of intra- or intermolecular covalent bonds (De Jong and Koppelman, 2002; Motoki and Seguro, 1998). It has been long used in the surimi industry for the setting or 'suwari' of salted fish pastes and was shown to improve the textural properties (breaking force and deformation) of surimi gels from Alaska pollock (*Theragra chalcogramma*) flesh (Kuraishi et al., 2001; Sakamoto et al., 1995). Besides fish proteins, transglutaminase has also been employed on wheat (Gerrard et al., 2001), milk (Bönisch et al., 2007) and meat (Kuraishi et al., 1997) proteins resulting in further strengthening of the protein network. Moreover, transglutaminase has been used for the production of protein-stabilized emulsion gels. Lee et al. (2006) showed that sodium caseinate and soy protein isolate emulsion gels, prepared by adding microbial transglutaminase, were comprised of a more organized protein network, which on storage led to less release of aroma than emulsions without the addition of transglutaminase.

3.3 Texture-modifying proteins

3.3.1 Caseinate

Caseinates are commercial forms of milk casein. Typical forms of caseinates are in the form of sodium and calcium salts. Sodium caseinate is dispersed in aqueous media of neutral pH forming particles of 20–50 nm, whereas calcium caseinate forms larger particles of 100–300 nm due to the binding influence of divalent calcium. Caseinates are very efficient oil-in-water emulsifiers (Dickinson et al., 1995), but they can be antagonized at the surface by small-molecule surfactants (Dickinson et al., 1999; Dickinson and Ritzoulis, 2000) in processes that may alter the emulsions structures, mostly via depletion interactions.

Caseinate can be involved in gel formation in a multitude of ways, as reviewed by Dickinson and Eliot (2003). Of interest is the thermoreversible character of caseinate gels; for example, sodium caseinate-stabilized emulsions under specific conditions have been reported to undergo thermoreversible gelation at about body temperature in the presence of calcium (Dickinson and Casanova, 1999; Eliot and Dickinson, 2003). Such approaches can be of interest in manipulating the rheology and texture of novel food products.

Glycono-δ-lactone-acidified caseinate-stabilized emulsions can produce foams of much higher overruns than typical whipped cream. The stabilization and rheology of such structures is related to direct interactions between interfacial protein, partial coalescence being relatively limited, and hence less temperature-dependent (Allen et al., 2006). This is in contrast to the stabilization of whipping cream, where a Pickering-type stabilization by partially coalesced droplets adsorbed at the air–water interface (bubble surface) is considered to be a prominent stabilization mechanism. Although the texture of such products is obviously different from the one of whipping cream, their reported sensitivity to pH and temperature-tolerance could point to interesting textural alternatives.

It should be pointed out that although both sodium and calcium caseinate are usually referred together as 'caseinates', their effect on the food's structure and mechanical properties (hence texture) are not essentially similar: Calcium caseinate is structurally influenced by the divalent cation. It was already mentioned that the particles of sodium caseinate and of calcium caseinate are not similar in size. Their effects in emulsion stability when they are used as emulsifiers are not the same (Srinivasan et al., 2001). An interesting property of calcium caseinate with potential impact in texture engineering is its ability to form anisotropic transglutaminase-induced gels under shear (Manski et al., 2007). The same researchers report that the same cannot be achieved by sodium caseinate. Apparently electrostatic interactions involving divalent calcium are involved in this interesting property.

Meat research can provide interesting hindsight on the effects of caseinate on the texture rather than simply on the mechanical properties of products. TEM observations suggest that caseinates can integrate successfully into the meat protein matrix of frankfurter-type sausage paste, binding in the meat protein matrix and fat (Atughonu et al., 1998). A direct result of this binding is the capacity of caseinate to alter the properties of such products. The addition of sodium caseinate as a fat replacer in low-fat sausages improved the texture profile analysis (TPA) measured parameters, making them similar to those of regular fat sausages (Yoo et al., 2007). The same study also reported that sodium caseinate reduces the emission of volatile compounds that occurs in the absence of fat, substituting fat in that way as well. This can be of importance, as odour can be a part of the overall sense that is texture. A complex setup integrating objective and subjective sensory testing alongside TPA suggests that sodium caseinate is one of the most effective texture modulators for frankfurter-type sausages, increasing the objective biting force, while it reduces the perceived elasticity of the product (Petridis et al., 2010). The effects of caseinate addition in sausages is reported to significantly affect fattiness, chewiness, elasticity, hardness and consistency, in a strong relation to fat content (Petridis et al., 2013).

3.3.2 Mucins

The mouth and oesophagus are protected by multiple layers of the squamous epithelium, which is itself protected and lubricated by mucus secreted from the salivary and other glands. The most characteristic component of mucus is the mucins, the major glycoprotein group of the gastrointestinal fluids. Mucins are large, highly glycosylated proteins, which may contain up to 80% or more carbohydrate. The typical structure of mucin, extensively covered in relevant reviews (e.g., Johansson et al., 2013), is that of a protein backbone, from which covalently bound sugar oligomers protrude into the aqueous phase, in what resembles overall a brush-like structure. These sugar moieties bind amounts of water, generating hydrocolloid-like properties to the salivary mucus. The mucins found in the mouth so far are MUC5B, MUC1, MUC16 and MUC7 (Johansson et al., 2013).

Although they are not a component of food, mucins appear to play a significant role in texture perception, as they can become a part of the bolus during mastication and mixing of the food with saliva. In that aspect, a masticated bolus is not simply food but

a new system comprising both food and saliva. In that case, mucins are expected to play a role in the bolus similar to that of structural proteins in the food and, hence, influence the rheology and texture during oral processing. The perceived texture of foods has been reported to depend on the total protein concentration of saliva for some semisolid materials (Engelen et al., 2007). The extent to which the two systems (food and saliva) are mixed during mastication is unclear, but recent works suggest that moisture content of boluses (and perhaps integration of mucins into the food matrix) is linearly increased at a rate dependent on the subject and food type (Motoi et al., 2013).

The effect of mucins on the structure and mechanical properties of solid foods is difficult to model, especially when one considers the challenges in monitoring the physicochemical, physiological and neurological processes during their mastication. Liquid emulsions, on the other side, present a very good starting point for such investigations, as one could expect saliva–food mixing to be easier. One of the most important findings concerning the perceived texture of the foods is that mucins can cause otherwise stable emulsions to flocculate in the mouth, thus radically altering their structure (Shi et al., 1999); this process was shown to occur on exposure of emulsions to saliva (van Aken et al., 2005), whereas it became immediately apparent that the preceding two observations had a common ground in salivary mucin, which has been considered responsible for this process (Vingerhoeds et al., 2005; Sarkar et al., 2009). Mucin binding at droplet interfaces appears to be dependent on the electrostatic interactions between the interfacial proteins and the mucins themselves (Silletti et al., 2007), although it is dependent on even small alterations in pH and can be reversible (Ritzoulis et al., 2012). Of the salivary proteins, MUC5B associates strongly to positively charged lysozyme-covered droplets and up to a degree to negatively charged β-lg-covered droplets, whereas MUC7 associates with β-lg-covered droplets (Silletti et al., 2010).

3.3.3 Muscle proteins

Muscle food proteins can be classified into three categories, based on their solubility, as follows: sarcoplasmic (water-soluble), myofibrillar (salt-soluble) and stromal (non-soluble). Myofibrillar proteins make up the largest proportion of total proteins, followed by sarcoplasmic and stromal, although this order may be different depending on animal age (Xiong, 1997; Hall and Ahmad, 1997).

The gelation of animal proteins is critical for the formation of desired texture in muscle foods (Park, 1995). Structured red meat and fish products are typical examples of foods in which protein gelation is responsible for the different levels of firmness, cohesiveness, elasticity, chewiness and so on (Hamann, 1987). The thermal gelation of myofibrillar proteins in various muscle foods (pork, beef, turkey, chicken, rabbit and fish) has been extensively studied by many researchers and the effects of various factors, such as heating rate and time, on several textural properties investigated (Lan et al., 1995; Yongsawatdigul and Park, 1996, 1999; Boyer et al., 1996).

Regarding sarcoplasmic proteins, their role in the gelation process of myofibrillar proteins is rather controversial. In surimi processing, for instance, their removal

during the leaching of fish mince with water was considered important because it was believed that if present in the mince, they would interfere with the gel-forming ability of myofibrillar proteins. However, in later studies Park et al. (2003) showed that addition of sarcoplasmic proteins significantly improved the gel properties of heat-induced surimi gels. Farouk et al. (2002) have also shown that sarcoplasmic proteins were important in determining the cohesiveness of cooked sausage batter. Detailed information about the contribution of sarcoplasmic proteins on the gelation of myofibrillar proteins is available in the review article by Jafarpour and Gorczyca (2012).

The most representative protein of the stromal group is collagen. The role of collagen in the development of texture of meat deriving from terrestrial animals and aquatic organisms, as well as after their subsequent cooking or freezing, is of paramount technological importance. Typically, meat from terrestrial animals exhibits a tougher texture than that deriving from fish, and this has been largely attributed to the higher collagen content of the former (Lampila, 1990; Hall and Ahmad, 1997). An exception to the category of aquatic organisms are cephalopods (e.g., squid, octopus, cuttlefish), which are known to have higher collagen content than fish, the content being higher in the head and tentacles than in the mantle (Thanonkaew et al., 2006; Katsanidis, 2004).

3.3.4 Gelatin

Gelatin is the water-soluble mixture of proteins and peptides that derives by partial hydrolysis of native collagen, the major protein in animal connective tissue (Karayannakidis et al., 2014). It presents a wide range of applications in the food industry sector and has been used as a means to improve the gelation, water binding, foaming and emulsifying properties of food products among others (Karayannakidis and Zotos, 2014). The most important technological property of gelatin is its ability to form thermoreversible gels, meaning that on cooling the colloidal solution (sol) forms a gel, whereas on heating, at temperatures below body temperature, the gel reverts into sol (Schrieber and Gareis, 2007; Karim and Bhat, 2008). It is this thermoreversibility process that gives gelatin its unique melt-in-mouth perception, which has yet to be matched by polysaccharides forming thermoreversible gels (e.g., agarose, pectin and carrageenans; Karim and Bhat, 2009).

The principal physical property of a gelatin preparation that determines its commercial value is gel strength, which is also known as Bloom value, when a standardized procedure is performed (Wainewright, 1977; Karayannakidis and Zotos, 2014). The Bloom value is defined as the weight (g) required to depress by 4 mm the surface of a 6.67% (w/v) aqueous gelatin gel, which has previously matured at 10 °C for 18 h (Karim and Bhat, 2008). Based on the preceding definition, it is obvious that the Bloom value of gelatin will increase with increasing rigidity. Generally, commercial gelatins can be divided into low Bloom (<150 g), medium Bloom (150–220 g) and high Bloom (>220 g; Anonymous, 2012). Table 3.1 shows the function of gelatin preparations of varying Bloom values in different food systems.

The main sources for the production of gelatin on a large commercial scale are the skins and bones of terrestrial animals (e.g., pig skins and bones and cattle hides and

Table 3.1 Application of gelatin in food products

Type of product	Function	Bloom	Level of usage (%)
Dairy products	Gelling agent, prevents syneresis, texture, thickening	150–250	0.2–1.0
Gelatin desserts	Gelling agent, texture, elasticity	175–275	7.0–9.0
Meat products	Meat binding, gelling agent, stabilization, texture, covering	175–275	1.0–5.0
Confectionery	Gelling agent, texture, elasticity, chewability, binding, stabilization, aeration	50–275	0.5–9.0

Anonymous (2012).

bones; GME, 2014). However, since the livestock disease outbreaks (bovine spongiform encephalopathy and foot-and-mouth disease) and due to several religious restrictions (Hinduism, Judaism and Islam), alternative sources of gelatin have gained momentum (Karayannakidis and Zotos, 2014). Over the last decade, fish processing by-products have received considerable attention as an alternative source of gelatin, and many research studies have been performed regarding the extraction of gelatin from the skins, bones, scales and heads of various fish species (Jongjareonrak et al., 2006; Shakila et al., 2012; Wangtueai and Noomhorm, 2009; Arnesen and Gildberg, 2006). However, the physical properties (Bloom strength, viscosity and melting point) of fish gelatin have been found to be slightly inferior when compared to mammalian gelatin, which basically reflects the differences in the temperature of animals' living habitat. Nevertheless, Choi and Regenstein (2000) have shown through sensory analysis that fish gelatin was more preferable than porcine gelatin due to the lower melting point of the former, which enabled a better release of aroma and provided a stronger flavour of the cherry juice that was used to prepare both gelatin gels.

3.3.5 *Microparticulated proteins*

The use of microparticulated proteins has received attention recently, as these materials are promising texture modifiers, especially concerning texture restoration of foods after the removal of fat. Microparticulation consists of the controlled aggregation of proteins or of other macromolecules or of self-assembled smaller molecules to form particles in the nanometer scale. Microparticulation can be achieved, among other methods, via simultaneous heating and shearing (Singer et al., 1990), homogenization (Onwulata et al., 2002), other shearing processes (Dissanayake and Vasiljevic, 2010), acidic extrusion cooking (Queguiner et al., 1992), spray-drying (Toro-Siera et al., 2013), whereas membrane treatment can assist the process (O'Mahony and Tuohy, 2013).

A volume of works has highlighted that removal of fat from a product tends to reduce its sensory rating (i.e., Ritzoulis et al., 2010 for the direct link between reduction of fat content in selected sausages and decrease in acceptability). One of the principal applications of microparticulated proteins is fat replacement with other edible components (e.g. Schenkel et al., 2011 for cheese). As proteins are in general of significantly lower caloric value than fats (Akoh, 1995), a good incentive exists for the use of proteins as fat replacers. Another field of application for microparticulated proteins lies on the substitution of starch. The mechanism by which microparticulated protein produces similar textural responses to fat or starch is still an open field. Some data does exist on the putative mechanism of sensory replacement of fat, for example, the observation that the structures formed by added microparticulated whey protein are fractally similar to the fat-containing yoghurt structure formation (Torres et al., 2012). The simple fact that the fat particles and the protein microparticles are roughly of the same size should not be overlooked.

The ratio of native to denatured protein appears to be of some importance to the properties of the end products. The denatured protein obviously results from the intense treatment, and in that form protein is expected to be involved in the aggregation process leading to the formation of microparticles. Microparticulated whey protein with a high native-to-denatured ratio provided yoghurts with high creaminess and viscosity, slow meltdown in the mouth, creamy flavour and low syneresis (Torres et al., 2011).

Microparticulated whey protein can be manipulated with pH, heat treatment and intense homogenization conditions to achieve desirable particle size distribution, charge and viscosity (Chung et al., 2014), or to induce gelling (Dissanayake et al., 2010). Inulin has been successfully incorporated into microparticulated whey protein, altering the latter's level of denaturation during microparticulation and also affecting aggregation level and solution viscosity (Tobin et al., 2010). Such approaches can open the way for the manipulation of the functionality of microparticulated protein.

Utilization of microparticulated whey protein on Caciotta-type cheeses is reported to reduce the perceived firmness, whereas it contributes to moisture retention in the cheese (Di Cagno et al., 2014). Utilization of microparticulated whey protein as a fat replacer in cakes yielded products with lower resilience than certain carbohydrate fat replacers, but it preserved the crumbliness of certain low-fat formulations in respect to the control sample (Psimouli and Oreopoulou, 2013). An interesting application of microparticulated biopolymers is that of an oil repellent: Kim et al. (2012) used wheat-flour microparticulated flour wheat bran to decrease the oil-holding capacity of doughnuts.

3.3.6 Antifreeze proteins

A group of proteins that indirectly affects the textural properties of frozen foods are the so-called antifreeze proteins (AFPs). AFPs are a group of proteins and peptides of low molecular weight that lower the freezing point of water and inhibit ice-crystal growth, including recrystallization (Feeney and Yeh, 1998). They were first isolated

from various marine fish species, such as Atlantic cod, winter flounder and smelt, and in later studies from plants, fungi and bacteria (Griffith and Ewart, 1995).

AFPs are reported to be effective in preventing ice-crystal growth in frozen food systems. Payne et al. (1993) studied the cryoprotective effects of Antarctic cod and winter flounder AFPs in meat during chilled (2 °C) and frozen storage (–20 °C). Although AFPs had no significant effect on meat samples subjected to chilled storage, their presence in the samples subjected to frozen storage resulted in significantly lower intracellular spaces when compared to the samples without AFPs, indicating the formation of small-sized ice crystals in the former case. In another study Zhang et al. (2007) showed that the texture of dough supplemented with concentrated carrot protein, containing 15.4% (w/w) carrot AFP, was softer and steadier during frozen storage when compared against the sample without AFP. Although AFPs from different sources are commercially available, their applicability in frozen foods has been limited due to cost issues (Feeney and Yeh, 1998).

3.4 Challenges and perspectives

The preceding brings together some issues of importance, or at least of interest, pertaining to the effect of proteins on the perceived texture of foods. One could try to summarize some issues of the current state of the art, with a mind on future perspectives in the following statements:

- As macromolecules, proteins may, under certain conditions, be used to modify or tune the mechanical properties of the foods. The imparted properties are due to a host of mechanisms involving bulk or interfacially adsorbed proteins (even in water-in-water emulsions) and may be heavily influenced by factors such as temperature, ionic strength and pH. Events such as mixing or phase separations between two or more (glyco)proteins/polysaccharides may dominate the mechanical behaviour of food.
- Mechanical properties are not necessarily directly related to the food texture; the latter is also influenced by other factors, always keeping in mind that a food bolus is a time-dependent, two-component mixture of the food and of saliva. Salivary glycoproteins mucins appear to play a major role in this process, whereas their stability regimens with food proteins (and food components in general) are only partially mapped.
- The effect of interactions between proteins and other components (i.e., proteins, polysaccharides, water, triglycerides) on the mechanical properties of foods is known in relative detail for some widely studied foods (e.g., dairy products and bread). Little is known for most other categories of food, even less so for the effect of such interactions on the texture of food. Similarly, with the exception of some major foods (e.g., dairy products), surprisingly little information is found in the literature on the structure of complex interfaces (e.g., aqueous protein gel–fat particle in sausages), and of their effect on the mechanical properties and, indirectly, on the texture of such foods.
- In principle, proteins could be customized in terms of hydrophobicity, dimensions and pH/ionic strength behaviour to match specific mechanical/textural requirements. The tools for such customization may involve denaturation, hydrolysis, genetic modification, covalent bonding, glycosylation and other techniques. One should always keep in mind, however, the possible health risks associated with some of these treatments.

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Enzymatic modification of dairy product texture

4

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4.1 Introduction

Dairy products represent one of the most extensive categories of products produced by the modern global food industry and one of the most scientifically and technologically complex, in terms of production and factors affecting their characteristics.

Along with flavour, texture is a key defining characteristic of many dairy products, and much of the diversity of dairy products arises from the fact that the constituents in milk can, following a range of biological or mechanical treatments, undergo a huge range of different interactions that ultimately determine the texture of the final products. For example, by applying different processes, liquid milk can be modified relatively easily to yield structures varying from that of a hard cheese to a yoghurt; how to achieve these conversions has been known for millennia, long before the underlying science was elucidated. In addition, cheese texture, and differences among varieties, is very dependent on the action of enzymes from different sources.

In recent years, a number of novel strategies for manipulating the texture of dairy products by enzymatic means have been explored. Probably the best characterized of these approaches involves the use of transglutaminase (TGase), which has applications in a number of different food systems and for which some commercial applications have been realized. However, there has also been extensive study of a number of other enzymes, including tyrosinases, peroxidases, laccase and others.

This chapter reviews the state of knowledge of the impact of these enzymes on milk proteins and provides a perspective on their future potential application in the dairy industry.

4.2 The texture of dairy products

Probably the key milk constituent of relevance to the texture of dairy products is protein; bovine milk contains two distinct families of proteins of very different properties, caseins and whey proteins, which are typically present in a ratio of 75–80:20–25. The caseins in bovine milk consist of four sub-types, α_{s1}-, α_{s2}-, β- and κ-casein, and are found in milk associated into colloidal structures called casein micelles, along with

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minerals collectively referred to as colloidal calcium phosphate (CCP). Typical diameters for casein micelles are in the range 100–300 nm, and the micelles are highly hydrated, containing 3–4 g of water per g of dry matter. The dry matter consists primarily of caseins, but also contains 5–7% inorganic material, collectively referred to as CCP. This CCP is present in the form of nanoclusters (2–2.5 nm in radius), which are stabilized on the surface by phosphoserine (SerP) residues from the caseins. Because caseins also show intermolecular association, a network can be created. As a result, particles the dimensions of a casein micelle can be created, consisting of >10,000 casein molecules and several hundred CCP nanoclusters. The micelles are stabilized in the aqueous environment of milk by the presence of κ-casein, which is a phosphoglycoprotein with an amphiphilic character, at the surface of the micelle, where it stabilizes micelles against aggregation through steric and electrostatic repulsion. The stabilization of the casein micelles by κ-casein has been described as that of a colloidal particle stabilized by a polyelectrolyte brush in a medium of rather high ionic strength. Overall, casein micelles can be considered as hydrated proteinaceous association colloids stabilized by a polyelectrolyte brush. The encapsulation of calcium phosphate in nanoclusters in the core of these micelles presents one of nature's truly great examples of natural nanoencapsulation.

This stabilization can be overcome by two routes: enzymatic hydrolysis of κ-casein to remove the protective 'hairs', or adjustment of pH to 4.6, the isoelectric point of casein, at which brush solvency is reduced and the outer 'brush' layer collapses. The former is the principle of rennet coagulation of milk, the first step in cheese making, whereas the latter is the basis of the acid coagulation leading to products such as yoghurt. Both processes transform milk into a gel, comprised of a three-dimensional network of aggregated casein micelles, but the gels have very different properties. In addition, other factors such as application of heat or addition of alcohol can affect the properties and aggregation of milk proteins; indeed, heating of milk in the presence of alcohol, followed by cooling, has been shown to produce aggregates of casein micelles (O'Connell et al., 2003). Treatment at high pressures can also result, depending primarily on pressure applied, in association or dissociation with casein micelles (Huppertz et al., 2006), whereas adjustment of milk to alkaline pH values results in dissociation of casein micelles (Vaia et al., 2006).

The other protein family in mammalian milk is the whey proteins, which in bovine milk consist of two major proteins, α-lactalbumin and β-lactoglobulin, with bovine serum albumin, immunoglobulins and other proteins being present at lower levels. In raw milk, these globular proteins are found in the serum phase and have little significance for texture. However, heating at temperatures above 75 °C results in very significant alterations to both the structure and textural significance of the whey proteins, driven principally by the denaturation of β-lactoglobulin; this protein contains a free sulphydryl group, which, when exposed by heat-induced unfolding of the molecule, can form new disulphide bonds with a range of milk proteins, including formation of homo-polymers (up to large macroscopic aggregates), complexes with α-lactalbumin and complexation with the casein micelles through binding to surface κ-casein or serum κ-casein.

This has a range of consequences for dairy product texture. For example, under optimal conditions of pH and ionic strength, solutions of isolated whey protein or β-lactoglobulin of sufficient concentration (typically >10% protein) can be induced to form gels on heating, and this ability to contribute to texture has resulted in whey proteins being regarded as very valuable food ingredients (van Vliet et al., 2004; Dissanyake et al., 2013; Cornacchia et al., 2014). Furthermore, the reaction of whey proteins with casein micelles is a key consideration in producing a nonsyneresing gel during the manufacture of yoghurt, for which milk is thus heated under conditions (e.g., 80–90 °C for 5–15 min) that are sufficient to induce almost complete whey protein denaturation. In addition, under certain conditions (low pH and targeted hydrolysis), whey proteins can form structures such as fibrils (Loveday et al., 2012), which can form gels at lower protein concentrations compared to native whey proteins (Loveday et al., 2009).

The significance of milk fat for dairy product texture is less than that of milk proteins, but the high saturated triglyceride content found in bovine milk fat means that, at refrigeration temperatures, milk fat, and products rich in milk fat, such as butter, are rather solid and poorly spreadable. This has necessitated development of a range of strategies to produce more spreadable butter, for example, altering the diet of cows to produce more unsaturated (and hence softer) fat, controlling the cooling profile of cream between pasteurisation and churning to get the best possible crystal structure in the final butter, and fractionation of milk fat by temperature-mediated crystallization. The specific textural properties of milk fat are also a reason why low-fat cheese products have struggled to match the mouthfeel and hence consumer acceptability of their full-fat counterparts.

Of the major components of milk, the disaccharide lactose has perhaps the least textural significance, except in contexts where it is present at levels beyond its saturation limit, and where it thus forms crystals (e.g., sweetened condensed milk); in such cases, specific technological interventions are required to control crystal type and size so as not to yield a sandy or grainy-textured product.

4.3 Role of indigenous milk enzymes

Milk contains a range of indigenous enzymes, including proteases, lipases, oxidoreductases, phosphatases and others; there are probably at least 100 enzymes present in milk, and several have been reported only fleetingly, with around a dozen having been studied in any detail to date (Fox and Kelly, 2006a,b; Kelly and Fox, 2006).

Due to the importance of protein for the texture of dairy products, it is perhaps not surprising that the impact of indigenous proteases on dairy product texture has received more attention than other enzyme types in this context (Kelly et al., 2006). Of the milk proteases, the best studied is the alkaline protease plasmin, which has a pH optimum of 7.5 and a preference for hydrolysis of β-casein, from which it produces a family of products known as γ-caseins and proteose peptones (Ismael and Nielsen, 2010). In addition, milk contains a variable level of somatic cells, the lysosomes of which contain a range of enzymes, including proteases such as cathepsins

B and D and elastase, the activity of which have been relatively little studied in milk (Haddadi et al., 2006); it is likely that the significance of such enzymes in good-quality milk is relatively low, but as the somatic cell count increases, particularly in cases of mastitis, their relative contribution is likely to greatly increase (Larsen et al., 2004, 2006, 2010; Somers et al., 2003).

It has been shown that hydrolysis of casein by plasmin can reduce the strength of rennet-induced gels through these proteolytic pathways (Srinivasan and Lucey, 2002; Somers et al., 2002) and can also reduce firmness and viscosity of yoghurt, along with reduced serum separation (Gassem and Frank, 1991). Mara et al. (1998) added purified plasmin to milk at a range of levels and, following incubation, showed that increased levels of plasmin-induced hydrolysis of caseins were associated with increased curd firming and cutting time and reduced curd firming rate and curd firmness, but had little impact on rennet coagulation time (RCT) or levels of curd moisture. It should be noted that the effect of added plasmin on milk properties may differ from that of indigenous enzyme, which is intimately associated with the casein micelles and thus may differ in terms of access to bonds for cleavage and interaction with serum-phase inhibitors.

Plasmin has also been reported to be a likely factor (along with physicochemical changes in milk proteins and the Maillard reaction) in the age gelation of ultra-high-temperature (UHT) milk (Chavan et al., 2011), although the exact mechanism underpinning this phenomenon is not completely understood (Datta and Deeth, 2001); nonetheless, the enzyme is highly heat stable, and peptide formation is generally correlated with unstable milk during storage (Gaucher et al., 2008, 2009).

Proteolytic enzymes derived from the lysosomes of somatic cells in milk may also impair the coagulation properties of milk and contribute to the poor quality of cheese made from mastitic milk (Revilla et al., 2007), and it has been reported that the lysosomal enzymes cathepsin D and elastase can coagulate milk under certain conditions (Larsen et al., 1996; Considine et al., 2002).

4.4 Enzymes in cheese

As mentioned earlier, the transformation of milk into cheese is an archetypal example of the use of enzymes to modify the texture of dairy systems, as it has been known for millennia that calf stomachs contain an agent (now identified as the enzyme chymosin) that has a highly specific action on the stabilizing effect of κ-casein in the casein micelle system, destabilizing the micelles by hydrolysis of the Phe_{105}—Met_{106} bond of κ-casein, which leads to gelation of milk through aggregation of the destabilized micelles into a three-dimensional network (McSweeney, 2004).

The fresh curd thus produced, when separated from the surrounding whey, then undergoes a series of complex biochemical reactions to develop flavour and texture, with relatively minor differences in exact make procedures, ripening conditions (e.g., temperature and humidity) and, critically, cultures and enzymes present or added to the milk or cheese, yielding a great diversity in cheese characteristics and hence varieties.

The texture of cheese develops in the first instance through the hydrolysis of casein by a range of proteolytic enzymes in the cheese, which eventually leads to peptides, amino

acids and flavour compounds; sources of these enzymes include the milk (i.e., plasmin as discussed earlier), residual coagulant that has not been lost in the whey, enzymes of the starter bacteria (typically lactic acid bacteria of the genus *Lactococcus* or *Lactobacillus*), enzymes of nonstarter adventitious lactic acid bacteria, and enzymes of other microorganisms that may be added (e.g., yeasts and moulds such as *Penicillium camemberti*). The early stage of hydrolysis erodes the paracasein network in the fresh curd, progressively softening the cheese through hydrolysis of bonds and reduction in the level of intact casein (Lucey et al., 2003; McSweeney et al., 2006). Although a number of studies have indicated that chymosin-mediated proteolysis plays a significant role in the softening of cheese texture (Wium et al., 1998), in more recent years it has been proposed to be less significant than the impact of calcium solubilization (O'Mahony et al., 2005). However, the impact may depend on the cheese variety in question, as Al-Otaibi and Wilbet (2006) reported that reducing the level of chymosin in white salted cheese did not directly impact on cheese textural properties and proposed that any impact on hardness due to initial protein hydrolysis was being confounded by other factors; in contrast, Madadlou et al. (2005) found that increasing rennet concentration reduced values of instrumental hardness of white cheese. In addition, Wium et al. (1998) proposed that the action of chymosin could have a specific effect on cheese texture that was not correlated with general levels of proteolysis, and Yun et al. (1993) reported that the choice of coagulant (on comparing use of *Endothia parasitica* protease, fermentation-derived chymosin and *Mucur meihei* protease) had a significant influence on the texture of mozzarella cheese, with *E. parasitica* coagulant yielding cheese with a softer texture than the other two enzymes.

The choice of coagulant has a significant influence on the texture of cheese, and a wide range of types of coagulant are used for cheese making today, including plant (e.g., *Cynara cardunculus*), microbial (e.g., *Mucor meihei*) and recombinant enzymes (Lane et al., 1997; Garcia et al., 2012). In recent years, a number of studies have examined the possible implication of use of camel, rather than calf, chymosin as a coagulant for cheese making (Govindasamy-Lucey et al., 2010; Bansal et al., 2009). Moynihan et al. (2014) showed that camel chymosin could be used to produce low-moisture part-skim mozzarella cheese that maintained key functional properties for a longer shelf life than conventional bovine calf chymosin, whereas Borsting et al. (2012) studied the impact of these two types of chymosin, along with starter choice, on reduced-fat Cheddar cheese and reported that camel chymosin resulted in a less bitter but harder cheese.

Lamb rennet paste was shown by Ferrandini et al. (2011) to yield a firmer Murcia al Vino cheese than calf rennet, whereas Pirisi et al. (2007) compared the impact of lamb rennet paste from different sources (traditional and industrial manufacture) on proteolysis and lipolysis during ripening of bovine milk cheese.

4.5 Enzymatic cross-linking of proteins

Texture of dairy products is mainly determined by the extent of physical and chemical interactions between polymeric components, in particular proteins. The positive effects of high-temperature heat treatment, which leads to increased hydrophobic interactions and incorporation of intermolecular disulphide bridges into the protein

network during yoghurt fermentation, is a well-documented fact today, as mentioned earlier in this chapter.

By comparison, enzymatic cross-linking of proteins, that is, enzyme-catalyzed formation of covalent bonds between reactive amino acid residues of protein molecules, represents a relatively new approach for the improvement of food structure. Alteration of the functional properties of milk proteins through enzymatic cross-linking may be utilized to manufacture dairy products with appealing texture, improved stability and water-holding properties, despite reduced-fat, reduced protein or dry matter contents.

Cross-linking enzymes have gained interest during the past few decades, mainly after the availability of Ca^{2+}-independent microbial TGase (Ando et al., 1989). TGase has been extensively investigated in meat (Marques et al., 2010), plant (Dube et al., 2007) and dairy (Huppertz, 2009; Jaros et al., 2006a; Loveday et al., 2013) protein applications. It has had GRAS (generally recognized as safe) status since 1998 and is recognized as a processing aid in EU regulations (Regulation (EC) No 1332/2008). TGase is currently used for protein cross-linking in industrial production of various food commodities for improved product texture, stability and overall profitability. In addition to TGase, which is a transferase, oxidative enzymes such as tyrosinases, laccases, peroxidases, and sulphydryl oxidases (SOXs) have also been shown to have the ability to introduce covalent cross-links between/within proteins with or without use of auxiliary substances (see recent reviews by Buchert et al., 2007, 2010; Gerrard and Cottam, 2012; Heck et al., 2013; Huppertz, 2009; Zeeb et al., 2014).

Milk proteins are probably the most studied substrates for enzymatic cross-linking, due both to their abundance in high purity forms and to their well-characterized molecular and physicochemical properties, which enables thorough assessment of enzymatic modifications. Caseins are superior substrates for enzymatic cross-linking compared to whey proteins because of the accessible structure of the former. The confined tertiary structure of whey proteins in their native form limits enzymatic catalysis, as the target amino acid residues may be embedded in the interior of the molecule and thus is inaccessible. Complete or partial denaturation of the globular structure increases the susceptibility of whey proteins to enzyme-induced cross-linking reactions (Eissa et al., 2006; Ercili-Cura et al., 2012; Partanen et al., 2011; Sharma et al., 2001).

Enzymatic cross-linking may occur between two different protein molecules (intermolecular cross-linking) as well as within a single molecule (intramolecular cross-linking; Figure 4.1). In both conditions, certain physicochemical and functional properties of protein molecules and the structural properties of the matrices they form are affected. However, from a technological point of view, intermolecular cross-linking is most prevalent and leads to modification of the textural properties in protein-stabilized systems.

4.5.1 Cross-linking enzymes

4.5.1.1 Transglutaminase

TGases (EC 2.3.2.13, glutaminylpeptide:amine γ-glutamyltransferase) catalyze an acyl transfer reaction between a protein-bound glutamine residue (acyl donor) and an ε-amino group of a protein-bound lysine residue (acyl acceptor), leading to

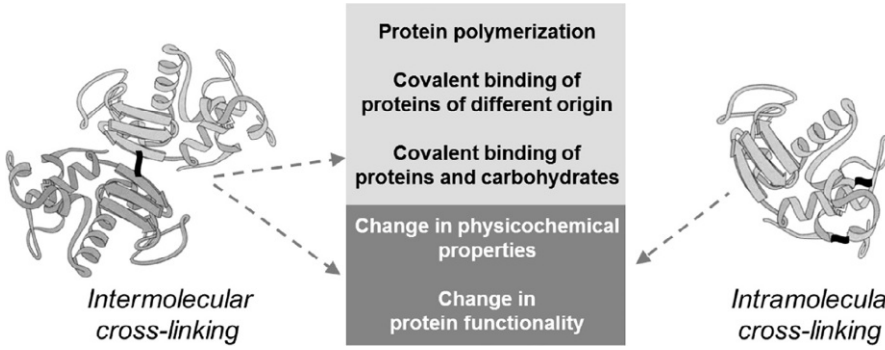

Figure 4.1 Schematic representation of enzyme-catalyzed covalent bonds between (intermolecular) or within (intramolecular) protein molecules and predicted outcomes.

(γ-glutamyl)-lysine isopeptide linkages. In the absence of amines, water serves as acyl acceptor, leading to deamidation of glutamines. Ammonia is generated during the TGase-catalyzed reactions (Folk and Finlayson, 1977; Kashiwagi et al., 2002). Accordingly, TGase can modify protein substrates by incorporating inter- and/or intramolecular isopeptide linkages and also by changing certain physicochemical properties through deamidation. The commercially available TGase from *Streptomyces* species shows optimum activity in the pH range 5–8 (Ando et al., 1989). In addition, it is Ca^{2+}-independent, shows a high reaction rate and has a low activity for deamidation (Kashiwagi et al., 2002). All those attributes make microbial TGase practical for use in a variety of food systems.

TGase has shown an excellent ability to cross-link caseins without required pretreatment and whey proteins after unfolding of the globular structure by means of, for example, heat, pressure or addition of reducing agents (e.g., dithiothreitol [DTT]; Huppertz, 2009; Jaros et al., 2006a). In milk, where caseins are found as association colloids, that is, micelles, κ- and β-caseins were shown to be the proteins most susceptible to TGase-catalyzed cross-linking, followed by α_s-caseins (Hinz et al., 2012; Hsieh and Pan, 2012). The effects of TGase-catalyzed cross-links in milk on the structure of dairy products made therefrom will be elucidated in the following sections.

4.5.1.2 Oxidative enzymes (oxidoreductases)

Oxidoreductases (oxidases, oxygenases, peroxidases) are enzymes that catalyze the transfer of electrons from one molecule (the oxidant, the hydrogen or the electron donor) to another molecule (the reductant, the hydrogen or electron acceptor). Although these enzymes have distinct biological roles in their native host organisms, for example, melanin synthesis, lignin formation/degradation, and protein folding, their pronounced lack of substrate specificity enables them to catalyze cross-linking reactions between biopolymers (such as proteins and carbohydrates). These enzymes are either still only being studied at laboratory scale (e.g., tyrosinase, laccase, SOXs) or are already established biocatalysts (e.g., peroxidase, glucose/hexose oxidases) for modifying the texture of foods.

4.5.1.3 Tyrosinase

Tyrosinases (EC 1.14.18.1, monophenol, *o*-diphenol:oxygen oxidoreductase) catalyze two distinct reactions: (i) hydroxylation of monophenols to *o*-diphenols (monophenolase or cresolase activity) and (ii) subsequent oxidation of diphenols to quinones (diphenolase or catecholase activity). Molecular oxygen is used in both steps as the electron acceptor (reviewed by Claus and Decker, 2006; Faccio et al., 2012b; Fairhead and Thöny-Meyer, 2012). Quinones are highly reactive species and can undergo nonenzymatic reactions, similar to the ones responsible for synthesis of melanins, or browning in fruits and vegetables (Bittner, 2006; Yang et al., 2009). In proteins, tyrosinase can oxidize the tyrosine residues to the corresponding quinones, which further react nonenzymatically with other tyrosines, cysteines, lysines or histidines in the same or a different molecule, resulting in inter- and/or intramolecular cross-links (Buchert et al., 2010; Faccio et al., 2012b). The most extensively investigated and used tyrosinase so far for research purposes is that from the mushroom *Agaricus bisporus*, mainly because of its commercial availability (Fairhead and Thöny-Meyer, 2012). Fungal tyrosinases show optimum activity at neutral or slightly acidic pH values, whereas an alkaline tyrosinase from *Trichoderma reesei* has also been reported (Selinheimo et al., 2006) and used for protein cross-linking (Mattinen et al., 2008a; Selinheimo et al., 2007b).

Tyrosinases of different origins have been used for cross-linking of milk proteins. Both caseins (Ercili Cura et al., 2010; Halaouli, 2005; Mattinen et al., 2008b; Monogioudi et al., 2009, 2011; Selinheimo et al., 2007b, 2008; Stanic et al., 2010) and whey proteins (Ercili-Cura et al., 2012; Mattinen et al., 2008b; Partanen et al., 2011; Thalmann and Lötzbeyer, 2002) have been cross-linked with various tyrosinases. Tyrosinase from *T. reesei* was found to be superior against *A. bisporous* tyrosinase in cross-linking efficiency (Ercili Cura et al., 2010; Selinheimo et al., 2007b).

4.5.1.4 Laccase

Laccase (EC 1.10.3.2, benzenediol:oxygen oxidoreductase) catalyzes reactions that proceed by oxidation of a substrate molecule (phenols, aromatic or aliphatic amines, thiols) to corresponding free radicals. The free radicals thus produced can further undergo nonenzymatic reactions, leading to polymerization but also depolymerization reactions (see reviews by Claus, 2004; Riva, 2006). Laccase-catalyzed modification of proteins proceeds via oxidation of tyrosine, tryptophan and possibly also cysteine residues. Mainly isodityrosine-type linkages were identified on cross-linking of proteins and peptides by laccase (Mattinen et al., 2005, 2006). Reactivity of laccases with proteins, however, is limited due either to the limited accessibility of the enzyme active site to the proteins or high redox potential of the proteins (Riva, 2006; Xu, 1996). Thus, small phenolic molecules, which are readily oxidized by laccase, are often used as mediators in laccase-catalyzed cross-linking of proteinaceous substrates. Laccases show wide variation in their MW and optimum activity conditions, depending on their origin; for phenolic substrates, the optimum pH can vary between 3 and 7 for fungal laccases (Xu, 1996).

Whey proteins have been polymerized with different origin laccases in the presence of chlorogenic acid (Faergemand et al., 1998), ferulic acid (Steffensen et al., 2008), vanilic acid (Ma et al., 2011), and apple or cherry phenolics (Tantoush et al., 2011). Similarly, caseins have also been cross-linked either in the presence or absence of phenolic mediators (Ercili Cura et al., 2009; Hiller and Lorenzen, 2009; Paananen et al., 2013; Selinheimo et al., 2008; Stanic et al., 2010; Steffensen et al., 2008). Laccases can also induce formation of heteropolymers by making use of naturally existing phenolic moieties in, for example, polysaccharides to couple with proteins. Hetero-cross-linking between hydrolyzed oat spelt xylan and α_s-caseins has been reported (Selinheimo et al., 2008). In addition to cross-linking, protein fragmentation sourced from laccase-induced free radicals has also been reported in various food systems (Ercili Cura et al., 2009; Lantto et al., 2005; Selinheimo et al., 2007a).

4.5.1.5 Peroxidases

Peroxidases are a diverse group of oxidoreductases that use hydrogen peroxide as an electron acceptor to oxidize a variety of substrates such as phenols, amines and thiols, with concomitant reduction of hydrogen peroxide to water (see review by Veitch, 2004). In the presence of hydrogen peroxide, peroxidase-catalyzed protein cross-linking proceeds mainly through the coupling of two phenolic radicals formed by tyrosine oxidation (Heijnis et al., 2011; Minamihata et al., 2010). Horseradish peroxidase (EC 1.11.1.7) is probably the peroxidase most widely used for cross-linking purposes.

Peroxidase-catalyzed cross-linking of caseins and whey proteins has been previously reported (Faergemand et al., 1998; Hiller and Lorenzen, 2009; Matheis and Whitaker, 1984). Recently, researchers from Wageningen University (the Netherlands) thoroughly elucidated the cross-linking of α-lactalbumin with horseradish peroxidase and characterized the obtained protein nanoparticles under various environmental conditions (Dhayal et al., 2014; Heijnis et al., 2010a,b, 2011; Saricay et al., 2012, 2013). Similar to laccases, peroxidase-catalyzed formation of hetero-conjugates between feruloylated arabinoxylans and β-caseins was reported (Boeriu et al., 2004).

4.5.1.6 Sulphydryl oxidases

SOXs (glutathione oxidase, EC 1.8.3.3 and thiol oxidase, EC 1.8.3.2) catalyze oxidation of thiols to disulphide bonds. Molecular oxygen is reduced either to hydrogen peroxide or water, depending on the structure and the origin of the SOX. The substrate specificity of SOXs is not strict and ranges from small thiol compounds such as DTT to protein-bound cysteine residues (Faccio et al., 2011). SOXs naturally exist in bovine milk. Bovine milk SOX has been reported to catalyze oxidation of cysteine and its analogues, some volatile thiols, peptides, and also thiols in proteins (Swaisgood, 1980), and has been exploited in elimination of 'cooked' flavour in UHT-treated milk (Swaisgood, 1977). At present, research on exploitation of SOXs as cross-linkers for food structure modification is rather limited. They have been most extensively studied in baking applications for possible formation of

SOX-catalyzed disulphide linkages in gluten and also additional nonenzymatic cross-linking catalyzed by produced hydrogen peroxide. Fungal SOXs have shown positive effects on dough characteristics when used together with glucose oxidase or hemicellulases (see review by Faccio et al., 2011) or in presence of ascorbic acid (Faccio et al., 2012a).

4.5.1.7 Glucose oxidase

Glucose oxidases (EC 1.1.3.4) catalyze the oxidation of glucose to gluconic acid and hydrogen peroxide (Vroeman, 2003). Peroxide generated due to the action of glucose oxidase has been suggested to oxidize cysteine residues, leading to formation of disulphide linkages in wheat dough systems (Rasiah et al., 2005). Glucose oxidase has shown cross-linking activity also in milk protein systems either alone (Hiller and Lorenzen, 2009) or in the presence of horseradish peroxidase and glucose (Chang and Zhao, 2012). The glucose oxidase/peroxidase system has potential especially in the baking industry as a dough structure improver.

4.6 Structural modification of fermented milk gels by enzymatic cross-linking

Tailoring the structure of dairy products by use of enzymatic protein cross-linking necessitates thorough understanding of the molecular and colloidal aspects of milk proteins and their interactions during and after coagulation. Extensive research has been conducted on using TGase as a protein cross-linker, and molecular and macromolecular effects have been documented. In comparison, the use of oxidative enzymes in structure modification of dairy gels has been investigated only in a few studies, although some of these have shown promising results in cross-linking milk proteins directly in the environment of milk or caseinate dispersions. It is rather more challenging to draw conclusions about the effects of oxidase-mediated cross-linking on formation and structure of protein networks, as the nature of bonds are less specific compared to that of isopeptide linkages created by TGase. Moreover, formation of reactive quinones or free radicals as the precursors of protein–protein linkages may result in colouring of the media, which limits the use of oxidative enzymes in food products (Buchert et al., 2010; Heck et al., 2013).

Treatment of milk with TGase before or during fermentation (or chemical acidification) results in increased gel firmness and elasticity in set-type and higher viscosity in stirred-type acid milk gels. Moreover, TGase-catalyzed cross-linking of proteins leads to improved water-holding capacity and hence decreased levels of serum separation in milk gels (Huppertz, 2009; Jaros et al., 2006a). Serum separation is a major quality defect in fermented dairy products, especially when fat or dry matter content is reduced. With the use of TGase (at an optimal dosage), non-fat yoghurt with physical and sensory properties that are comparable to those of full-fat yoghurt can be produced without the need for additional protein or stabilizers (Lorenzen et al., 2002; Özer et al., 2007). In stirred-type yoghurts, TGase treatment is also advantageous, as desired

viscosity levels can be achieved at lower protein or total solids contents. In fact, a small extent of protein cross-linking has been shown to increase the viscosity of stirred yoghurt considerably, allowing a 1.2% decrease in protein content compared to a conventional control (Bönisch et al., 2007a). Some of the most recent publications on TGase treatment in set- and stirred-type yoghurts are summarized in Table 4.1; for earlier publications, please refer to the reviews of Huppertz (2009) and Jaros et al. (2006a).

The effects of the TGase-induced protein cross-linking on gel structure depends highly on the dosage of the enzyme/incubation period, in other words, to the degree of protein polymerization. Whether the enzyme was left active during the course of gel formation or inactivated prior to the addition of the acidulant/starter cultures was also found to be an important parameter for determination of the final gel properties (Jaros et al., 2006b; Lorenzen et al., 2002). A degree of polymerization of 20–25% was shown to result in increased firmness, as analyzed both by small- and large-deformation rheological measurements in set-type (Jaros et al., 2006b) and increased viscosity in stirred-type (Bönisch et al., 2007a) gels. However, a further increase in degree of polymerization caused reversal of this behaviour in set-type gels when the enzyme was inactivated before acidification (Jaros et al., 2006b, 2010, 2014). Extensive cross-linking was suggested to result in impaired gel firmness due to restriction of proper rearrangements during gel formation (Jaros et al., 2006b, 2010).

On the other hand, when TGase was active during the course of acidification, extensive cross-linking before and during acidification led to a continued increase in firmness, as determined by large-deformation measurements (penetrating probe), whereas the effect was suppressed for elastic modulus, as determined by rheological measurements (Ercili-Cura et al., 2013; Jaros et al., 2006b). Due to the colloidal stability of casein micelles and the high casein concentration inside the micelle, it is mainly intramicellar cross-linking that takes place in milk (Huppertz and De Kruif, 2008; Huppertz et al., 2007; Moon et al., 2009). At high enzyme dosages, extensive intramicellar cross-linking apparently affects small-deformation rheological measurements in a more complex manner compared to the firmness as measured by a texture analyzer. In fact, Jaros et al. (2014) recently demonstrated that extensive cross-linking in an acid casein solution results in large and inflexible casein aggregates that do not contribute to elastic modulus, compared to moderately cross-linked aggregates, which can still be incorporated into the protein network efficiently.

Despite the intensive research over the last few decades, efficient use of TGase in industrial applications is yet to be further improved. In practice, treatment of yoghurt milk with TGase simultaneously with the fermentation is a more cost- and time-efficient method compared to pretreating the milk with TGase for a certain time followed by inactivation (by additional heat treatment) prior to fermentation. It is, however, impossible to inactivate the enzyme by the former method. Even though TGase activity is significantly reduced below pH 5 (Ando et al., 1989), it has been shown that residual activity led to increasing gel strength and viscosity during storage in set-type (Lorenzen et al., 2002; Yüksel and Erdem, 2010) and stirred yoghurts (Guyot and Kulozik, 2011; Şanli et al., 2013; Wróblewska et al., 2011). If the structural strengthening exceeds the tolerable levels, this may result in unacceptable sensory properties, especially for stirred yoghurts.

Table 4.1 Effect of TGase on technological properties of set and stirred type yoghurts

Product	TGase treatment	Effects on firmness and viscosity	Reference
Set-type yoghurt (3% fat)	TGase (1 U/g protein) was added to milk before or after heat treatment and incubated for 0, 10, or 60 min at 50 °C before addition of the starter bacteria	Gel strength and viscosity of the yoghurts were significantly higher when TGase was not inactivated. Increase in firmness and viscosity during storage was not statistically significant	Şanli et al. (2011)
Ayran (a traditional drinking yoghurt from diluted milk) (2% fat, 6% solids non-fat)	Heat treated ayran milk was incubated with TGase (1 U/g protein) for 0, 10, and 60 at 50 °C before addition of the starter bacteria	Significant increase in viscosity with increasing incubation time was achieved. Viscosity continued to increase during storage which was not observed in control. TGase treatment prevented serum separation	Şanli et al. (2013)
Set-type yoghurt with 10% or 14% solids non-fat with varying fat contents (0%, 2.5%, 4%)	TGase (~5 U/g protein) was added to heat treated milk and pre-incubated for 0 or 90 min at 42 °C before addition of the starter bacteria. TGase was inactivated after 90 min for one batch	Comparable firmness and water holding capacity was attained in non-fat yoghurts pre-treated with TGase to that of yoghurts containing fat or higher solids non-fat. When not inactivated, TGase-treated yoghurts showed increasing gel strength during storage	Yüksel and Erdem (2010)

Stirred-type yoghurt prepared from UHT milk (2% fat)	TGase (~1 U/g protein) was added together with the starter bacteria (fermentation was carried out at 37 or 42 °C)	The increase in viscosity of the TGase-treated yoghurts during storage was threefold when fermented at 37 °C, while it was 11-fold at 42 °C. Sensory panel showed considerably increased mouth thickness for TGase-treated yoghurts during storage	Wróblewska et al. (2011)
Set-type yoghurt with varying protein content of 3.0–3.7% (2% fat)	Yoghurt milk was subjected to either thermal or high pressure treatments. TGase (2.2 U/g protein) was added simultaneously with the starter bacteria	Significantly higher gel strength and water holding capacity were attained at a low protein content by combining high pressure and TGase treatments	Tsevdou et al. (2013)
Set-type yoghurt from goat's milk (~3.5% fat). Protein was standardized to 5.3% by using skim milk powder	Yoghurt milk was TGase treated (1.8 U/g protein) for 0.5–7 h at 40 °C. Enzyme was inactivated before acidification by D-glucono-δ-lactone	Combination of enrichment of goat's milk with skim milk powder and TGase treatment resulted in sixfold higher gel firmness compared to gels from pure goat's milk and comparable firmness to gels from cow's milk	Ardelean et al. (2012)

Guyot and Kulozik (2011) recently suggested a novel and efficient use of the enzyme; a skim milk powder, which was produced from TGase pretreated milk (in which the enzyme was inactivated before spray-drying), was used for protein fortification in yoghurt production. Stirred yoghurts produced by the addition of TGase pretreated skim milk powder to milk had viscosity values similar to those achieved by the addition of twofold higher amounts of regular skim milk powder. Moreover, the viscosity of the yoghurts containing TGase pretreated skim milk powder remained constant during storage (Guyot and Kulozik, 2011).

Structural modification of milk protein gels by enzymatic cross-linking using several oxidative enzymes has also been studied. In the existence of ferulic acid, pretreatment of sodium caseinate solution with laccase resulted in increased gel firmness and a finer gel network in acid-induced gels (Ercili Cura et al., 2009). Hiller and Lorenzen (2009) reported that treatment of milk with laccase (together with chlorogenic acid) or glucose oxidase resulted in a significant increase in viscosity leading possibly to gelation, depending on the incubation conditions.

Fungal tyrosinases of different origin, that is, *T. reesei* and *A. bisporus* tyrosinases, have also been added to raw or heat-treated milk and the effects on the structure of subsequent acid-induced gels compared to that of TGase (Ercili Cura et al., 2010). Acid gels from *T. reesei* tyrosinase-treated raw milk had a higher elastic modulus and increased gel firmness compared to the control gel, as well as gels from raw milk treated with *A. bisporus* tyrosinase or TGase. In heated milk, however, only TGase treatment increased the gel strength as measured by penetration tests, whereas *T. reesei* tyrosinase also clearly affected the loss modulus and resulted in a finer gel microstructure, which was comparable to that of TGase-treated milk gel. These results emphasized the role of colloidal structure of milk in determining the effects of enzymatic cross-linking on gel formation and possible alterations in other physical interactions due to dissimilar mode of action of different enzymes (Ercili Cura et al., 2010).

In another report, mushroom tyrosinase was shown to increase the viscosity of heat-induced gels prepared from a composite slurry of whey protein isolate (WPI) and calcium caseinate, which was microparticulated together with alginic acid (Onwulata and Tomasula, 2010). Chang and Zhao (2012) recently showed a novel use of horseradish peroxidase together with glucose oxidase and glucose for modifying the functional properties of sodium caseinate. As mentioned earlier, cross-linking of proteins by horseradish peroxidase necessitates the presence of peroxidase. Chang and Zhao (2012) used glucose oxidase and glucose for *in situ* production of necessary peroxidase for protein modification and reported formation of covalently linked casein polymers and a substantial increase in solution viscosity.

4.7 Structural modification of cheese by enzymatic cross-linking

Research regarding the use of TGase in cheese production has progressed only relatively recently, mainly after the discovery of the presence of a heat-labile TGase inhibitor in milk (De Jong et al., 2003) and evidence of the enhancement of TGase activity

in raw milk in the presence of glutathione (Bönisch et al., 2007b, 2008; Miwa et al., 2002). Heating milk to high temperatures is normally avoided during cheese making as this impairs the renneting properties greatly (Singh and Waugana, 2001). Hence, TGase treatment of cheese milk was not practical. Currently a TGase preparation (Activa®YG, Ajinomoto Co., Inc.), which contains yeast extract as a source of glutathione, is commercially available for use in raw or pasteurized milk.

Use of enzymatic cross-linking technology in cheese making has been less straightforward compared to yoghurt-type products, also due to impairment of rennet coagulation properties of casein micelles after TGase treatment (Bönisch et al., 2008; Huppertz and de Kruif, 2007; O'Sullivan et al., 2002; Özer et al., 2012). TGase-induced impairment of rennet coagulation was shown to be due to the effect of cross-linking on the enzymatic hydrolysis of κ-casein and, to an even greater extent, on the rate of aggregation of *para*-κ-casein micelles, retarding the development of curd firmness (reviewed in Huppertz, 2009). Several authors have suggested the use of TGase and rennet simultaneously, at an enzyme dosage not higher than 3 U (per g of milk protein) to reduce the impairment of coagulation properties and to maximize the curd yield (Bönisch et al., 2008; Özer et al., 2012). Yüksel et al. (2011) recently showed that, when TGase was added 5 min after the addition of rennet, a coagulum with increased gel firmness and water-holding capacity was attained. The initial milk pH, renneting temperature, order of TGase and rennet addition and the TGase dosage are important parameters to be optimized for advantageous use of enzymatic cross-linking in soft and semihard cheeses (Yüksel et al., 2011; Özer et al., 2012, 2013).

When used simultaneously with the coagulating enzyme, at an optimized dosage, TGase leads to increased curd yield, due to enhanced retention of milk serum in the coagulated protein network and also partly due to increased incorporation of whey proteins to the curd (Bönisch et al., 2008; Cozzolino et al., 2003; Di Pierro et al., 2010; Özer et al., 2013). Increased curd yield has high economic value in cheese production. However, when attained in traditional ways, for example, by altering the cutting time, elevated moisture content leads to softening of the cheese texture, which is undesirable for semihard and hard cheeses (Aaltonen et al., 2014). Enzymatic cross-linking during formation of the coagulum may limit the softening, as the protein network will be stabilized by additional covalent bonds. On the other hand, when left active during ripening, continued cross-linking may result in gradual hardening of the cheese, which may be a negative quality attribute.

Recently, Aaltonen et al. (2014) proposed a novel use of TGase in Edam cheese production. Accordingly, the ultrafiltration (UF) retentate of milk was TGase-treated and standardized with raw milk before renneting. Despite the increase in moisture content, softening did not occur in cheese curd. The authors standardized the TGase-treated UF retentate with raw milk to make use of the indigenous TGase inhibitor in milk as a means of controlling residual cross-linking activity. Cheese produced from TGase-treated milk did not harden during 24 weeks of ageing and showed only a limited decrease in hardness when compared with the control cheese (Aaltonen et al., 2014). Softening of cheese texture during ripening is related to ongoing proteolysis, and TGase treatment has been previously suggested

to interfere with proteolytic breakdown of the casein network in cheese (di Pierro et al., 2010; Özer et al., 2013).

There are no published reports on use of cross-linking enzymes other than TGase in cheese production so far.

4.8 Application of other enzymatic strategies

4.8.1 Enzymatic deamidation of milk proteins

A recent development in enzymatic modification of milk proteins is enzymatic deamidation, involving the hydrolysis of amide side groups of protein-bound Gln and Asn residues, leading to the conversion to Glu and Asp residues, respectively, and the concomitant release of ammonia. Although chemical deamidation of proteins, peptides and free amino acids, as well as enzymatic deamidation of small peptides and free amino acids, have been widely studied, enzymatic deamidation of proteins has been limited due to the lack of availability of suitable enzymes. However, a protein-glutaminase (PG) capable of deamidating protein-bound Gln residues was purified from *Chryseobacterium proteolyticum* and can be used for enzymatic deamidation of proteins. PG has a molecular mass of ~20 kDa and a pI of 10.0 and shown optimum activity in the pH range 5–7 and at a temperature of ~50 °C (Yamaguchi et al., 2001).

Treatment of skim milk with PG leads to a reduction in turbidity and an increase in viscosity, with electron microscopy indicating the dissociation of casein micelles into considerably smaller particles, as also measured by particle size analysis (Miwa et al., 2010). Levels of nonsedimentable casein and calcium increase as a result of the PG-induced disruption of casein micelles, but levels of nonpermeable (5 kDa) calcium remained constant, indicating that calcium phosphate nanoclusters and calcium caseinate interactions are not disrupted by treatment with PG (Miwa et al., 2010). This leads to the conclusion that PG-induced dissociation of casein micelles probably arises from the disruption of casein–casein interactions, as a result of the increased net-negative charge due to the conversion of Gln to Glu residues. Set yoghurt produced from PG-treated milk had lower firmness and higher adhesiveness, but also considerably less syneresis (Miwa et al., 2014). Microstructural evaluation of yoghurt from PG-treated milk showed a considerably more homogeneous microstructure compared to that from control milk, with the absence of large pores typically observed in the set yoghurt from untreated milk (Miwa et al., 2014).

In addition to milk, the treatment of WPI with PG has also been studied, and in this case, considerable effects of deamidation were also observed. Analysis by circular dichroism and fluorescence spectroscopy highlighted changes in protein conformation arising from PG treatment of WPI solutions (Miwa et al., 2013). After heat treatment at 85 °C for 30 min, large protein aggregates were observed in control 1.5% WPI solutions, whereas aggregation was limited in heated PG-treated WPI solutions, possibly due to increased intermolecular repulsion as a result of increased net-negative charge. Heat-set WPI gels (12%) showed considerably less syneresis when the WPI was pretreated with PG, whereas gels with considerably lower strength were also produced in the presence of up to 200 mM NaCl; at higher NaCl concentrations, heat-set gels from

PG-treated WPI had higher firmness than those from control WPI (Miwa et al., 2013). The observed influence of NaCl on the firmness of WPI gels from control and PG-treated WPI again highlight the importance of increased intermolecular repulsion in protein aggregation.

4.8.2 Enzymatic deglycosylation of milk proteins

Of the caseins, κ-casein is the only to be posttranslationally glycosylated through O-linked glycosylation of threonine residues. A wide variation in the degree of glycosylation of κ-casein molecules is observed, ranging from zero to six carbohydrate moieties per protein molecule. In addition, the size of the carbohydrate moiety differs strongly, from one to four monosaccharide units (for review, see Dziuba and Minkiewicz, 1996). Due to the fact that *N*-acetylneuraminic acid (NANA) can be the terminal unit of the saccharide units, glycosylation can contribute to the charge of κ-casein and thus of the micelle surface. In this respect, it is important to realize that the pK_a of NANA is ~2.6, and it thus retains its negative charge at the isoelectric point of casein, for example, as encountered during the manufacture of yoghurt of acid casein.

Due to the fact that glycosylation only occurs in the hydrophilic C-terminus of κ-casein, treatment of milk with soluble or immobilized neuraminidase is efficient in removing virtually all NANA from milk (Mehaia and Cheryan, 1983; Mehaia, 1987; Cases et al., 2003). Treatment of milk with neuraminidase had only a small, but significant, effect on its heat stability (Robitaille and Ayers, 1995). Such treatment also did not affect levels of sedimentable protein in milk or the zeta-potential or solvation of casein micelles, but did have strong effects on acid coagulation properties of milk (Cases et al., 2003). For milk treated with neuraminidase, acid-induced gelation commenced at a higher pH and yielded gels of considerably (approximately fourfold) higher stiffness at pH 4.2 (Cases et al., 2003). It is possible that these differences are related to absence of the negatively charged NANA moieties in neuraminidase-treated milk.

4.8.3 Enzymatic dephosphorylation of milk proteins

Caseins derive some of their unique features from the fact that they are subjected to posttranslational phosphorylation; as a result, they are able to stabilize the calcium phosphate nanoclusters present in the casein micelles and thus facilitate the transport high levels of bioavailable calcium phosphate to the neonate. A number of studies have evaluated the effect of enzymatic dephosphorylation on the functional properties of caseins. In most of these studies, suspensions of sodium caseinate or acid casein in buffers were subjected to dephosphorylation. Under suitable conditions, virtually complete dephosphorylation of caseins is achieved (Van Hekken and Strange, 1993, 1994). Enzymatic dephosphorylation leads to a reduction in net-negative charge on the caseins, as the negatively charged SerP-residues are converted to uncharged Ser residues. As a result, solubility of dephosphorylated casein in the pH range of 5–6 was lower than that of control casein (Van Hekken and Strange, 1993). In

addition, dephosphorylated whole casein (Van Hekken and Strange, 1993) or β-casein (Darewicz et al., 1999; Darewicz and Dziuba, 2001) was found to be considerably less sensitive to calcium-induced insolubilization.

Chymosin-induced coagulation of dephosphorylated casein in the presence of calcium was observed to proceed slower and lead to the formation of softer gels (Yamauchi and Yoneda, 1978; Van Hekken and Strange, 1994). Similar effects were observed in artificial casein micelle systems, where increases in RCT, but also reductions in syneresis, were noted (Pearse et al., 1986).

Foaming properties of dephosphorylated casein were found to be lower than those of unmodified casein, with both lower foam volume and lower foam stability observed (Van Hekken and Strange, 1993). In addition, less stable emulsions were also formed from dephosphorylated casein than from unmodified casein (Van Hekken and Strange, 1993). However, emulsions formed from isolated α_s-casein or β-casein were shown to be considerably more stable to creaming than their control counterparts (Lorenzen and Reimerdes, 1992). More recently, McCarthy et al. (2013) showed that partially dephosphorylated β-casein resulted in less efficient emulsification than the untreated protein, but the globules coated in the modified protein were more stable against calcium-induced aggregation, whereas the dephosphorylated protein failed to form a gel, unlike the control protein.

4.9 A perspective on potential future trends

Milk proteins are the class of food proteins with highest functionality. However, market trends towards palatable products with a reduced-fat content, reduced calorie density or increased protein content drive demands on functionality to levels not achievable with native milk proteins. In these cases, enzymatic modification of the proteins using a wide range of enzymes has provided ample opportunity for attaining novel and improved functionalities through, for example, controlled hydrolysis, cross-linking or the modification of specific amino acids. Although proteolysis is long used and widely applied, the use of cross-linking enzymes in the dairy industry is still in its infancy. Furthermore, specific modification of amino acids through deglycosylation, dephosphorylation or deamidation has thus far only received limited attention in dairy research. However, the potential of such approaches is clear and will most certainly propel further bodies of research and potential industrial application. Finally, the emergence of new enzymes available to the dairy researcher in future years will undoubtedly result in new and exciting opportunities for modifying milk proteins to create new textures and stabilities.

4.10 Sources of further information and advice

As this chapter has shown, the principal source of information on the activity of these enzymes and their potential application in dairy systems is the scientific literature; an extensive list of relevant publications (cited where appropriate in the preceding text) is

provided following. The principal other source of information regarding those enzymes that have reached commercial application is the companies supplying such enzymes; the principal supplier in this regard is Ajinomoto (http://www.ajinomoto.com/en/), who supply commercial preparations of TGase.

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Oils and fats in texture modification

5

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5.1 Introduction

For many years, humans have scrutinized the health impact and implications of food sources; however, today, nutrients are not only evaluated for their positive role but also meticulously and closely scrutinized for their negative or even potential hazardous effects.

The role of all food ingredients in human health is inescapable. Fats, carbohydrates, and proteins directly influence the growth and maintenance of the human body. They are not only the main ingredients affecting human health but also the major factors involved in sensory and physicochemical characteristics of food products.

Fats, as significant components of nutrients, have long been mainly noted for being a major source of energy and the key factor in causing the feeling of satiety (O'Brien, 2008). Little by little, their other roles as the main source of essential fatty acids and the carriers of fat-soluble vitamins were also recognized. Like any other newly discovered or manufactured product, they have set in motion a wide range of discussions, considerations, and researches. However, the increasing rate of obesity and its consequent increase in cardiovascular diseases in society has driven scientists to determine and probe into the significant factors involved and to focus on the genetic and environmental causes of this modern plague (Sandrou and Arvanitoyannis, 2000). The results call for significant changes in diets, decreasing the consumption of dietary saturated fats and hence improving the consumption of unsaturated vegetable fats.

5.2 The role of fat in food systems

5.2.1 Nutritional values

The nutritional value of fats is an undeniable reality that, despite all the hypotheses and arguments concerning healthy foods, still has been reviewed and seriously considered by many researchers. Fats are the most calorie-dense compounds in food, weighing in at more than twice the calories per gram of either proteins or carbohydrates.

Fats are *partially* responsible for the feeling of satiety in individuals, and the studies conducted have proven their role in this feeling. Furthermore, fats set the necessary conditions for the transfer of the fat-soluble vitamins K, E, D, and A and facilitate their

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proper absorption in various parts of the body. Fats are involved in the composition of cell membranes; without a proper membrane, cellular functions are impaired. As for the brain, fats not only exist in the structure of brain cells, but they can also be found in the composition of the sheathe surrounding and isolating myelin, which helps accelerate the propagation of impulses. Some of the other functions of fats are to help with hormone synthesis and to help maintain supple and healthy skin.

5.2.2 Sensorial functions

We could have easily forgotten all about ice cream if the industry had never thought about the application of fat replacers. The unique mouthfeel you receive through ice cream consumption is the result of a gradual melting down of butter fat. During the freezing phase, fat globules cover the ice crystals and prevent the subsequent sandy sensation in ice cream. Moreover, the greater the fat content, the better holding capacity for air when whipped. This condition is not confined to ice cream.

Sensorial features of a product are the most significant factors affecting the perception of products by customers. The presence and level of fat in a product affect the appearance features, taste and aroma, mouthfeel, and most important, the food texture. The shiny appearance of many products, suggesting the presence of fat and hence increasing the enjoyment of consumers or the fat-induced mouthfeel, which is also known as creaminess, makes the presence of fat in products imperative. Fats impart a wide range of characteristics to food, including desirable appearance, flavor, aroma, texture, and mouthfeel.

The feeling of fat in products depends on the two factors of taste and texture. Some of the features deeply affected by decreasing the amount of fat in products are the intensity, duration, and finally, balance among different senses of taste and aroma (Bayarri et al., 2006). The existence of fat causes a gradual release of taste and aroma while chewing food, whereas a decrease in its level results in a sudden discharge of such compounds. That is why in low-fat products the tastes of salty, sour, bitter, acidic, and so on are more pronounced. Furthermore, lowering the fat content negatively affects the proper condition for displaying the effects of taste- and aroma-inducing compounds, which are mostly fat soluble. Hence, the aromatic and taste profile would be different from that of fat-rich products.

As for the product texture, the feelings suggested by the existing fat when the first morsel enters the mouth or first bite is chewed and eventually swallowed are the elements affecting the decision of a consumer to continue the consumption of that particular product. Lubrication capacity of fats is the constituent influencing the proper mouthfeel and creaminess of food products. For this very reason, food products such as crackers, which have a dry texture in nature, are best served with spreads. The palatable mouthfeel of ice creams and mayonnaise sauces is a consequence of this compound.

Fats are effective in the firmness of food products. In emulsion-based products, through affecting the composition and as a result the viscosity of food products, the presence of fat influences their whole texture.

The type of fat used determines the melting point of the product in the mouth; a wrong choice of fat might result in a rejection of the product by the consumers. More sensitively, this issue is evident with products such as chocolate, cracker spreads, and ice creams. Melting point is the temperature at which a substance is turned from solid into liquid. Should the selected chocolate not melt and change shape at a favorable speed in the mouth, it cannot convey the required mouthfeel to the consumer.

5.2.3 Shelf life extension

Shelf life of a food product is defined according to its nature and expectations of the product during storage. In mayonnaise, the physical separation of oil from the product is considered a sell-by date indication. It is expected that peanut butter should not go through any changes in texture and consistency during storage. Oil separation, the occurrence of a blooming process, and appearance of a thick and dry layer on the surface result in product rejection by consumers. Creation of a sour taste in yogurt before its shelf life expiration and lack of a pleasant mouthfeel in low-fat sausages are instances of the effects of an insufficient amount fat in food products.

A decrease in the amount of fat in products may also bring about food spoilage in multiple ways. Lack of fat in a product accentuates the amount of a product's water, increases the a_w, and hence sets the condition for the spoilage of food products. That is why food spoilage due to mold is one of the dangers threatening low-fat products (Roller and Jones, 1996).

In addition, other events that might happen to low-fat food products are the occurrence of chemical spoilage due to the effective role of water in food environment and the accelerated rancidity of fats. Physical spoilage, which is preceded by chemical spoilage, is a phenomenon during which the physical properties of foods such as texture and appearance go through changes. For instance, decreasing the fat content in a product adversely affects its texture. Food texture analysis and sensorial measurements of a product are among the common methods for evaluating texture. An insufficient amount of fat leads to hardening of the texture, instability in the emulsion structure of products, lack of uniform mouthfeel while chewing and finally, a decrease in the product cohesiveness.

5.3 Fat replacement necessity

Given the discussion so far, we are faced with a metaphorical scale where on one hand we have all the nutritional values and functional significance of fats, and on the other we observe the medical documents pointing out the detrimental effects of fats on the human body; a part of the growing trend of obesity, cardiovascular conditions, and diabetes afflicting societies has to do with unhealthy diets.

Striking a balance between the two sides of the scale has driven the scientists and the practitioners of the food industry to develop with compounds that reduce fats and improve public health, but are also capable of reproducing the positive features of

fat-rich products. In other words, the enhancement of low-fat products with the minimum changes in their chemical, physical, and sensorial properties in addition to the least impact on the products' shelf life are among the major concerns and goals in using fat substitutes.

Fat substitutes refer to all the bulking agents and compounds that in one way or another simulate the presence and behavior of fat in food products (Roller and Jones, 1996). An ideal and suitable fat substitute reduces the fat and overall calories of a product and at the same time retains all the properties of the original fat-rich product.

5.4 Application of fat replacers

With confirmation of the application of fat replacers as an indispensable part of food products, it seems imperative to determine the suitable type of substitute that is capable of reproducing the missing features of the original product. Varying approaches have been proposed for categorizing fats. What follows is a sample of such categories (Roller and Jones, 1996).

Fat replacer: any compound used for replacing fats
Fat substitute: a synthetic compound designed for weight-by-weight replacement of fats with a normally similar structure to that of fats with the exception they are resistant to hydrolysis by digestive enzymes
Fat mimetic: a replacer that needs a great amount water for its function
Low-calorie fat: a synthetic triglyceride that has bonded an unusual fatty acid with the glycerol backbone and hence decreased the amount of calories produced
Fat extender: a system of fat replacers in which, besides the standard fats, there are other accompanying compounds

Another categorization, based on the structure of fat replacers, puts them in the categories of carbohydrate fat replacers, fat-based fat replacers, and protein-based fat replacers.

Carbohydrate fat replacers: they are the largest of the three groups. Most of the members of this group, more than being called fat replacers, mainly serve the functions of improving the textural features of a product such as firmness, mouthfeel, and retention of the accessible moisture of products (Lucca and Tepper, 1994).

All members of this group are plant-based polysaccharides of such varying types as modified starches, fibers, cellulose, and resins. These compounds are well capable of enhancing and increasing the viscosity, texture, and bulk so that a very similar mouthfeel like that of a fat-rich product is achieved. In most cases, these compounds may not be used on a gram-by-gram basis instead of fats.

Protein-based fat replacers are compounds that are created specifically to replace fats and include compounds with fat-like structures and similar mouthfeel to that of fats (Napier, 1997). Like the previous category, they cannot be used on a gram-by-gram basis to replace fats. The compounds in this category include microparticulate protein (marketed under the brand names Simpless and Trailblazer) and modified whey protein (marketed under Dairy-Lo).

Fat-based fat replacers are substances that look and feel like fat and have physical and thermal properties similar to fat. They can be distinguished in emulsifiers and lipid analogs. Mono- and diglycerides (marketed as Dur-Em, Dur-Lo, etc.) help the dispersion of fat in watery mediums, thereby allowing less fat to be used in a product (Napier, 1997). Lipid analogs include two types of products; the first is usually referred to as low-calorie fats and the second as fat substitutes.

Low-calorie fats are triglycerides with a fatty acid composition different from that found in fats so as to contribute fewer calories. Fat substitutes are esters of fatty acids with a backbone other than glycerol (e.g., sucrose), so enzymes in the human gut cannot cleave the fatty acids. Therefore, these fat replacers do not contribute any energy.

These categories provide the closest mouthfeel to that of fats and therefore have the most acceptable taste to consumers. One of the most well-known ingredients in this group is Salatrim.

The best perception of fattiness is, however, related to a combination of several poorly defined parameters such as adhesiveness, viscosity, and cohesiveness (Khalil, 1998). It is, therefore, a necessary approach to use a combination of fat replacers to recover all the characteristics produced by the fat in foods.

5.4.1 Modification of sensorial characteristics

The first sensory response to fats involves olfactory perception—through the nose or mouth—of fat-soluble volatile flavor molecules. These compounds impart the flavor and aroma characteristics to many foods (Drewnowski, 1992).

Decreasing fat content of a product results in the limitation of necessary context for the aroma-inducing molecules, and therefore it can be observed that one of the problems in low-fat products is lack of sufficient aroma and uniform release of aroma over time; there is a uniform profile in the release of aroma.

Flavor compounds emulsified with lipid-like materials that can replace the missing pleasant mouthfeel sensations are being increasingly used in low-fat food formulations. In addition, polysaccharides have been shown to slow down the flavor release as much as fats do. Incorporation of polysaccharides into a low-fat system might give flavor-release properties similar to that of a full-fat system.

Modification of rheological properties of low-fat products through adding fat replacer compounds may render a controlled release of aroma- and flavor-inducing compounds. In products with lower viscosity and strain sensitive textures, the release of the aromatic substances is even more accelerated. Introducing fat-replacing compounds to a product, in addition to improving the texture, may also contribute to improving the aromatic features of a given product; because aromatic substances are effective in giving a specific feeling of texture, this sensorial cycle finds a synergistic effect.

Subsequent oral perception of fat is determined by food texture as sensed by oral cavity during chewing and swallowing. More viscous stimuli are generally perceived as more desirable and rich in fat content. Accordingly, the illusion of fat mouthfeel can be created through the use of thickeners that enhance the perceived smoothness or creaminess of the food product (Frøst and Janhøj, 2007). However, it should be

mentioned that the studies carried out by some researchers revealed that the feeling of creaminess of the product is not solely achieved by establishing proper viscosity reproduced by the fat replacers. They concluded that shear thinning induced by the hydrocolloids present in the product also play a pivotal role in the sense of creaminess (Akhtar et al., 2006). Generally, two major factors are responsible for a favorable mouthfeel and consequently in selection of the right fat replacer that has a high-viscosity-creating capability and low pseudoplasticity of the product. These two features, together with the sensory features such as flavor and aroma, can guide in choosing the right fat replacer.

The lubrication capacity of fats has also made them an outstanding ingredient for food applications. The tribological property of foods is discussed in Section 5.5.2.2.

5.4.2 Rheological characteristics recovery

Texture is the major indicator of whether fat is present in a food or not (Rolls, 2012), and it could be also considered the first victim in fat reduction. Any small changes in the formulation of food products will certainly affect the rheological characteristics of the original version. Reduction of fat would leave dramatic influences on the rheology of full-fat products. Manipulating the rheological characteristics of food should take place in such a way that other major features such as processing, stability, and sensory are not harshly influenced. That is the multifunctional characteristic of fats.

Modification of the rheological properties in emulsion-based low-fat foods is mainly dependent on the type of emulsion system. For the oil-in-water emulsion systems, as the most common system in foods, textural properties are essentially relevant to the improvement of continuous phase. Application of the thickening agents, especially the hydrocolloids with the ability to control the viscosity of the continuous phase and subsequently the whole system, is the usual operation carried out in these cases.

The process would be a little more difficult for the water-in-oil emulsions. Manipulation of the water droplet structure, changing the oil phase characteristics, manipulating the oil–water interface, and finally, modification of the process according to the characteristics of the new formula are some suggested solutions that would affect and improve the rheology of the whole system (Roller and Jones, 1996).

Although most of the time, all the attention is drawn to the fact that the addition of fat replacer should be able to recover all the missed rheological characteristics of the product, it should take into account that the sensorial characteristics of food are the major parameters that influence the consumers' total acceptance. The balance between the two characteristics—rheology and sensory—is of great importance.

In a study on the textural attributes of low-fat yogurt, results revealed that the application of whey protein concentrate (WPC) and the combination of WPC-microparticulated whey protein as fat replacers could imitate the textural properties of the full-fat version (Sandoval-Castilla et al., 2004).

Liu et al. (2007) reported that application of pectin weak-gel as fat mimetic in low-fat mayonnaise shows similar textural characteristics as the full-fat sample (Liu et al., 2007). The textural attribute driven from the sensory evaluation was well correlated

with the results obtained by means of texture analyzer. However, the correlation of the sensory analysis and the rheological data driven from the rheometry measurements appeared random. They suggested that the application of the rheometer geometry could not be a good criterion of the oral processing.

5.5 Evaluation food texture fattiness

5.5.1 Sensory methods

Szczesniak (2002) proposed that texture is a sensory characteristic because only a human can perceive and describe it. Application of the instrumental methods can measure certain physical parameters, which then is required to be interpreted in terms of sensory perception (Szczesniak, 2002).

In its conventional way, certain attributes are evaluated by a group of panelists using scales that are related to standard samples. Quality descriptive analysis and free choice profiling are two methods of sensory evaluation that are commonly used in experiments. However, none of them has considered the dynamic nature of oral processing and sensory texture perception (Wilkinson et al., 2000). Novel techniques in sensory evaluation such as progressive profiling and time intensity have appropriated the oral processing duration and events. Despite the powerful idea behind these novel methods, the time-consuming feature involved in the process is a limitation in their application (Wilkinson et al., 2000). Temporal dominance of sensations (TDS) brings information about differences between products, attributes, and evolution through time. The nature of the information is similar to that obtained via the time-intensity (TI) method. In addition, the TDS approach is capable of evaluating several attributes simultaneously and revealing the sequences of the dominant sensations. However, memorizing all the attributes simultaneously and also quoting descriptors in the same order for all samples are some drawbacks mentioned for this method (Ng et al., 2012).

Despite the essential role of fat in food texture and the overall sensory perception, consumer acceptance is often not directly related to fat level (Tobin et al., 2013). So it is shown that the sensory evaluation could not be the most appropriate method in fattiness measurement. Moreover, the instinct uncertainties of sensorial evaluations, that is, its dependence on the environmental and individual conditions, is of great importance. It should, however, be mentioned that sensory evaluation is still the integral component in all product development processes.

Development of novel techniques in sensory evaluation has made it a suitable method in the assessment of fattiness. Wendin and Hall (2001) evaluated the influence of fat, thickener, and emulsifier on different formulas of salad dressing. They used dynamic (time-intensity) and descriptive sensory measurements to characterize the sensory properties of products. There was a good relation between the results obtained through the dynamic and descriptive sensory in the fattiness attribute, and also strong relations were observed between the sensory and the instrumental rheological properties (Wendin and Hall, 2001). Deegan et al. (2014) could prove the influence of the homogenization process on the perceived fattiness and smoothness of

reduced-fat cheeses by confocal laser scanning microscopy images. They showed that smaller and more distributed fat globules result in higher interactive interface with mouth, and therefore, higher fattiness is sensed through descriptive profiling sensory evaluation (Deegan et al., 2014).

In an interesting study, Johansen et al. (2008) evaluated the capability of image analysis techniques to predict the sensory properties of low-fat yogurt and cream cheese. For both dairy products there was a good relation between the image features and textural properties. However, the prediction of perceived creaminess was more accurate in cream cheese than in yogurt (Johansen et al., 2008). This example shows the capability of nondestructive methods in the food creaminess measurement.

5.5.2 Instrumental methods

5.5.2.1 Rheological characteristics

Perception of fattiness, or as most recently named as the sixth sensation, could be through flavor or texture properties perceived during oral processing. From the moment food is placed in the mouth until it is swallowed, oral processing has been considered as the following six stages: (1) first bite, (2) comminution, (3) granulation, (4) bolus formation, (5) swallowing, and (6) residue (Stokes et al., 2013).

Depending on the nature of textural mouthfeel attributes, some of them are perceived on the first bite of food (hardness, brittleness, and viscosity), some during chewing (creaminess, fattiness, and smoothness), and the others through residue and aftereffects of food consumption (astringency and afterfeel).

But does the rheology have the capability to measure all the textural attributes? Developing relationships between the sensorial characteristics of food and the rheological measurements is one of the fields of interest that many researchers have focused on. For instance, more often than not, the viscosity value at 50 s^{-1} shear rate is considered as the thickness perceived by the consumer. However, this common hypothesis has failed due to the dynamic nature of oral processing. Changes that occur in the length scale of food during oral processing have resulted in dividing the measuring techniques into two distinct categories; sensorial attributes perceived at the time of placing food into the mouth are evaluated using the rheological methods. The characteristics perceived at the later stages of oral processing are measured by means of rheological and/or tribological methods (depending on the stage and the nature of involved stresses scales; Figure 5.1).

Tactile mechanosensation plays a pivotal role in the perception of texture; however, it is hard to measure this property using the rheological instruments. In addition, textural perception is a dynamic process at different stages of which a special sensorial attribute will be perceived. It is, therefore, difficult to draw any conclusion about an attribute based on a parameter determined in a limited range of rheological measurements (Prakash et al., 2013).

Consequently, it is required to move to a deeper level to measure the fattiness in foods. Tribological characteristics, which will be discussed in Section 5.5.2.2, are the response and the solution of scientists in this matter.

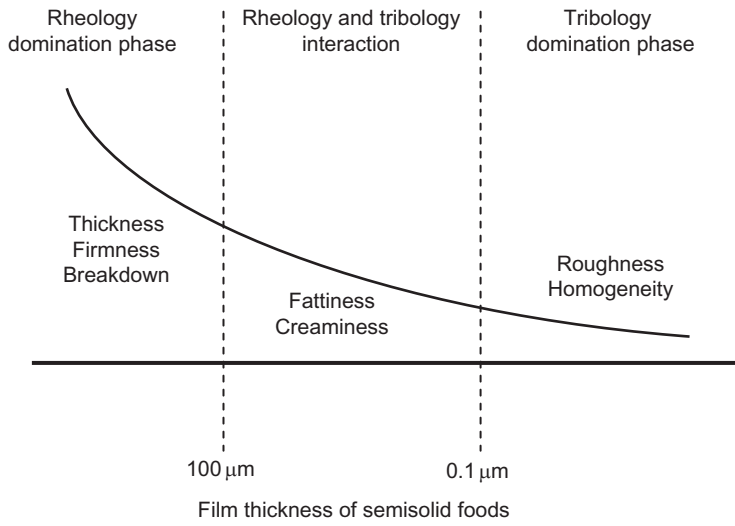

Figure 5.1 Transition in film thickness from rheology domination phase to tribology domination phase. Also shown are textural attributes that govern each phase.

5.5.2.2 Tribological characteristics

Tribology is the study of friction and lubrication between surfaces that are in contact with each other in a relative motion. It is a major part in the evaluation of food texture because the texture is influenced by not only the structure and rheology but the surface properties as well (Stokes et al., 2013). The papillae placed on tongue have a special role in the perception of sensorial attributes. The interaction between these papillae and food in the presence of saliva as lubricant is the main basis of tribological approach in oral processing. That is the main reason why recent studies have found their way from the rheological points to the tribological ones (de Wijk and Prinz, 2005).

As shown in Figure 5.1, the variation in film thickness during the consumption of foods leads to a shift in the measurement techniques from the rheological to the tribological. Based on the perceived attributes, some of them are perceived by rheological methods. For example, the thickness that is a criterion of the bulk properties in the food system is measured using rheological methods. But for the attributes like fattiness, it is considered to be determined by the rheology of the bolus formed and also the surface properties measured by tribology (de Wijk et al., 2011).

The oral tribology studies are categorized into three parts: imitative approaches, empirical approaches, and fundamental studies.

The imitative studies are performed using animal tongues to mimic the frictional properties of human tongues. However, according to the variability and the unknown chemistry of these surfaces, application of the elastomer substrates has become of more interest (Stokes et al., 2013).

In the empirical techniques, the differences between treated samples are determined, and they are also compared with the results obtained through the sensory evaluation (Stokes et al., 2013). This method is commonly used in tribological studies, and good correlations have been found between the friction coefficients obtained by this method and some sensorial attributes like fattiness acquired through sensory evaluations. There are, however, some doubts cast regarding this method's predictive capability of sensory properties. The different varieties of surfaces and geometries applied in the empirical approach are some sources of uncertainties existing in this method.

The fundamental method is the application of well-defied substrates and geometries in evaluation of lubrication and film thickness. Application of ball-on-disk tribometer in a mixed rolling and sliding contact and using at least one polydimethylsiloxane (PDMS) substrate is the most common configuration in this approach (Stokes et al., 2013). The microengineering capability of the PDMS surface and its sufficiency to adsorb saliva proteins are two parameters that have made it an appropriate choice in this case.

The researchers attempt to uncover the relations between the rheological and tribological properties of foods. The dynamic nature of oral processing and, therefore, the changing length-scale of food particles through this process have made rheology more dominant over the first stages of the process and the tribology more important through the further steps.

5.6 Novel techniques in texture recovery of low-fat food systems

Many researchers have thought about novel techniques for improvements in low-fat food characteristics.

As the texture and flavor properties are dependent on each other, application of each method, which leads to the modification of each of these attributes, will influence the other one as well. For example, the addition of hydrocolloids to a low-fat food system directly results in texture modification and subsequently improvement in intensity and duration of flavor sensation.

Water-in-water emulsion systems are a result of thermodynamic incompatibility of macromolecules with interesting structural characteristics. The thermodynamic properties beyond this system have been studied by some researchers (Tolstoguzov, 2003). From a physical point of view, when two biopolymers are mixed and one or both of them are uncharged or have similar electrical charges, a relatively strong repulsion would appear between the two biopolymers. The two components form a two-phase solution, as their concentration exceeds a certain level. One phase is rich in one type of two polymers and depleted in the other one, and the opposite behavior is seen for the second phase (McClements, 2006).

The phase diagram resulted from these blends is dramatically affected by different parameters such as pH, ion strength, and temperature.

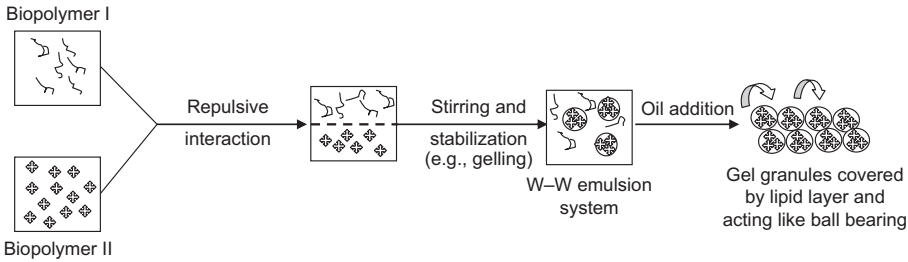

Figure 5.2 Capability of W–W emulsion systems to act as a ball bearing in low-fat food systems.

Applying different preparation conditions will lead to a variety of microstructures, which would be set at a kinetically stable state by changing the solution/environmental conditions (Tolstoguzov, 2006). By attempting to texturize these biopolymer blends, many scientists hope to develop novel sorts of low-fat foods with high similarity to the original high-fat versions. The nonpolar food components present in the W/W system (e.g., lipids) may be dispersed in one of the phases and/or adsorbed at the interfacial layer. The lipid adsorbed within the interfacial layer will make a thin layer, which in sufficiently concentrated biopolymer blends would lead to a honeycomb-structure formation. Small granules filled with aqueous phases and covered by lipid thin layer can play the role of a ball bearing (Tolstoguzov, 2006) and therefore, intensify the degree of perceived creaminess in the food product (Figure 5.2).

Many food products benefit from structured emulsion-filled gel systems. All the changes that occurred in the based emulsion will directly affect the rheological and sensorial characteristics of the emulsion gel system (Sala et al., 2007). Lowering the fat content in the emulsion system will reduce the palatability of food. The presence of hydrocolloids in the emulsion-based system, not only as a stabilizer but also as a fat replacer, can effectively influence the food texture. The interactions between the hydrocolloids and the gelling agent will determine the final gel characteristics.

Workers at Research Institute of Food Science and Technology (RIFST), Iran, and Phillips Hydrocolloid Research Center in China are studying the potential of emulsion-filled gel systems stabilized by hydrocolloids as fat replacers. The electrical charge on the hydrocolloids and gelling agent will determine the attractive or repulsive interactions in the gel system, which eventually will lead to a stronger or weaker food texture.

As mentioned earlier, one of the most important properties damaged by fat reduction in foods is the intensity and duration of flavor perception. Any approach in the entrapment of flavorants used to slow down their release will be an important step toward full-fat product imitation. Electrospinning is a newly introduced method in food technology. Its application in this field is under study and improvement.

Electrospinning is the process of using electrostatic forces to distort a pendant droplet of polymer solution into fine fibers followed by deposition onto a grounded collector (Ghorani et al., 2013). Figure 5.3 shows a schematic representation of this process.

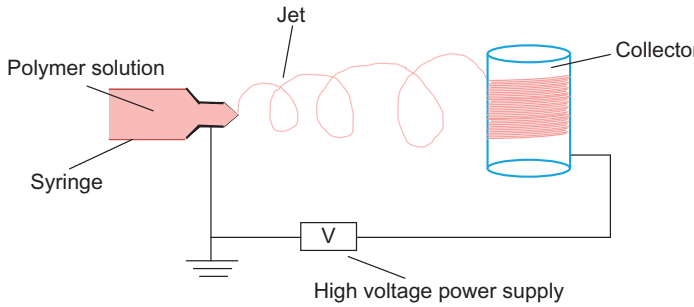

Figure 5.3 Schematic illustration of electrospinning setup.

The polymer in liquid phase is extruded from the needle tip at a constant rate by a syringe pump, forming a droplet at the tip. When a small volume of polymer liquid is exposed to an electric field, the droplet is first stretched and then deformed into a structure known as a Taylor cone (Reneker et al., 2000; Yarin et al., 2001). When the electric field reaches a critical value at which the electrical forces overcome the surface tension of the droplet from the tip of the Taylor cone, a charged fluid stream or jet of the polymer solution is ejected (Doshi and Reneker, 1995).

The jet is then elongated partly by means of a whipping action during its transit from the tip to the collector. The jet is also subject to drag forces, which can also be expected to contribute to its attenuation. Electrostatic force, drag force, gravity, columbic repulsion force, surface tension, and viscoelastic force act on the charged jet (Ding et al., 2006). Thinning of the polymer jet continues until the solvent is evaporated and continuous fibers are deposited on the grounded collector (Shin et al., 2001).

Entrapment of fatty odor in an appropriate system with gradual release capability is the idea behind the understudy project of electrospinning at RIFST. By application of this method, one of the major problems concerning low-fat foods (i.e., a nonuniform profile of flavor release) is expected to be solved.

5.7 Conclusion

One of the most important challenges in the low-fat food industry is the problems encountered with the texture of systems. A wise insight of several aspects in selection and application of fat replacers is required to have the best recovery in low-/reduced- or even zero-fat foods. Varying the formulation and/or the process involves effects on the rheological behavior, physicochemical properties, and subsequently shelf life of low-fat foods. Furthermore, profound awareness should be taken about the sensorial characteristics of the final product. The latter demonstrates the complexity and the delicacy of the subject.

Application of strategies such as W/W emulsion systems and emulsion-filled gels and also novel techniques like electrospinning are promising new gates of research and development to the low-fat food production area.

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Part Three

Novel processing techniques for food texture modification

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Improved thermal processing for food texture modification

6

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6.1 Introduction

Food texture has been defined by the International Standards Organization (ISO) in their standard vocabulary for sensory analysis as 'All the rheological and structure (geometrical and surface) attributes of a food product perceptible by means of mechanical, tactile, and where appropriate, visual and auditory receptors' (ISO, 2008). Texture of food materials plays a key role in consumer acceptance and market value. Texture features are considered important from both quality assurance and food safety perspectives (Wilhelm et al., 2004). Smith (1947) listed nine specific parameters contributing to overall food quality, of which five are linked to the concept of food texture (Kramer, 1975). Texture is a key quality parameter used in the fresh and processed food industry to assess consumer acceptability. Among the texture characteristics, hardness (firmness) is one of the most important parameters, which is often used to determine the freshness of food. Springiness, cohesiveness, adhesiveness and gumminess are significant properties for the texture evaluation for meat-based products. Textural quality attributes of food may be evaluated by descriptive sensory or instrumental analyses (Chen and Opara, 2013a). Although flavour is commonly found to be an important sensory factor responsible for the preference of foods, texture is often cited by consumers as the reason for not liking certain foods (Cardello, 1996).

Thermal processing of food and food products is one of the most important unit operations in the food industry. It is of great interest due to its numerous processing and preservation applications. The desired microbial food safety can be achieved over a wide range of temperature–time combinations. However, thermal processing also results in changes in physical, chemical and various organoleptic properties of foods (Rattan and Ramaswamy, 2014), including texture. Apart from food preservation applications, thermal processing of food is employed to modify food texture of various food products. Increasing consumer demand for 'fresh-like' processed plant-based foods has resulted in research being carried out on methods to improve the texture of thermally processed products. Methods employed include low-temperature blanching (<70 °C) before sterilisation, calcium infusion before thermal processing, pH adjustment during processing and exogenous pectinmethylesterase (PME) infusion before thermal processing (Sila et al., 2008). Analysis of consumer complaint data on food products indicates that there is scope for considerable improvement in textural properties of foods that are presently produced (Wilhelm et al., 2004). This chapter

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reviews various mechanisms of softening of foods and recent developments in thermal processing to achieve desired food texture modification.

6.2 Mechanisms of texture modifications during thermal processing

Many food scientists, engineers and technologists measure mechanical properties to investigate subjective texture, whereas material scientists have developed rheological and fracture mechanics approaches to understanding the properties of food materials in general. Food texture can also be evaluated using techniques such as sensory, oral processing and non-invasive techniques (Chen and Opara, 2013b).

In general, the effect of thermal processing on food texture is influenced by temperature, shear, pressure and processing time (Lal Dar and Light, 2014). The thermal processing temperature and time combination used strongly influences texture development. Food texture modifications results from physicochemical changes in cell-wall materials, for example, gelatinization of starch, protein denaturation, solubilization of pectic material and degassing in the case of blanching of fruit and vegetables. More details of these mechanisms are provided in the following sections.

6.2.1 Solubilization

Changes in texture during processing and storage are mainly related to biochemical conversions in cell wall and middle lamella components. These accumulative changes result in tissue softening and flavour development in cooked food products. One such component is pectin. The plant cell wall is a network of cellulose fibres embedded in a matrix of hemicelluloses and pectins that encase the cells and strengthen the plant body (Figure 6.1; Torres et al., 2009). Pectin found in the plant middle lamella plays a crucial role in cell–cell adhesion, and, moreover, is brought into solution more easily and is more chemically reactive than the other cell-wall polymers. One of the main structural components of pectin is homogalacturonan, a linear chain of α-(1,4)-linked galacturonic acid residues that can be methoxylated. Pectin solubilization can occur naturally due to enzymes (e.g., PME and polygalacturonases [PG]) or application of heat. Enzymatic pectin degradation by PME and PG proceeds in two steps: (i) pectin is partly demethylated by PME resulting in the production of methanol, pectin with a lower degree of methylation and polygalacturonic acid, and (ii) the latter will be depolymerized by PG. However, at elevated temperatures, high methoxylated pectin is prone to non-enzymatic conversions via depolymerization and demethoxylation reactions. The β-eliminative depolymerization is mainly responsible for the extensive softening of low-acid fruits and vegetables during heat treatments. Pectin depolymerization is one of the main reasons for texture deterioration of fruits and vegetables during thermal processing. This depolymerization leads to pectin solubilization and, consequently, decreased cell–cell adhesion, resulting in tissue softening (Sila et al., 2006).

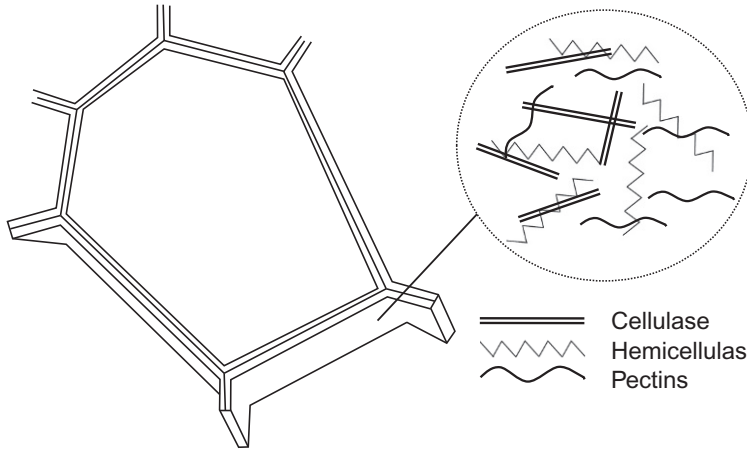

Figure 6.1 Primary cell-wall structure showing cellulose fibres in a matrix of hemicelluloses and pectins (Torres et al., 2009).

De Roeck et al. (2009) studied the effect of combined high-pressure/high-temperature (HP/HT) treatments on the chemical demethoxylation and β-eliminative depolymerization of pectin to investigate the impact of HP/HT treatments on the texture of fruits and vegetables. Apple pectin solutions at pH 6.5 were subjected to different HP/HT combinations (500, 600 and 700 MPa/90, 110 and 115 °C). At atmospheric pressure, both zero-order reaction rate constants increased with increasing temperature. At all temperatures, demethoxylation showed a higher rate constant than β-elimination. However with combined HP/HT treatment, β-elimination was retarded or even stopped, whereas demethoxylation was stimulated. β-Elimination is one of the main causes of thermal softening, and low methoxylated pectin can enhance tissue strength by forming cross-links with calcium ions present.

According to Vervoort et al. (2012) texture softening of thermally processed and HP-processed carrots can be mainly attributed to mechanical membrane damage and the associated turgor loss. Thermal processing of carrots was also preceded by a pre-heating phase at 40 °C, which enhanced PME activity. Furthermore, severe thermal pasteurisation conditions can in their turn induce β-elimination, which provides additional softening, compared to the mild or severe HP pasteurisation. Knockaert et al. (2011) reported comparable results for mild and severe pasteurisation. However, the increased cooking time of common beans is attributed to the presence of more ferulic acid bound to soluble pectin in the beans, which may cause changes in cell adherence, thereby inhibiting cell separation when the beans are cooked (Siqueira et al., 2013).

Gas represents a substantial part of the volume of fruit tissues; during heating the dissolved gas, which is enclosed in tissue cells and/or in the intercellular space, is released and supports the breakdown of a cell structure. Gas release from tissue above 90 °C can influence the texture of heated tissue (Yu et al., 2011). For example, rapid

initial softening followed by a much slower rate of softening during the retort process was observed for diced carrots (Peng et al., 2014).

6.2.2 Starch gelatinization

When heated in the presence of water, starch undergoes an irreversible process termed gelatinization. Various changes are observed, including swelling of granules, water absorption, loss of crystallinity and amylose leaching (Donald, 2004). Starch gelatinization is an endothermic process that corresponds to the loss of starch crystallinity in the starch granules under particular heat and moisture conditions. During thermal treatment, starch granules imbibe water and swell considerably, resulting in gelatinization at around 60–80 °C. This leads to an expansion of cell size and volume, cell separation and cell-wall distension, depending on the starch content. Not all granules in any one sample gelatinize at the same temperature; rather, gelatinization occurs over a temperature range of about 8–15 °C units (Briffaz et al., 2012). Starch gelatinization in excess water follows pseudo first-order kinetics in complex food systems. Conversely, gelatinization follows first-order kinetics only beyond a certain degree of gelatinization, corresponding to the initial gelatinization of amorphous starch regions (Gibert et al., 2010).

The textural variation of plant tissues is attributed to the presence of biopolymers like starch, cellulose, pectin, and so on and the cellular and molecular organisation of the plant materials. Amylose imparts definite characteristics to starch, affecting its quality. Purified amylose forms stiff gels as a result of hydrogen bonding between molecules and may also contribute to retrogradation during the cooling phase after heating (Sajeev et al., 2008). Rattan and Ramaswamy (2014) showed that with heating of potato particles, the pectin substances degrade, and gelatinization of potato starch takes place which, ultimately softens the potato tissue. Hydrothermal treatment is a processing technique that provides unique textural characteristics linked to hydration, swelling and gelatinization in starchy vegetables.

6.2.3 Protein denaturation

Protein denaturation can be defined as structural change in response to extreme conditions of temperature, pH, pressure or salt concentration, which renders the molecule incapable of performing its original biological function. Protein denaturation is one of the main mechanisms for altering the texture of foods containing protein. Kong et al. (2007a) measured shear force using a multiple thin-bladed Kramer-type texture fixture (MTB). The MTB consists of an upper part of 10 thin blades and a lower part comprising a support base with slots (Kong et al., 2007b). This probe was fitted to a Texture Analyser TA-XT2. Observations of the changes in shear force of salmon during heating at 100 °C were divided into four phases: (1) rapid toughening phase during which the shear force increased from raw muscle to the first peak, (2) rapid tenderization phase during which the shear force decreased from the first peak to a minimum, (3) slow toughening phase during which the shear force increased again to a second peak, and (4) slow tenderization phase during which the tissue gradually

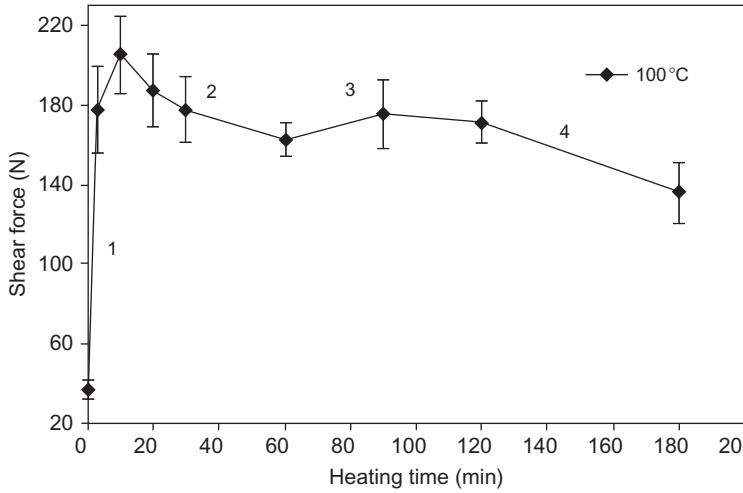

Figure 6.2 The change of shear force of salmon fillet (Kong et al., 2007a).

became soft as heating time increased (Figure 6.2). The authors suggested that the long heating time of the salmon sample negatively affected the tenderness of salmon.

Ma and Ledward (2004) studied the effects of 800 MPa of high pressure applied at different temperatures (20–70 °C) for 20 min on beef texture. The authors showed that collagen was reasonably resistant to pressure and it was denatured only at a temperature range of 60–70 °C. However, myosin was relatively easily unfolded by both pressure and temperature, and when pressure denatured, a new and modified structure was formed of low thermal stability. Although this new structure had low thermal stability at ambient pressure, it still formed in both the meat and myofibrils when pressure was applied at 60 °C.

According to Ko et al. (2007) a significant decrease in hardness was exhibited in the stripes of heat-treated winter mushrooms, which became more pronounced with an increase in either heating temperature or time. Compared to vegetables and fruits, mushrooms do not have rigid cell walls as support, but are composed of glucan and chitin. When mushroom are heated, proteins denature, and permeability increases, leading to the loss of cellular solutes and water resulting in softened cells. Khan et al. (2014) developed a ready-to-eat salted duck meat product using a HP heat process. They found that collagen began to denature at a temperature range of 70–80 °C in heat-only and cooked control samples resulting in shrinkage of myofibrillar structures which contributed to increased hardness. According to Xu and Li (2014) the plasmodesmata play a key role in maintaining cell-wall rigidity and cell integrity. Plasmodesmata consist of pores proteins, which include cytoskeletal proteins, such as actin and myosin. Most of plant cells are interconnected through plasmodesmata in cell walls. During heating, some pore proteins start to fold causing the closing of pores at 40 °C and subsequently breakdown at about 60 °C. The damage of the pore protein system

is accompanied with fundamental increases in cell-wall permeability and then the instant collapse of the cells resulting in softened texture.

6.3 Methods to modify food texture

6.3.1 Blanching/preheating and pH adjustment

Preheating foods before thermal processing is one of the most effective ways of modifying the final texture of food products. Preheating at temperatures of 50–70 °C for $\geq$30 min is recommended to improve texture for most fruits and vegetables. This time and temperature combination activates cell-wall-bound PME demetoxylates, which form ionically cross-linked pectin complexes, reducing the β-elimination reaction and hence reducing the susceptibility to softening reactions. Also, a combination of this method with calcium impregnation facilitates cation influx, which also boosts PME activity.

Blanching of carrots at 73 °C for 20–30 min as a pre-heat treatment for canning results in firmer texture than a blanching pre-treatment of 100 °C for 4–5 min (Lee et al., 1979). This can be attributed to the effects of PME, which is activated by the low-temperature blanching and inactivated by the HT blanching. Slices of broccoli, lettuce and mustard precooked for 30 min at temperatures below 60, 70 and 70 °C were found to be firmer than those directly cooked without precooking. Optimum temperatures for precooking were 50, 60 and 60 °C, respectively, which were coincidental with optimum temperatures of activity of PME extracted from the fresh tissues (Wu and Chang, 1990). Similar effect of blanching on firmness of final product was reported for green beans and tomato. This temperature activation of PME has been investigated by measuring the formation of methanol in intact tissue of green beans and tomatoes (Anthon and Barrett, 2005). Calcium infusion combined with preheating at 60 °C for 40 min has been effective in retaining texture firmness (Sila et al., 2005).

Sample pH during thermal processing has a profound effect on the firmness of final products. Higher or alkaline pH has been used as method to retard food texture softening during thermal processing. The effect is attributed to susceptibility of pectin to texture softening due to desertification at lower pHs due to β-elimination depolymerization of pectin during heating (Van Buren and Pitifer, 1992). Pepper rings packed in low-acid brines (1% and 1.2% acetic acid) were firmer and had less water-soluble pectin (WSP) than those packed in high-acid brine (Howard et al., 1994).

6.3.2 HP pre-treatment

High-pressure processing (HHP) has been widely applied in the food industry to increase food safety and extend the shelf life of food products. It usually employs a water-based solution as a medium to transmit nearly instantaneous and isostatic pressures up to 800 MPa. Furthermore, HHP can be used to modify the physicochemical parameters of food matrices to produce more healthy and nutritional products because it eliminates the adverse effects of heat severity (Tao et al., 2012). Moreover, it has

been found to activate PME for texture preservation in fruits and vegetables (Sila et al., 2008). Thus, to maintain texture firmness in food products, HHP can be used as pre-treatment to thermal processing of foods.

Duvetter et al. (2006) showed that the highest rate of PME-catalysed pectin de-esterification was found when high pressures in the range 200–300 MPa were applied at temperatures in the range 50–55 °C. According to Sila et al. (2007) PME was inactivated above 50 °C; however, it was stable at 600 MPa pressure especially at low temperatures (<40 °C). The catalytic activity of carrot PME is highly dependent on the temperature and pressure applied. Verlent et al. (2004) investigated the enzymatic reaction of purified tomato PME on pectin during a combined HP/HT treatment (0.1–600 MPa/20–65 °C) at pH 8.0 and pH 4.4. The optimal temperature for tomato PME activity at atmospheric pressure was approximately 45 °C at pH 8.0 and 35 °C at pH 4.4, respectively. At both pH 8.0 and pH 4.4, the optimal temperature increased at higher values at elevated pressure. At both pH values, the catalytic activity of tomato PME was higher at elevated pressure than at atmospheric pressure.

Sila et al. (2004) pretreated carrots under different HP conditions prior to thermally processing in the range of 90–110 °C. For a given thermal treatment, the rate constant (k-value) of texture degradation decreased with increasing pre-treatment pressure. A HP pre-treatment (200–500 MPa) at 60 °C for 15 min was more efficient to improve texture compared with the same pre-treatment at 20 and 40 °C, respectively. High pressure combined with calcium impregnation conferred more beneficial effects. Moreover, Sila et al. (2005) found a ninefold reduction in the thermal softening rate of HP pretreated (400 MPa, 60 °C, 15 min) calcium-soaked carrots (0.5% w/v) compared to conventionally pre-processed samples (90 °C, 4 min).

Marigheto et al. (2004) used a combination of nuclear magnetic resonance water proton relaxometry and optical microscopy to investigate the effects of high pressure on parenchyma tissue of fresh strawberries. Higher pressures of 100 MPa were shown to rupture membranes and cause major water redistribution. Cell-wall damage was apparent at pressures of 300 MPa. Texture was affected by the rupture of membranes and the accompanying microscopic redistribution of water as well as by the gelatinization of cell biopolymers. The pressure-induced denaturation of cell proteins is another factor influencing texture in food products (Sila et al., 2008).

6.3.3 Use of firming agents

6.3.3.1 Calcium ions

Calcium treatment has been used to prevent the softening in many fruits and vegetable during thermal processing. Its ability to bind with demethoxylatedpolyuronides and formation of cross-links between free carboxyl groups of a pectin chain and calcium ions has led to the widespread use of calcium as a technique to prevent texture softening. Although calcium use brings desirable effect to texture, excess use of calcium can alter the taste of products as well as promote the β-elimination reaction. However there is lack of precise knowledge on the mechanisms conferring tissue stability, and the functionally relevant pectic polymers after calcium infusion remain unknown (Sila et al., 2008).

Different methods of calcium infusion including passive osmotic infusion, vacuum-assisted infusion, pressure-assisted infusion and vacuum-assisted infusion with fungal PME improved the firmness of strawberries (Duvetter et al., 2005). Also calcium chloride was found to have a positive effect on the texture of foods when used in combination with thermal treatments. The combination of high pressure with $CaCl_2$ pre-treatment led to a marked increase in the texture of both pressure-assisted thermal pasteurisation and thermally processed samples. It is likely that the application of HP pre-treatment results in increased diffusion of calcium during $CaCl_2$ treatment. This results in increased linkages of calcium with demethylated pectin molecule, which can be attributed to cell membrane permeabilization (Rastogi et al., 2008).

According to Howard et al. (1994) pepper rings packed in brine containing $CaCl_2$ were firmer and had higher bound calcium and less water-soluble pectin than peppers packed in brine containing no $CaCl_2$. Peng et al. (2014) studied texture degradation of carrot dices in different solutions (distilled water, 0.1% and 1.4% $CaCl_2$ solutions) under temperatures ranging from 80 to 110 °C. The effects of preheating (60 °C for 20 min) enhanced the texture of the final products, and the improvement in texture became more apparent when $CaCl_2$ was added. The texture degradation rate increased at higher temperature. The authors also carried out trials using an isotonic carrot tissue solution to avoid possible ion leakage of carrot tissue during heating, but no significant differences were found between the texture of carrots immersed in the isotonic solution and distilled water after thermal treatments. In a study Rico et al. (2007) investigated the effect of calcium lactate (15 g/L) in combination with a heat-shock (25 and 50 °C) as an alternative to chlorine washing to maintain the shelf life of ready-to-eat carrots. They observed that the use of calcium lactate combined with heat-shock is a promising washing method for fresh-cut carrots to improve texture and nutritional value in addition to avoiding the use of chlorine washing. Cryo-scanning electronic microscopy of carrots demonstrated the effectiveness of combined heat-shock and calcium lactate in maintaining the turgor of cortex tissue cells and reduced the extent of lignification at cutting-edge areas as shown in Figure 6.3 resulting in lower degree of shrinkage after 10 days storage at 4 °C.

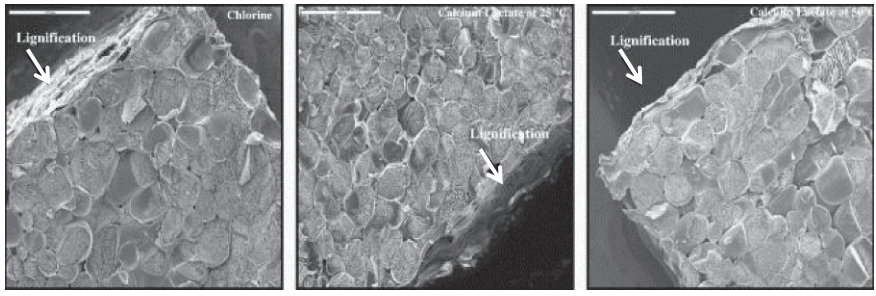

Figure 6.3 Lignification observations in cryo-SEM micrographs of sliced carrot tissues in the peeler-wounded area (orientation 1) at day 10 in samples treated with chlorine (120 mg/L), calcium lactate (15 g/L) at 25 °C and calcium lactate at 50 °C (Rico et al., 2007).

6.3.3.2 Phenolics and hydrocolloids

Cell wall carries a small percentage of ferulic acid and associated phenolic groups. These groups can be cross-linked by action of peroxidase and hydrogen peroxide. The resulted cross-linked structures form intercellular bridges such as diferulate and *o*,*o*′-dityrosine, thus connecting polymers in a tight network. Thermal textural stability is linked to diferulic acid in many products such as bamboo shoots, sugar beets and Chinese water chestnuts (Waldron et al., 1997). Thermal processing of Chinese water chestnuts causes starch gelatinization and the rounding up of cells, but adhesion and texture is maintained (Sila et al., 2008). According to Parker et al. (2003) the loss of strength of Chinese water chestnuts was coincident with the loss of 8,8′-diferulic acid, aryltetralin form. Dehydrodimer, 8,8′-diferulic acid, may be particularly concentrated at the edge of the cell faces, which gives evidence for thermal stability of cell–cell adhesion and hence firmer texture in Chinese water chestnuts.

Hydrocolloids can improve texture by generating intercellular bridges in the pores, by complementing the plant cell-wall network or by forming bonds between the added hydrocolloids and the cell-wall components (Sila et al., 2008). Hydrocolloids have been used to improve quality of button mushroom by adding xanthan gum before canning (Gormley and Walshe, 1986).

6.3.4 Cooking methods

6.3.4.1 High-temperature sous-vide cooking

Sous-vide cooking is defined as ‘cooking under controlled conditions of temperature and time inside heat-stable vacuumed pouches’ (Schellekens, 1996). Sous-vide is increasingly used to process ready-made meals or prepare ready-to-use ingredients for applications in the food service industry. This method differs from traditional cooking methods in two fundamental ways: the raw food is vacuum-sealed in heat-stable, food-grade plastic pouches, and the food is cooked using precisely controlled heating methods, usually at low temperatures for an extended period of time to enhance shelf life. The sous-vide method is generally performed at relatively low temperatures of 55–60 °C for an extended period of time of 72 h. Applying a high temperature in the sous-vide method may accelerate the softening of carrot textures due to β-elimination and the solubilization of pectin. HTSV (high-temperature sous-vide) is a useful method to provide ready-to-use carrots as ingredients for food restaurants due to its softened texture (Hong et al., 2014).

6.3.4.2 Deep frying

Frying is one of the oldest food preparation processes dating back to early 1600 BC. Due to water evaporation and protein denaturation, structural changes in food can be seen as dimensional changes. During frying the rheological properties of the fried food product are significantly affected (Mittal, 2009).

The texture of fried products is mainly characterised by the formation of a surface crust. This crusty texture is a consequence of changes in the external layers of the product at a cellular level. These physicochemical changes include the physical damage caused by cutting the product, the formation of a rough layer with a release of

intracellular material, starch gelatinization, protein denaturalization, water evaporation, expansion, tissue browning and, finally, oil ingress. According to Heredia et al. (2014) samples showed an initial stage of softening or firmness loss from the maximum value of firmness registered at time zero, followed by a second stage where maximum force tended to increase as the surface crust appeared. However, it should be noted that the mode of frying affected the first stage of softening. In deep-oil frying a sharp decrease of the maximum force was produced in the first 2 min, which is related to a fast gelatinization of surface starch, a characteristic of deep-oil frying, along with the partial denaturalization of proteins; whereas in hot-air frying the firmness loss took place in a more gradual way, reaching a minimum value between 15 and 21 min depending on the pre-treatment applied. Another difference is that crust formation started to appear earlier in the deep-oil fried potatoes (after 2 min) due to the high evaporation rate, than in the hot-air fried ones (after 18 min). It is important to point out that the transition from the first stage (softening) to the second one (crust formation) takes place when the minimum force value is achieved.

6.3.4.3 Ohmic heating

Ohmic heating uses the principle of electrical resistance to generate heat. Heat is produced directly within the fluid itself by Joule heating as alternating electric current (I) is passing through a conductive material of resistance (R), with the resultant energy generation causing a temperature rise (Engchuan et al., 2014).

According to Farahnaky et al. (2012) the hardness of all samples decreased with heating time (Figure 6.4). They indicated that similar to hardness, the compression energy and gradient of all samples reduced as a function of processing time. The textural properties show similar softening patterns with a rapid rate of softening at the beginning, followed by a slow rate of softening that starts at a cooking time of about 10 min. Different studies have revealed that when vegetable tissues were processed at high temperatures (>90 °C), the tissue firmness reduced in a biphasic manner, rapidly in the first few minutes and then more slowly over the duration of the processing time. After each cooking treatment, tested tissues lost a large part of their initial hardness. It is clear that texture softening caused by the ohmic treatment at 380 V was greater than of other cooking treatments, and this can be related to a more complete destruction of cellular structures. Turgor component of texture caused the rapid phase of firmness loss of carrot. Also these researchers reported the changes in carrot pectin characteristic as the possible reason for the second phase of firmness loss. At the beginning of the cooking process, heat is transferred to the inner tissue layers. As soon as individual cells reach a temperature of about 50 °C, cell membranes start degrading, and the cellular turgor shows a reasonably rapid decay, whereas cell-wall pectin molecules are not yet affected. Decomposition of intracellular mucilage that takes place by heating treatment resulted in weakened cell wall, softened texture and decreased fracturability.

6.3.5 Aseptic processing

Aseptic processing can be defined as the processing and packaging of a commercially sterile product into sterilised containers followed by hermetic sealing with a sterilised closure in a manner that prevents viable microbiological recontamination of the sterile

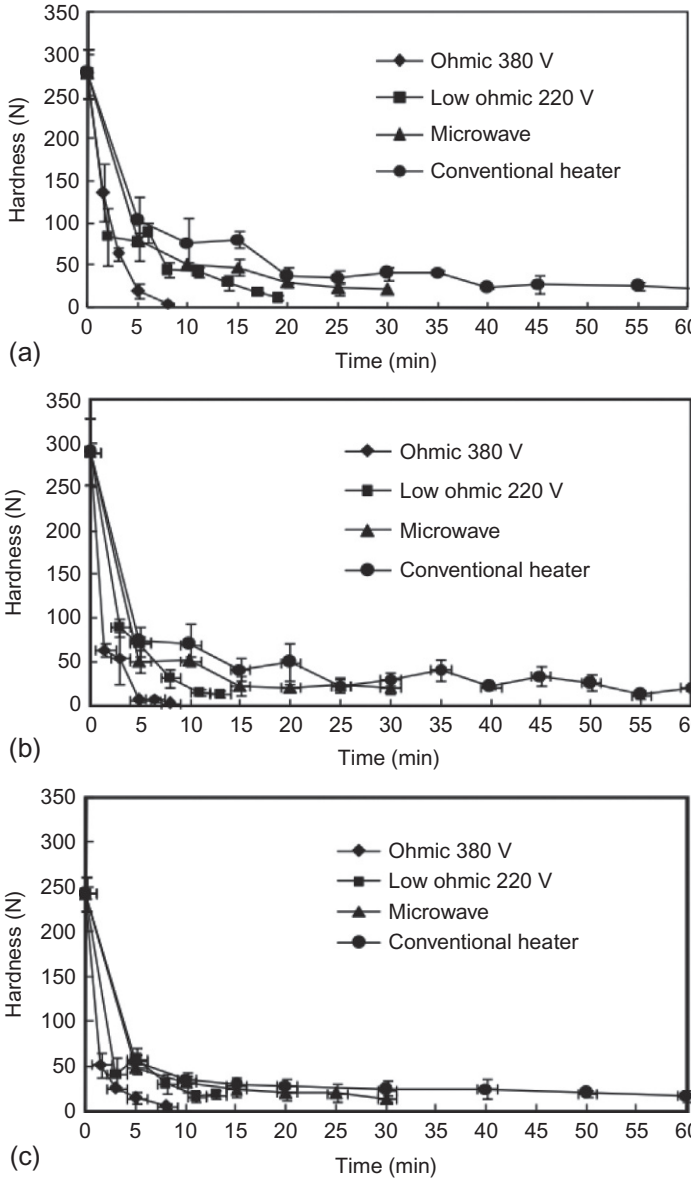

Figure 6.4 Hardness of (a) carrot, (b) red beet and (c) golden carrot versus cooking time by ohmic, microwave and conventional heating methods.

product (Betta et al., 2011). The benefits of aseptic processing over conventional canning include longer shelf life, wider packaging sizes, wider container materials and improved nutritional and sensory properties. Anderson and Walker (2011) measured the texture as work per unit mass required to shear individual mushrooms. According to the authors, aseptic processing improved texture by 3.9% and 4.6% for whole and

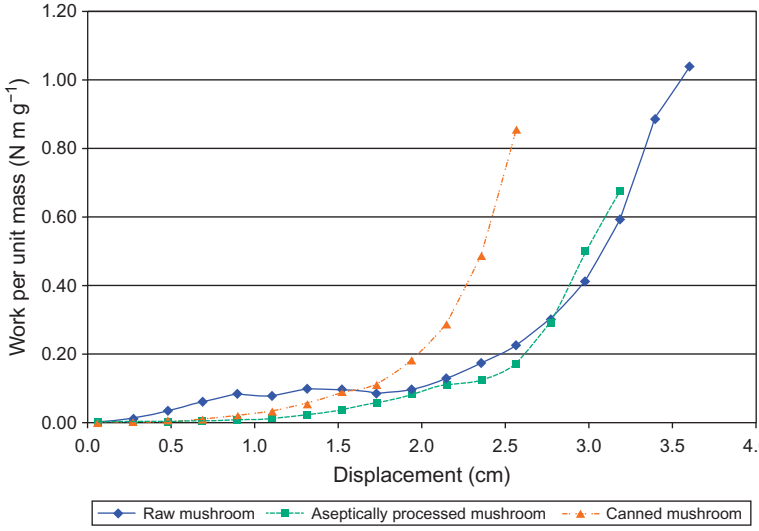

Figure 6.5 Texture plots for similarly sized individual raw, aseptically processed and canned whole mushrooms (Anderson and Walker, 2011).

sliced mushrooms, respectively, compared to conventional canning. Moreover, the texture of typical aseptically processed mushrooms matched more closely with the texture of a typical raw mushroom versus a canned mushroom (Figure 6.5).

However, according to Dawson et al. (1991), aseptic processing results in significant moisture losses and toughening of chicken breast meat, which may be attributed to protein denaturation and myofibrillar shortening. The aseptic HT, short-time process extracts and solubilizes collagen, which results in a tougher final meat texture.

6.4 Conclusions

Texture characteristics of food products are strongly related to consumer food preferences. Thermal processing results in changes to food product properties including texture, flavour and organoleptic properties. Changes in food texture are influenced by physicochemical changes occurring in the cell-wall materials such as reduction in hydrostatic pressure, gelatinization of starch, protein denaturation and solubilization of pectic material in the middle lamella, cell separation and an associated loss of turgor. However, the increasing demand for 'fresh-like' processed foods has prompted additional research on methods of improving the texture of thermally processed products. Recently investigated methods include low-temperature blanching/preheating prior to sterilisation, pH adjustment and also infusion of firming agents such as calcium ions, phenolics, hydrocolloids and PME before thermal processing. Moreover,

recently thermal processing has been modified to obtain the desired softened texture through sous-vide cooking, deep frying and ohmic heating. These methods of texture modification for thermally processed foods should be further investigated prior to scale-up trials for commercial evaluation.

6.5 Future trends

Texture is one of the most important factors determining preference of foods with solid or semisolid states. The impact of processing including temperature, level of shearing and pressure on texture was been widely investigated. Many studies have been carried out to explore textural variation in a wide variation of thermally processed foods. However, developing new food products with desired textural properties is challenging. Thermal processing of foods softens the texture of food products, which may be desirable or undesirable depending on the product profile and market. Recent advances offer the potential for innovative technologies for non-destructive food texture measurement based on acoustic and optical approaches that provide 'real-time' or 'on-line' texture measurement for fresh and processed foods. However, there is still currently a lack of common international standards for food texture measurement, which often makes it difficult to trace and compare outcomes of processing methods even in the same food product and using the same instrument (Chen and Opara, 2013b).

Future research should be aimed at investigating microstructural changes in food products at different variables of thermal processing such as time, temperature and pressure. Additional research is also required to improve understanding of pectin solubilization in plant-based foods. In particular, there is lack of knowledge on the effect of processing on the texture of plant-based foods and the related correlations with pectin conversions. Moreover, mechanisms of pectin solubilization, starch gelatinization, protein denaturation and loss of turgor should be analyzed in depth for novel thermal processing technologies such as microwave and ohmic heating.

Techniques suggested in this chapter can be used in combination to have synergistic effects, resulting in more desirable texture in the final product. Techniques such as combining the infusion of PME with the impregnation of gelling and/or firming agents including calcium ions may be further developed and exploited to improve the mechanical properties of foods. Although HP pre-treatment has been studied to improve the texture of food products, its effect on microstructure of food product requires more study. Moreover, there is need to develop new non-thermal processing technologies that can be effective in extending shelf life of food products without significantly affecting the textural properties of foods. Also, genetic manipulation of cell-wall polymers could be used to study modification of cell-wall enzymes, thereby improving or maintaining the texture quality of final products. These technologies also need to be cost effective and should be scalable to larger industrial operations.

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Structure and texture development of food-emulsion products

7

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7.1 Introduction

In recent decades, the manufacture and marketing of healthier versions of many food products have been on the rise due to growing concerns about rising levels of diet-related chronic diseases, such as obesity, heart disease, hypertension, diabetes, and others (Buttriss, 2013; Krystallis et al., 2008; Nagai et al., 2013; Robinson et al., 2013). Consumer acceptance of these healthier products is often poor because they are perceived as being less palatable or less appealing than conventional products (Berg et al., 2003; Raghunathan et al., 2006). Thus, there is still a need to develop healthful foods that satisfy both nutritional and sensory criteria. For example, many *emulsion-based* low-calorie food products have been reported to lack the complex taste, texture, and appearance of their full-calorie counterparts (Chojnicka-Paszun et al., 2012; Soukoulis et al., 2010; Tomaschunas et al., 2013; van Aken et al., 2011). This is because taste, texture, and appearance are critical aspects of good eating pleasure (Sorensen et al., 2003; Vadiveloo et al., 2013; Wadhera and Capaldi-Phillips, 2014).

The textural and mouthfeel attributes of a food product are perceived during mastication and are covered by the science of *food oral processing* (Hutchings and Lillford, 1988; Stieger and van de Velde, 2013). *Texture* is defined as the "sensory and functional manifestation of the structural, mechanical, and surface properties of foods detected through senses of vision, hearing, touch, and kinesthetic" (Szczesniak, 2002). *Mouthfeel* is defined as the sensations arising from the interactions of ingested food, mixed with saliva, with the receptors in the mouth that respond to tactile stimuli during mastication (Smith and Margolskee, 2001). Sensory feedbacks generated by different receptors in the mouth during mastication are integrated within the brain and lead to the overall perception of texture and mouthfeel of a food product. Of the many receptors, mechanoreceptors are responsible for food texture perception (Stieger and van de Velde, 2013). More information on oral food processing can be obtained in recent articles in this area (Chen, 2009; de Wijk et al., 2011; Foegeding et al., 2011; Foster et al., 2011; Koc et al., 2013; Stieger and van de Velde, 2013). The physical and structural properties of a food product have a significant influence on their perceived texture and mouthfeel. In view of this, healthful food products should be methodically designed, formulated, and manufactured so that they possess excellent sensory attributes to encourage consumers to choose a healthy eating lifestyle.

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Many high-calorie food products that are commonly consumed exist as oil-in-water (O/W) emulsions, for example, batters, beverages, cheeses, creams, desserts, dips, dressings, sauces, and soups (Le Reverend et al., 2010; Ma and Boye, 2013; McClements, 2005). These products consist of fat droplets dispersed within an aqueous medium (McClements, 2005; Santana et al., 2013). The properties and spatial arrangement of the fat droplets within a food matrix have an appreciable influence on the physicochemical properties and sensory attributes (appearance, aroma, taste, mouthfeel and texture, and shelf stability) of the final product (Chung and McClements, 2013; Ciron et al., 2012; Stieger and van de Velde, 2013; van Vliet et al., 2009). Consequently, the architecture of food systems can be designed to control the sensory attributes of products or to create new eating experiences. This chapter focuses on the properties of O/W emulsions that influence the structural organization, rheological, and sensory properties of emulsion-based food products.

7.2 Effect of emulsion properties on structural and textural properties

In general, emulsions are categorized according to the type of immiscible materials that make up the dispersed and continuous phases. Emulsions consisting of an oil phase dispersed within a continuous aqueous phase are known as O/W emulsions, whereas emulsions consisting of an aqueous phase dispersed within a continuous oil phase are known as water-in-oil (W/O) emulsions (Figure 7.1). Milk, cream, desserts, dips, sauces, soups, and dressings are all examples of O/W emulsions, whereas butter and margarine are examples of W/O emulsions. Most existing emulsion-based food products fall into these two categories of emulsions. Nevertheless, the classification of emulsions based on their structural organization has grown more complex recently due to the development of a wide range of structured emulsions aimed at addressing specific challenges. These structured emulsions can be classified according to the

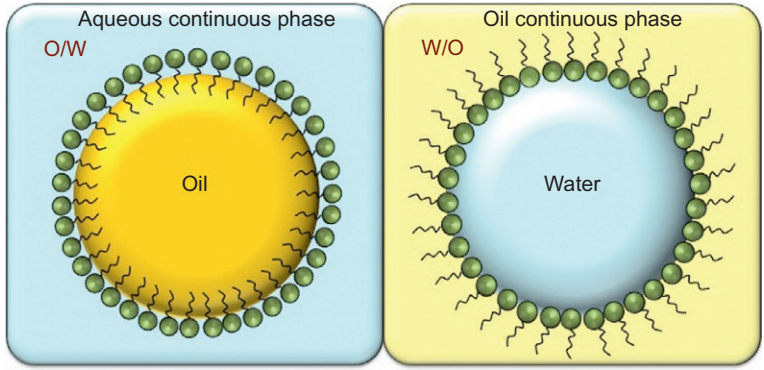

Figure 7.1 Schematic diagram of *simple emulsions*—oil-in-water (O/W) emulsion and water-in-oil (W/O) emulsion.

composition of the different dispersed phases or to the size of the dispersed-phase droplets. Some complex emulsions can contain two (or more) phases of dispersed droplets, and they are categorized according to the location of the primary (innermost) dispersed phase. Examples of multiple emulsions are oil-in-water-in-water ($O/W_1/W_2$), water-in-oil-in-water ($W_1/O/W_2$), and oil-in-water-in-oil ($O_1/W/O_2$) emulsions (Dickinson, 2011; Jimenez-Colmenero, 2013; McClements, 2012a). Complex emulsions can also be classified according to particle sizes (e.g., *nanoemulsions*—10–100 nm radii, *miniemulsions*—100–1000 nm radii, and *macroemulsions*—1000 nm–1000 μm radii; McClements, 2012b; Santana et al., 2013).

The major steps involved in emulsion formation have been reviewed in detail elsewhere (McClements, 2005; Santana et al., 2013). The methods and conditions used significantly determine the physicochemical properties of the emulsions created (Huck-Iriart et al., 2011; McClements, 2005; Neirynck et al., 2009; Santana et al., 2013). After formation, the different phases and their spatial arrangement also have significant influence on the physicochemical and sensory properties of emulsion-based food products, as will be discussed in the following sections.

7.2.1 Influence of dispersed phase

The influence of dispersed-phase properties, such as droplet concentration, size, charge, and interactions, on the structural organization, rheology, and sensory properties of food emulsions is highlighted in this section.

7.2.1.1 Oil phase volume fraction

It is widely known that the amount of oil present in a food affects its overall sensory quality. Some common examples include the eating experience of low-fat milk to full-fat milk or low-fat food (sauces or yogurt) to their full-fat versions (Chojnicka-Paszun et al., 2012; Tomaschunas et al., 2013). Oil concentration influences the perceived whiteness (lightness, L^*) of food products due to the impact of the fat droplets on light scattering (Chung et al., 2013c). It also influences the rheology and/or textural properties (viscosity or thickness) of foods due to the impact of the fat droplets on the resistance of emulsions to fluid flow (Chojnicka-Paszun et al., 2012; Chung et al., 2013d; Soukoulis et al., 2010; Tomaschunas et al., 2013; van Aken et al., 2011; Vingerhoeds et al., 2008). Mathematical equations have been developed to relate the lightness and viscosity of emulsions to droplet concentration (Chung et al., 2013c; Derkach, 2009; McClements, 2005). Typically, the lightness of an emulsion increases steeply from 0% to 5% fat droplets, and then increases more gradually at higher fat contents. On the other hand, the viscosity of a (nonflocculated) emulsion increases gradually with fat droplet concentration from around 0% to 30% fat droplets, but then increases steeply at higher levels due to close packing effects. It has been demonstrated that emulsions with higher oil content are perceived as creamier or more appealing in appearance and texture (Chojnicka-Paszun et al., 2012; Chung et al., 2013d; Tomaschunas et al., 2013).

7.2.1.2 Oil droplet size

The size of the fat droplets in an O/W emulsion may also influence the appearance, rheology, and sensory properties of food emulsions. The lightness of an emulsion tends to be maximum when the droplet dimensions are similar to the wavelength of light, but decreases for smaller or larger droplets. The rheological properties of food emulsions may also be strongly affected by fat droplet size. Droplets with smaller sizes allow more efficient packing of the dispersed phase within complex food systems that contain other nonfat particles, such as starch granules, protein aggregates, or air bubbles. For example, studies on low-fat yogurts found that smaller fat globules were more easily incorporated into protein gel networks, which led to different microstructures and creamier yogurts compared to control (full-fat) yogurts (Ciron et al., 2012). Another study found that small fat droplets were incorporated into the interstitial spaces between starch granules and led to different microstructures and viscosities (Chung et al., 2012b). The size of the fat droplets may also directly influence the sensory perception of a food product (Engelen et al., 2005a,b; Imai et al., 1995, 1997, 1999). Studies have reported that particles exceeding a certain size (e.g., microcellulose >23 μm; albumin >40 μm; casein >50 μm; Imai et al., 1997, 1999) and exhibiting certain shapes (noncircular, irregular, or with sharp edges) or hardness (Engelen and Van Der Bilt, 2008; Engelen et al., 2005a,b) have a tendency to impart "gritty" and "grainy" textures in some food products. This phenomenon is important when replacing the fat droplets in reduced-calorie food products with nonfat particles.

7.2.1.3 Droplet charge

The fat droplets in most food products have an electrical charge depending on the type of emulsifiers used, environmental conditions (such as pH and ionic strength), and ingredient interactions (such as polymer or ion absorption; McClements, 2005). The droplet charge, which is usually characterized in terms of the ζ-potential versus pH profile, influences the interactions of oil droplets with each other and with other nonfat charged species (such as proteins, polysaccharides, or mineral ions). Two droplets of similar charge will repel each other, whereas two droplets of opposite charge will attract each other. These interactions therefore affect the microstructure, rheology, and stability of the overall system (Dickinson, 2010a; Mao and McClements, 2013b; van Aken et al., 2011).

7.2.1.4 Droplet interactions

Fat droplets in an emulsion interact with each other and with other nonfat components (e.g., minerals, proteins, or polysaccharides) through different molecular or colloidal interactions. These interactions can promote stability or instability and influence the rheology and textural properties of the overall systems (Huck-Iriart et al., 2011; Mao and McClements, 2013a; Tangsuphoom and Coupland, 2008). Some of the most important interactions are van der Waals, electrostatic, steric, hydrogen bonding, hydrophobic, and depletion interactions (McClements, 2005). These interactions are in turn influenced by droplet characteristics (e.g., size, charge, and dielectric

constant) and the continuous phase environment (e.g., ionic strength, pH, and dielectric constant). If the attractive interactions are stronger than the repulsive interactions, then droplet *flocculation* (association of two or more droplets) or *coalescence* (merging together of two or more droplets) may occur. These forms of droplet aggregation will result in product instability, which may adversely affect sensory quality (e.g., oily appearance, perturbed flavor, and "oily" and "gritty" texture). On the other hand, controlled fat droplet flocculation can be used to increase the viscosity and creaming stability of some systems, which may be useful for the development of reduced-calorie products (Dickinson, 2010a; Mao and McClements, 2013a; McClements, 2012a).

7.2.1.5 Droplet interface

Fat droplets are usually surrounded by a layer of emulsifier molecules that separates the oil phase from the aqueous phase and protects the droplets from aggregation (Figure 7.1). This thin layer of emulsifier has a pronounced influence on the physicochemical and sensory properties of emulsion systems, for example, their rheology, stability, and flavor attributes. Manipulation of interfacial properties can therefore be used to influence the overall physicochemical and sensory properties of food products (Benjamin et al., 2011; Paraskevopoulou et al., 2009; Seta et al., 2014; Surel et al., 2014). Interfacial properties may be altered by using different emulsifiers, by coabsorbing other substances (such as polyelectrolytes), or by altering environmental conditions. A recent study showed that altering the properties of interfacial layers composed of proteins led to various textures in O/W emulsions (Surel et al., 2014). Emulsions coated with native whey protein or native casein micelles had fluid-like characteristics, and the fat droplets were not connected, whereas emulsions coated with aggregated (heated) whey protein alone or in combination with caseins had gel-like characteristics.

7.2.2 Influence of continuous phase

Emulsion-based foods are complex systems that contain not only the oil phase but also a variety of different components in the continuous phase, such as salts, acids, sugars, proteins, polysaccharides, minerals, and air bubbles. The presence of these components also influences the microstructure, physicochemical, and sensory properties of food products. For example, addition of starch, fibers, and/or polysaccharides is known to increase the viscosity of a system (Chung et al., 2012b; Flett et al., 2010; Ma and Boye, 2013), and this increase can enhance fat-related sensory attributes (e.g., thickness, creaminess, and smoothness), as well as other textural attributes of foods (Arancibia et al., 2011; Chung et al., 2013d; Fernandez et al., 2010; Flett et al., 2010). The degree of influence of the nonfat particles depends on the type, concentration, conformation, and interactions of these components. It is therefore necessary to determine the physical and chemical properties of the different components to better understand their contributing effects on the overall properties of the system. Mathematical models have been created to predict the influence of nonfat particles on the rheological properties of emulsions (Chung et al., 2012b; McClements,

2005; Servais et al., 2002). These mathematical models are useful for new product formulation and development to predict their rheological properties. Some of them are described in detail in the cited references.

7.3 Novel structured emulsions

As mentioned earlier, there is increasing demand for more healthful food choices, which has led to interest in the use of structural design approaches to fabricate these types of products. A number of structural design approaches that may be useful for formulation of reduced-calorie food emulsions with desirable textural attributes are highlighted in the following sections.

7.3.1 Microclusters

The spatial arrangement and interaction of fat droplets have an appreciable influence on the viscosity and textural properties of emulsion-based foods. For example, flocculated emulsions have higher viscosities than nonflocculated ones containing the same amount of fat droplets. This viscosity increase is attributed to the increase in the effective volume fraction occupied by the particles in the system as a result of aqueous phase trapped within the flocs (Mao and McClements, 2013a; McClements, 2012a; Tangsuphoom and Coupland, 2008). Droplet flocculation may even lead to a three-dimensional particle network that leads to gel-like or paste-like textures, depending on droplet concentration, environmental conditions, and the

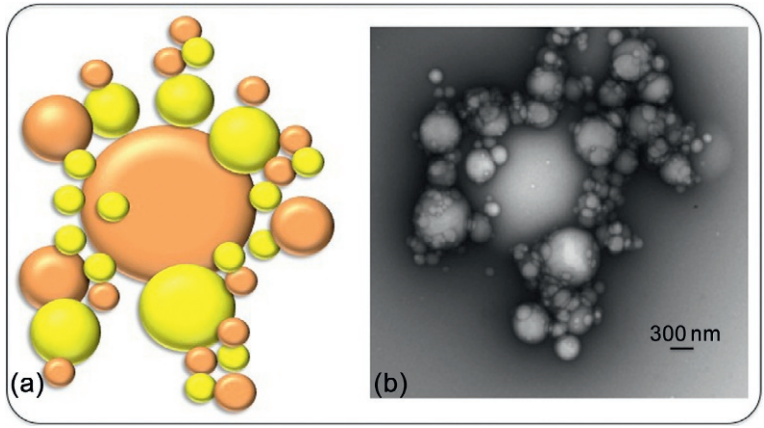

Figure 7.2 (a) Schematic diagram and (b) transmission electron micrograph of *microcluster emulsions* consisted of 40% lactoferrin- to 60% β-lactoglobulin-coated lipid droplets in water continuous phase (pH 7). (The different colored droplets each represent the two protein-coated lipid droplets.)
Micrograph was contributed by Mao, Y.Y. (Food Science Department, University of Massachusetts, Amherst).

nature of flocculation (Figure 7.2; Mao and McClements, 2012, 2013b). Controlled flocculation of O/W emulsions can therefore be used to create reduced-fat products with similar textural attributes as high-fat products.

The formation of droplet microclusters due to flocculation can be induced by modulating the attractive and repulsive colloidal interactions in emulsions. Flocculation can be made to occur by either increasing the attractive interactions and/or decreasing the repulsive interactions between fat droplets, with the precise mechanism depending on the nature of the colloidal interactions in the system. The resulting microclusters formed due to flocculation may be *homoaggregates* (contain similar particles) or *heteroaggregates* (contain dissimilar particles; Dickinson, 2010a; Mao and McClements, 2013b). A study found that heteroaggregates consisting of casein micelles and carrageenan could be used to enhance the creaminess and viscosity of a low-fat food product, which was attributed to the colloidal size of the microclusters formed (Flett et al., 2010).

As mentioned earlier, flocculation can be induced using various approaches depending on the precise nature of the system. Some of the major approaches are as follows:

- Electrostatic repulsion can be decreased by altering the pH to reduce the surface charge or by increasing the ionic strength to screen the surface charges (Farshchi et al., 2013; McClements, 2012a).
- Hydrophobic attraction can be promoted by increasing the surface hydrophobicity of droplets, for example, by heating globular protein-stabilized fat droplets above their thermal denaturation temperature (Dickinson, 2010a; Lam and Nickerson, 2014).
- Depletion attraction can be increased by adding nonadsorbing biopolymers or other nonadsorbing colloidal particles to the aqueous phase to increase the osmotic pressure acting between droplets (Dickinson, 2010a; Farshchi et al., 2013).
- Bridging flocculation can be induced by adding oppositely charged polymers or mineral ions to form ionic bridges between different droplets (Dickinson, 2010a; Guzey and McClements, 2007).

Recent studies have also examined the effect of mixing two emulsions together that contain oppositely charged fat droplets, that is, one negative and one positive (Mao and McClements, 2012, 2013a,b). The mixed emulsions had much higher viscosities than the individual nonflocculated emulsions they were prepared from. The heteroaggregates formed have potential application in reduced-fat products that need to have high viscosities or paste-like textures similar to those of full-fat products.

7.3.2 Filled-hydrogel particles

Another structural design approach that can be used to create novel structures and textures in emulsion-based products is the fabrication of filled-hydrogel particles. These systems consist of fat droplets embedded within hydrogel microspheres (Figure 7.3). The volume occupied by the hydrogel phase leads to an increase in the net volume fraction of the particles in the system, which leads to the formation of high-viscosity systems at low-fat contents. In addition, this type of structural organization delays the release of nonpolar flavor molecules from fat droplets, which can be useful in achieving flavor release profiles from reduced-fat products that mimic those of high-fat

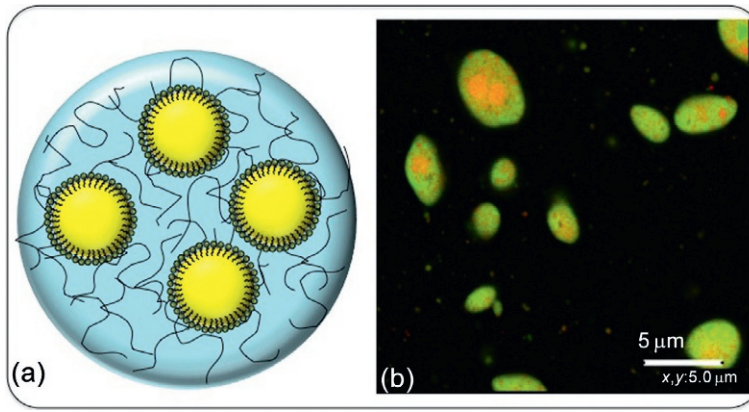

Figure 7.3 (a) Schematic diagram and (b) confocal laser scanning micrograph of *oil-filled-hydrogel particles* containing sodium caseinate as the dispersed gel matrix and high methoxyl pectin as the continuous phase.

products. Filled-hydrogel particles may also be useful for targeted delivery or controlled release of lipophilic components (Jones and McClements, 2012; Matalanis and McClements, 2013; Shewan and Stokes, 2013). Hydrogel particles can be made from biopolymers (e.g., proteins or polysaccharides) that form gel matrices under controlled conditions (e.g., heating, cooling, pH adjustment, salt, or enzyme addition) (Firoozmand and Rousseau, 2013; Matalanis et al., 2010). Several methods are available to form filled-hydrogel particles, including injection methods, molding methods, emulsion-templating methods, and controlled biopolymer phase separation methods (Burey et al., 2008; Chung et al., 2013a; Firoozmand and Rousseau, 2013; Matalanis and McClements, 2012; Shewan and Stokes, 2013). Most of these methods involve mixing an O/W emulsion with a biopolymer solution and then adjusting system conditions to induce hydrogel microsphere formation (McClements, 2012a).

Controlled biopolymer phase separation has been used in our laboratory to create oil-filled-hydrogel microspheres with appreciably higher viscosities than conventional emulsions with the same fat content (Chung et al., 2013a,b; Matalanis and McClements, 2013; Matalanis et al., 2011). In general, biopolymer phase separation can be divided into segregation-based (thermodynamic incompatibility) or aggregation-based (complex coacervation) methods (Burey et al., 2008; Chung et al., 2013a,b; Matalanis and McClements, 2012, 2013; Matalanis et al., 2010; Rediguieri et al., 2007).

We have used segregation-based methods to fabricate oil-filled-hydrogel particle systems for reduced-fat model emulsion systems (Chung et al., 2013a,b). When two different biopolymer solutions are mixed together above a critical concentration, they tend to separate into two phases that contain different compositions due to thermodynamic incompatibility. The phase-separated system is then mixed by blending or injection to form water-in-water (W_1/W_2) hydrogel particles. The phase that occupies the smaller-volume fraction normally becomes the dispersed phase (W_1), whereas the other phase becomes the continuous phase (W_2). Oil-filled-hydrogel

particles can be fabricated by mixing an O/W emulsion with the W_1/W_2 emulsion under conditions where the oil droplets partition into the dispersed aqueous phase (W_1). The $O/W_1/W_2$ emulsions formed are often unstable due to the tendency for the dispersed phase to coalesce and phase separation (Matalanis and McClements, 2012; Matalanis et al., 2010; Norton and Frith, 2001). The instability can be reduced by inducing network formation within the continuous phase or the dispersed phase using cross-linking agents or acidification (Burey et al., 2008; Matalanis and McClements, 2012; Turgeon et al., 2003). It can also be reduced by increasing repulsive interactions between the hydrogel particles (Matalanis et al., 2010; Rediguieri et al., 2007). The overall fabrication process involves a number of different steps including: (i) mixing the two biopolymer solutions together and then inducing phase separation at neutral pH, (ii) followed by promotion of W_1/W_2 formation by reducing the pH, and (iii) finally crosslinking the dispersed phase (Matalanis and McClements, 2012; Matalanis et al., 2010). This method involves a number of steps that are time consuming and may be unsuitable for commercial applications within the food industry. A simplified controlled biopolymer phase separation was recently established that may be more suitable for practical applications (Chung et al., 2013a,b). This simplified method involves fewer steps and requires a shorter time to fabricate the final $O/W_1/W_2$ emulsion. Both methods, nonetheless, can be used to fabricate filled-hydrogel particles that are stable and with higher viscosities than the individual biopolymers or emulsion systems (Chung et al., 2013a; Matalanis et al., 2011).

7.3.3 Multiple emulsions

Conventional O/W emulsions consist of fat droplets dispersed within an aqueous continuous phase. More complex systems, such as multiple emulsions, can be fabricated for specific purposes, for example, reduced calories, separation of reactive components, taste masking, or controlled delivery (Dickinson, 2011; Jimenez-Colmenero, 2013; Sapei et al., 2012). Multiple emulsions are emulsified emulsions that typically consist of small droplets embedded within larger droplets that are dispersed within a continuous phase (Figure 7.4). The two major types of multiple emulsions suitable for food applications are $W_1/O/W_2$ or $O_1/W/O_2$ emulsions (Dickinson, 2011; Jimenez-Colmenero, 2013; McClements, 2012a; O' Dwyer et al., 2013; Sapei et al., 2012). The two similar phases (i.e., W_1 and W_2 or O_1 and O_2) may have different compositions and properties (McClements, 2012a; Nisisako, 2008). The fabrication of multiple emulsions has been covered in detail in a number of articles and so will not be discussed further here (Dickinson, 2011; Jimenez-Colmenero, 2013; McClements, 2012a; Muschiolik, 2007; Nisisako, 2008).

A number of potential applications of multiple emulsions in food products have been described. Giroux et al. (2013) employed W/O/W emulsions for encapsulation of vitamin B_{12} for use in cheese fortification. The authors found that high encapsulation efficiency was achieved (~96%) using double emulsions, and vitamin loss was prevented during *in vitro* gastric digestion. Another study described the use of O/W/O

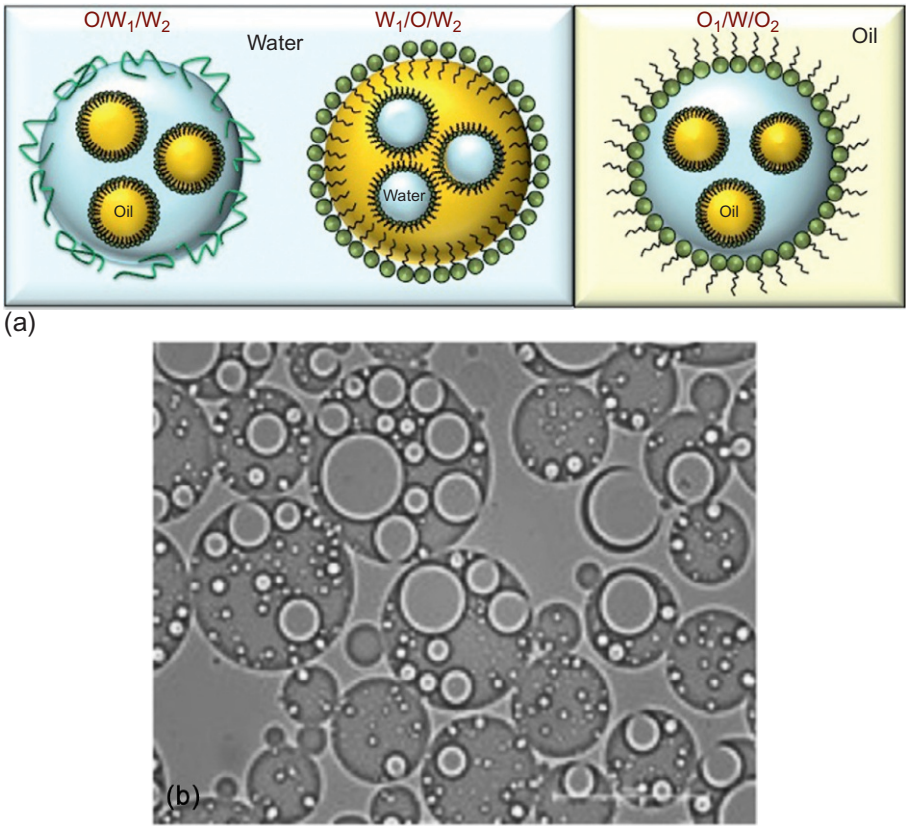

Figure 7.4 (a) Schematic diagram of various types of *multiple emulsion*—oil-in-water-in-water ($O/W_1/W_2$) emulsion, water-in-oil-in-water ($W_1/O/W_2$) emulsion, and oil-in-water-in-oil ($O_1/W/O_2$) emulsion. (b) Optical micrograph of a W/O/W emulsion.

emulsions to enrich spreads containing omega-3 oils (O' Dwyer et al., 2013). The oxidative stability of the enriched spreads was enhanced, whereas the texture and rheology of the spreads depended on the type of omega-3-rich oils added.

7.3.4 Multilayer emulsions

As mentioned earlier, the interfacial layer surrounding fat droplets plays an important role in influencing the microstructure and physicochemical properties of emulsions. The fat droplets in conventional emulsions are typically stabilized by a single layer of emulsifier molecules. Nevertheless, the properties of the interfacial layer can be manipulated to produce emulsions with improved properties. One method to modify the interfacial layer is by building laminated coatings around the droplets (Figure 7.5) using layer-by-layer deposition (Chun et al., 2013; De Temmerman et al., 2012; McClements, 2012a; Shchukina and Shchukin, 2012; Zeeb et al., 2014). The

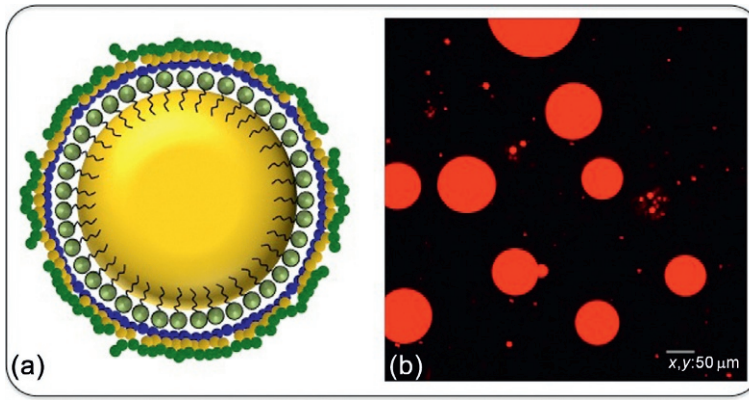

Figure 7.5 (a) Schematic diagram of a droplet in a *multilayer emulsion*. (b) Confocal micrograph shows fat droplets covered by a β-lactoglobulin-alginate coating (pH 3.5). Note the biopolymer layer is too small to see with confocal microscopy.

characteristics and properties of the layers, including the charge, thickness, permeability, and integrity, can be tailored for specific functions, like controlled-release mechanism, by using appropriate emulsifiers and by adjusting the conditions of the systems as well as the preparation conditions (McClements, 2012a; Shchukina and Shchukin, 2012; Trojer et al., 2013; Zeeb et al., 2014). It is important to carefully deposit the layers around the droplets to prevent bridging flocculation that can lead to instability. The use of multilayer emulsions for controlled release of volatile compounds has recently been investigated, and some promising results have been reported (Benjamin et al., 2012, 2013; Mao et al., 2013). The release of the volatile compounds could be delayed under simulated oral conditions or triggered by pH, salts, or artificial saliva. More details on the formation of multilayer emulsions have been covered in the cited references (De Temmerman et al., 2012; McClements, 2012a; Shchukina and Shchukin, 2012; Zeeb et al., 2014).

7.3.5 Air-filled emulsions

An alternative approach to formulate reduced-calorie food emulsions is to replace some of the fat droplets with micron-sized gas bubbles (microbubbles). These gas bubbles provide some of the desirable attributes of fat droplets (such as enhanced lightness, viscosity, and mouthfeel), without contributing calories (Dickinson, 2010b). The gas bubbles must be carefully formulated to ensure that they remain stable within the product during storage, transport, and processing. The resulting triphasic systems consist of gas bubbles and fat droplets dispersed within a continuous aqueous phase (Figure 7.6; Green et al., 2013; Le Reverend et al., 2010; Tchuenbou-Magaia and Cox, 2011; Tchuenbou-Magaia et al., 2010). Several studies have investigated the feasibility of forming microbubbles and incorporating them into emulsion systems. The microbubbles (1–10 µm diameter) are typically stabilized

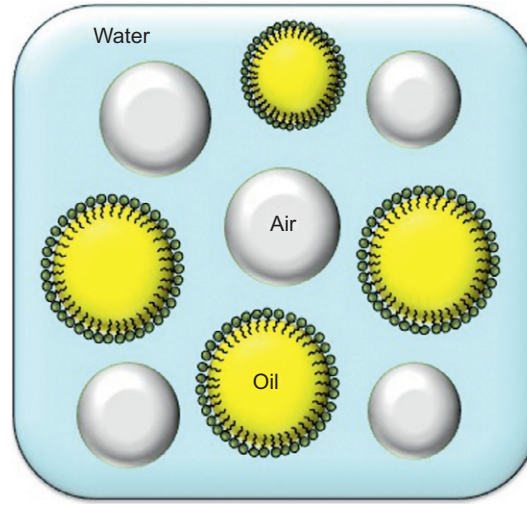

Figure 7.6 Schematic diagram of *air-filled triphasic emulsions*.

by cysteine-rich proteins that form a protective membrane at the air–water interface, for example, hydrophobins, egg albumin, or bovine serum albumin (Green et al., 2013; Tchuenbou-Magaia et al., 2011). Studies done on model salad dressings showed that triphasic emulsions had similar rheological properties as those made of conventional O/W emulsions but with lower fat contents. The air bubbles formed were stable during storage for up to 4 months at room temperature (Tchuenbou-Magaia and Cox, 2011). These preliminary studies strongly indicate the feasibility to incorporate air bubbles into food systems. Air-filled food emulsions may be suitable for the creation of reduced-fat products with improved sensory quality.

7.4 Food structure and textural properties assessment

The determination of food structure and textural properties by instrumental methods is particularly important during product development to rapidly optimize product formulation. A wide range of analytical methods has been developed to evaluate the physicochemical and sensory properties of foods. Instrumental methods help to identify key factors that influence the sensory attributes of foods and provide quantitative information that helps predict the sensory performance of foods. These instrumental methods, however, cannot totally replace sensory panel evaluations due to the extreme complexity of the human sensory system. In this section, a number of analytical methods available to measure the *structure* and *textural* properties of food emulsions will be briefly given. Sensory attributes, such as appearance, aroma, and taste, that are also important in determining the quality of food products are reviewed elsewhere.

7.4.1 Food microstructure analysis

The structural organization of the different components within a food emulsion plays a major role in determining its physicochemical and sensory properties. Consequently, it is important to determine the microstructure of emulsion-based food systems. The information obtained may provide knowledge of the mechanisms responsible for the textural (sensory) properties of a system. The most commonly used analytical instruments to characterize emulsion microstructure are based on microscopy, for example, optical microscopy, confocal laser scanning microscopy, scanning electron microscopy, or transmission electron microscopy (Chung et al., 2013b; Mao and McClements, 2012; McClements, 2005; Schuster et al., 2012; Tippetts et al., 2013). Some micrographs taken from various microscopy instruments are shown in Figures 7.2–7.5.

7.4.2 Texture and mouthfeel

Rheological instruments are the most commonly used analytical tools to quantify the textural attributes of fluid and semi-solid food products, such as viscosity, viscoelasticity, plasticity, and fracture properties (Fischer and Windhab, 2011; Fischer et al., 2009; Stokes et al., 2013). Even though most of the instruments used (viscometers or rheometers) do not closely simulate oral mastication conditions (de Wijk et al., 2011; Stokes et al., 2013), they often give results that do correlate with sensory perception (Chung et al., 2013d; Melito et al., 2013; Mossaz et al., 2010). In the following sections, the principles of a number of instrumental methods designed to mimic oral mastication are discussed.

7.4.2.1 Large deformation flow behavior

Large deformation measurements are typically performed on a viscometer or rheometer that aims to mimic the shear flow or disruption of a food system. From these measurements, the *apparent viscosity* of samples, calculated as the shear stress divided by the rate of strain, is derived, and it is commonly used to indicate the "thickness" of fluid to semi-solid emulsion-based food products. These products often exhibit non-Newtonian behavior, that is, their viscosity is dependent on rate of strain (Chung et al., 2013c; McClements, 2005; Rao, 2007a). Besides apparent viscosity, *yield stress*, *consistency*, and *flow index* can also be derived from shear stress versus shear rate measurements. Many of emulsion-based food products exhibit "plastic-like" behavior, where below a critical applied stress no flow is measured and above it samples behave as viscous fluids (McClements, 2005; Miri, 2011; Rao, 2007a). Appropriate strain rates and measuring conditions must be selected to simulate conditions (such as flow rates and saliva dilution) in the mouth during mastication. This is particularly significant when measuring foods that contain digestible starches as they are degraded by amylase during mastication (Chung et al., 2013d). A shear rate of 50 s^{-1} is often reported to give a good correlation with the perceived initial "thickness" of foods, but this value does not always give a good correlation because of the

complexity of the processes occurring within the mouth (Bistany and Kokini, 1983; Richardson et al., 1989; Stokes et al., 2013).

7.4.2.2 Small-deformation viscoelastic properties

Some emulsion-based products are semi-solids or solids. The rheology of these products is often characterized in terms of their *dynamic shear modulus* (G), which has as an elastic component G' (the storage modulus) and a viscous component G'' (the loss modulus; Genovese et al., 2007; Miri, 2011; Sandolo et al., 2010). Alternatively, the dynamic shear modulus can be reported as a *complex modulus* (G^*) and a *phase angle* (δ) (Miri, 2011; Rao, 2007b). Small-deformation measurements, like large deformation measurements, are typically not carried out under conditions that closely simulate conditions during mastication of foods within the mouth. Nonetheless, appropriate operating conditions should still be selected during measurements, for example, an oscillation frequency that corresponds to the shear rate experienced by foods in the mouth.

7.4.2.3 Tribology

Some alternative instrumental methods that more closely simulate conditions during mastication have been developed in recent years. During mastication, a thin film of food is formed between the tongue and palate, which influences the perceived frictional forces and lubrication in the mouth. It is therefore important to simulate these components using instrumental methods (Kokini and Cussler, 1983; Selway and Stokes, 2013; Stokes et al., 2013). Thin film rheology, also known as "tribology," has been increasingly used to simulate the oral behavior of food products and to correlate to sensory perception (Chen and Stokes, 2012; Dresselhuis et al., 2008; Prakash et al., 2013; Selway and Stokes, 2013; Stokes et al., 2013). In this method, an instrument known as a "tribometer" is used to form a thin film of sample and measure its thin film rheology. A simple tribometer consists of a ball and a disk between which a thin film of sample is placed. The instrument then measures the coefficient of friction of the thin film as the strain rate is varied by rotating the ball and disk at different speeds. Tribology measurements can provide information (friction, lubrication, and adhesion properties) about food products that cannot be deduced from conventional bulk rheology methods (large- or small-deformation measurements). Tribology measurements have also been related to in-mouth textural perception, for example, creaminess perception (Chen and Stokes, 2012; Dresselhuis et al., 2008; Stokes et al., 2013). More detailed information on the principles of tribology are available in the literature (Chen and Stokes, 2012; Prakash et al., 2013; Stokes et al., 2013).

7.4.2.4 Extensional/elongational flow rheology

Elongational flow has also been recognized as an important element during oral mastication of foods (De Bruijne et al., 1993). Food materials are squeezed between the tongue and palate as they move together during mastication, and strong elongational flow is created. Hence, in an ideal instrumental method, it is important to include both elongational and shear flow to more accurately simulate oral mastication and quantify the textural properties of foods (Le Reverend et al., 2010; Terpstra et al., 2007). Some

instrumental methods do include both types of flow, such as tribology (see earlier), squeezing flow rheology (Campanella and Peleg, 2002; Chatraei et al., 1981; Corradini and Peleg, 2005; Pelot et al., 2013), and "imperfect" squeezing flow rheology (Damrau and Peleg, 1997; Suwonsichon and Peleg, 1999; Terpstra et al., 2007). More detailed information of each of these methods can be found in the references cited earlier.

Recently, a combined squeezing flow and shear viscosity method has been developed in our laboratory to more accurately simulate oral mastication of semi-solid foods (Chung et al., 2012a, 2013d). The squeezing flow method compresses food samples between two parallel plates to induce biaxial elongational deformation (Campanella and Peleg, 2002; Chatraei et al., 1981; Corradini and Peleg, 2005; Engmann et al., 2005). The sample is compressed from an initial sample height to a final sample height by lowering the upper plate at a controlled rate. At the final height, the upper plate is halted to allow stress relaxation of the sample followed by a decompression step (Campanella and Peleg, 2002; Corradini and Peleg, 2005). The output of this test is in the form of force–height; force–time and/or height–time relationships, from which several rheological parameters are derived (Campanella and Peleg, 2002; Corradini and Peleg, 2005; Engmann et al., 2005). The *combined squeezing flow-shear viscosity* method utilizes two horizontal parallel plates of a rheometer with the sample placed between them. The sample undergoes a series of sequential compression-shear-decompression motions to simulate in-mouth mastication (Figure 7.7). The normal and shear forces are measured on the upper plate during measurements (instead of force–height, force–time relationships), which

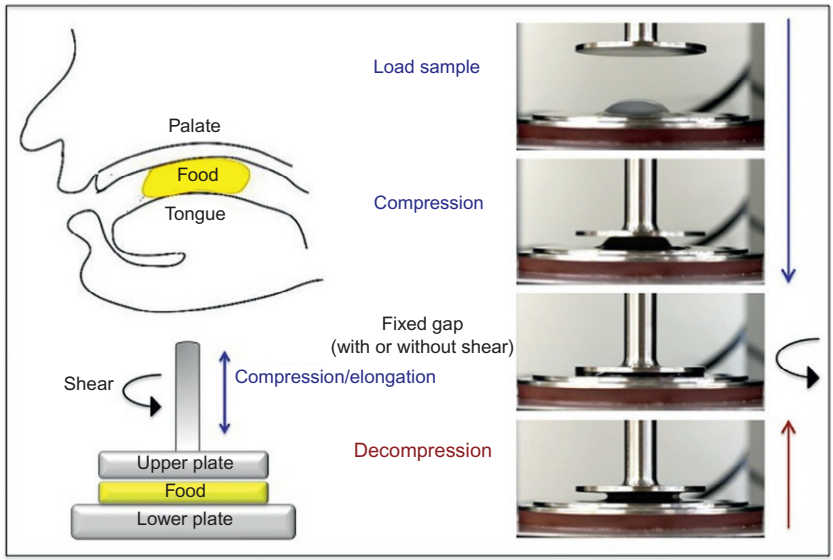

Figure 7.7 Schematic representations and photographic images of the combined squeezing flow-shear viscosity method designed to simulate the tongue and palate in the mouth during mastication.

provide information about the rheological properties of the sample, namely *maximum peak force* related to consistency; *maximum trough force* related to adhesiveness, *residual stress* related to yield stress, and *shear viscosity* (related to apparent viscosity; Chung et al., 2012a, 2013d). Saliva (artificial or fresh) can be added to the sample prior to starting the measurement to more closely simulate oral conditions (Bellamy et al., 2009; Chung et al., 2013d; Stokes et al., 2013). Good correlation between measurements obtained using this instrumental method and sensory evaluations performed on some model sauces has been reported (Chung et al., 2013d).

7.5 Summary

We have briefly discussed the need to understand the properties of emulsions and their influence on the textural properties of emulsion-based food products. This knowledge is indispensable in formulating and fabricating new healthy food products that possess good sensory attributes. The challenges in manufacturing healthy food products (low in fats and overall calories) that are palatable and appealing can be overcome by using structural design approaches. Some instrumental methods used to test and identify the key textural properties were also covered, as it is important to test the products during development stages, and sensory evaluation may not always be accessible.

7.6 Future trends

There has been a growing trend in developing healthier foods to try to combat diet-related chronic diseases, for example, overweight, obesity, diabetes, hypertension, and heart disease. The food industry has been actively producing and marketing reduced-fat and reduced-calorie emulsion-based products. Nevertheless, these products are often not accepted by consumers due to the loss of their desirable sensory attributes when fat droplets are removed. In view of this, it is important for the food industry to understand the fundamental principles that are involved in influencing the sensory properties of food emulsions, rather than manufacturing them entirely using a trial-and-error method. An improved understanding of this area would enable the food industry to rationally formulate and manufacture healthier foods with improved sensory attributes. Advances in the development of structured emulsions with novel properties may lead to the production of foods that are low in fat or carbohydrates without compromising their sensory properties. Moreover, the use of structural design principles may create new eating experiences, for example, with the incorporation of air bubbles into food products.

7.7 Sources of further information and advice

A vast literature is available for the food industry to understand the influence of microstructure and physicochemical properties of emulsions on the textural properties of food-emulsion products. Some of this literature has cited throughout the text. Some

recent articles and/or books have reviewed the relationship between emulsion structure and the physicochemical and sensory properties of food emulsions (Chung and McClements, 2013; Le Reverend et al., 2010, 2013; Norton et al., 2013; Stieger and van de Velde, 2013). It is also important to understand how foods are masticated within the mouth (oral processing) to better understand how taste and textures are perceived. There is a growing literature on oral processing and sensory perception, which will be useful to the food industry for formulating healthier food products (Foegeding et al., 2011; Foster et al., 2011; Koc et al., 2013; van Vliet et al., 2009).

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Controlled phase separation for texture modification

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8.1 Introduction

8.1.1 Challenge raised by mixing ingredients

Creation of manufactured food products with particular textures depends on the carefully designed mixing and interaction of many food ingredients. The purpose of combining the different food ingredients is to prepare food products with specific characteristics. But there is the complication that, after mixing food substances such as carbohydrates and proteins in aqueous media under certain condition of concentration, pH or ionic strength, the components redistribute themselves to reach eventually the most favourable thermodynamic state. Depending on the net charge, molecular weight, shape, size, conformation and flexibility of the biopolymer species present, and also on the ionic strength and pH of the medium, the interaction of food ingredients can be either associative or segregative. In associative redistribution the carbohydrate and protein components attract each other, whereas in segregative redistribution they tend to repel each other. In the latter situation, the redistribution is called phase separation, and its occurrence changes both the microstructure and macrostructure of the food matrix and also, potentially, its textural properties. By controlling the phase separation in the food system, it is in principle possible to determine the properties of the final product. Thus, understanding the phase behaviour of a mixture of the main functional ingredients in a food system is a guide to predicting the textural properties of the finished product.

8.1.2 Early sightings of phase separation in biopolymer solutions

Observation of the interactions between protein and carbohydrate, in particular gelatin and potato starch in aqueous media, goes back a century (Beijerinck, 1896). Beijerinck, in his study on the phase behaviour of mixed starch + gelatin solutions (Beijerinck, 1910), discovered that the mixing of 10% starch solution and 10% gelatin solution results in dispersed droplets of gelatin in a bulk aqueous phase of starch. He reported that heating and extensive mixing did not cause structural changes of this water-in-water emulsion. Then, it was shown that phase behaviours of starch and gelatin solutions are not the same for cereal starch and potato starch (Ostwald

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and Hertel, 1929b). Later it was found that the phase separation of macromolecules in aqueous solution is actually a common phenomenon (Dobry and Boyer-Kawenoki, 1947, 1948).

8.1.3 Modern studies of mixed biopolymers in solution

The systematic investigation of the thermodynamic incompatibility of protein + polysaccharide solutions had its origin in the interest of the scientists in the USSR Academy of Science (Russia) to produce artificial and formulated foods (Grinberg and Tolstoguzov, 1997). In recent years, the importance of phase separation phenomena in determining food shelf-life stability and the structuring of processed food has become increasingly well understood. The observed segregative phase separation is typically attributed to thermodynamic incompatibility between carbohydrate and protein in aqueous solution. The thermodynamic compatibility of different kinds of proteins and polysaccharides has been investigated extensively, even including a multinational project with financial support from the Commission of European Community entitled 'Mixed biopolymers–mechanism and application of phase separation' (EURTD program CT 961015). A large quantity of experimental information is now available on the compatibility of food biopolymers (Grinberg and Tolstoguzov, 1972; Antonov et al., 1975; Morris, 1990; Samant et al., 1993; Ledward, 1994; Dickinson and McClements, 1995; Polyakov et al., 1997; Tolstoguzov, 1998, 2002; Semenova and Dickinson, 2010).

The use of the gelatin as one of the key ingredients in food formulations has stimulated a vast amount of research on systems contain gelatin and different polysaccharides (Clark et al., 1983; Gotlieb et al., 1988; Kasapis et al., 1993a; Papageorgiou et al., 1994; Abdulmola et al., 1996; Foster et al., 1996; Michon et al., 1996; Al-Ruqaie et al., 1997; Antonov and Goncalves, 1999; Alves et al., 2000; Lau et al., 2000; Lorén and Hermansson, 2000; Butler and Heppenstall-Butler, 2001; Edelman et al., 2001; Gilsenan et al., 2003; Haug et al., 2004; Tromp et al., 2004; Nickerson et al., 2006; Wolterink et al., 2006; Harrington and Morris, 2009). It is now believed that, in general, thermodynamic incompatibility of proteins and polysaccharides in concentrated systems is the rule rather than the exception (Tolstoguzov, 1997) because over 100 different protein/polysaccharide/water systems have been shown to exhibit thermodynamic incompatibility (Grinberg and Tolstoguzov, 1997).

8.2 Thermodynamics of (bio)polymer solutions

Thermodynamics of polymer solutions can be used to address the thermodynamic incompatibility of protein + polysaccharide solutions (Huggins, 1941, 1942; Flory, 1942). It has been postulated that, in mixed polymer solutions, the entropy of mixing is low, and so the equilibrium state is mostly governed by enthalpic contributions from segment–segment interactions (Flory, 1953). But extension of the application of the

thermodynamics of polymer solutions to biopolymer mixtures is not straightforward because all proteins and many polysaccharides are polyelectrolytes. They have much more complex structures than synthetic polymers. In the aqueous state, change of solution properties like pH and ionic strength can have a pronounced effect on the phase behaviour of the solution. Nevertheless, the change of entropy and enthalpy on mixing in synthetic polymer solutions, which determines the change of Gibbs free energy, can be adopted to explain the qualitative features of segregative phase separation in biopolymer solutions.

8.2.1 Gibbs free energy

Within a system at constant pressure, physical stability is determined by the Gibbs free energy (G):

$$G = H - TS \tag{8.1}$$

where H is the enthalpy, a measure of the heat content of a system, T is the absolute temperature, and S is the entropy. In a system of fixed mass and composition, the most stable state (the state of equilibrium) has the lowest Gibbs free energy as a result of a compromise between low enthalpy and high entropy.

8.2.2 Gibbs free energy of mixing of liquids

In a mixture, by defining the pure unmixed substances as the reference state, the Gibbs free energy of mixing is denoted by:

$$\Delta G_{\mathrm{M}} = G_{\mathrm{mixture}} - G_{\mathrm{pure}} \tag{8.2}$$

In the same way the entropy of mixing ΔS_{M} and the enthalpy of mixing ΔH_{M} can be defined. ΔS_{M} is a measure of the change in degree of randomness (disorder) of the system on mixing. ΔH_{M} is the heat that is consumed ($\Delta H_{\mathrm{M}} > 0$) or released ($\Delta H_{\mathrm{M}} < 0$) as a result of mixing.

Thus the Gibbs free energy of mixing can be denoted as:

$$\Delta G_{\mathrm{M}} = \Delta H_{\mathrm{M}} - T\Delta S_{\mathrm{M}} \tag{8.3}$$

- If ΔG_{M} is highly positive, mixing is unfavourable and the components tend to exist as two unmixed liquids (phase separation).
- If ΔG_{M} is highly negative, mixing is favourable, and the components are integrated into one phase.
- If $\Delta G_{\mathrm{M}} \approx 0$, the components are partly miscible, depending on the composition.

8.2.3 Flory–Huggins theory

In the Flory–Huggins theory (Huggins, 1941; Flory, 1942) the polymer solution is imagined as a lattice whose cells are filled randomly by solvent molecules or polymer segments (Work et al., 2004). By assuming that the polymer segments and the solvent molecules are similar in size, the theory calculates the number of ways that polymer molecules can be placed on the lattice. The Flory–Huggins theory expresses the thermodynamic quantities of the solution in terms of the combinatorial entropy of mixing and the reduced Gibbs-energy parameter, the so-called χ interaction parameter.

The entropy of mixing consists of combinatorial and non-combinatorial contributions. The non-combinatorial term comes from the interaction of polymer with the solvent and is much harder to quantify. The combinatorial entropy is expressed as:

$$\Delta S_M = -R(n_1 \ln \Phi_1 + n_2 \ln \Phi_2) \tag{8.4}$$

where R is the universal gas constant, n_1 and Φ_1 are the number of moles and volume fraction of polymer, respectively, and n_2 and Φ_2 are the number of moles and volume fraction of solvent, respectively.

The enthalpy of mixing is related to a sum over the interaction energy between the individual segments. It is given by:

$$\Delta H_M = z\Delta\varepsilon N_1 \Phi_2 \tag{8.5}$$

where z is the lattice coordination number, $\Delta\varepsilon$ is the energy of contact formation between a polymer segment and solvent molecule, N_1 is the number of solvent molecules, and Φ_2 is the solvent volume fraction.

By adding together the combinatorial entropy of mixing and the enthalpy of mixing, the Gibbs free energy of mixing is expressed as

$$\Delta G_M = RT(N_1 \ln \Phi_1 N_2 \ln \Phi_2 + \chi N_1 \Phi_2) \tag{8.6}$$

where χ is the Flory–Huggins interaction parameter. This interaction parameter characterizes the molecular interaction energy between the components of a given system. In a polymer + solvent system it is characterized by

$$\chi = z\Delta\varepsilon/kT \tag{8.7}$$

where k is the Boltzmann constant.

8.2.3.1 Flory–Huggins parameters

In a system consisting of two biopolymers and a solvent, like gelatin (1) + starch (2) + water (0), three separate interaction parameters (three Flory–Huggins parameters) are involved, χ_{01}, χ_{02} and χ_{12}. The quantities χ_{01} and χ_{02} represent the enthalpic contributions to the free energy arising from the formation of solvent–protein and

solvent–polysaccharide interactions, respectively. The quantity χ_{12} represents the contribution from protein–polysaccharide interactions

$$\chi_{01} = (2\varepsilon_{01} - \varepsilon_{00} - \varepsilon_{11})z/kT \tag{8.8}$$

$$\chi_{02} = (2\varepsilon_{02} - \varepsilon_{00} - \varepsilon_{22})z/kT \tag{8.9}$$

$$\chi_{12} = (2\varepsilon_{12} - \varepsilon_{11} - \varepsilon_{22})z/2kT \tag{8.10}$$

The quantity ε_{ij} is the contact energy of the molecules i and j. It arises from the energy of dissociation and re-association of the bonds among the molecules. It could be among the same molecules, ε_{22}, ε_{11}, ε_{00}, or among different ones, ε_{21}, ε_{01}, ε_{02}. In biopolymer solutions due to the large size difference between the macromolecules and the solvent molecules, χ_{12} is the most significant factor controlling the stability of the ternary mixture with respect to phase separation (Magnin and Dumitriu, 2005); the parameter χ_{12} therefore sets the conditions defining the phase diagram in a biopolymer solution (Dickinson, 1994).

8.3 Susceptibility of biopolymer solutions to demixing

8.3.1 Low entropy of mixing

Each individual biopolymer component, that is, protein or polysaccharide, can usually dissolve into water on its own, but often the mixture of both components undergoes demixing. This is because the entropy of mixing of biopolymers is much lower compared to mixing of small molecules. Biopolymers have high molecular weights in comparison with small molecules, and in consequence they have much lower mole fractions in the solution, which favours miscibility with the solvent, but not with other polymers. Miscibility of the biopolymer solutions can normally be achieved when the heat of mixing is negative ($\Delta H_M < 0$). In molecular terms this can be accomplished by specific interaction between molecules, that is, hydrogen bonding. However, in most cases the attractive energy between pairs of the same monomer is stronger than for unlike pairs; this behaviour implies $\Delta H_M > 0$, and as the small entropy term ΔS_M is not sufficient to overcome the enthalpic contribution ΔH_M, a very small positive enthalpy change is enough to cause demixing.

8.3.2 Excluded volume effect

The excluded volume of a molecule is defined as the effective volume of the molecule that is inaccessible for other molecules in the system to occupy (Kuhn, 1934; Flory, 1949). The concept of excluded volume in biopolymer solutions causes work for biopolymer molecules influencing their space occupancy in the solution (Tolstoguzov, 2000). At a high concentration of protein + polysaccharide and a pH around the p*I* of the protein in a low ionic strength, if the unlike biopolymers do not have specific

affinity toward each other, the excluded volume of the biopolymers plays a crucial role; it determines the phase separation threshold of the system. On exceeding the phase separation threshold by addition of more biopolymer molecules into the solution, phase separation is initiated. This is because the increased number of overlapping excluded volumes, induced by the high concentration of biopolymers, restricts the space occupancy of the molecules in the system. Molecules try to maintain their excluded volumes, but it is more favourable for the system to separate the dissimilar species away from each other and to accumulate each similar species in its own phase (de Kruif and Tuinier, 2001).

8.3.3 Biological origin

Tolstoguzov (2006) postulated that the incompatibility of proteins and polysaccharides is a well-engineered tool, designed by nature to support and promote the evolution of life. In nature, at a very elementary stage of life, polysaccharides are considered to function as construction blocks of the cell wall, building up the immune and defence system of the living cell. As a cell comes in contact with unwanted foreign biopolymers, for instance, digestive enzymes (proteins) secreted by rival cells, the thermodynamic incompatibility of the cell wall (polysaccharide) would induce phase separation with the foreign macromolecule, and by this means the cell would be protected. Presumably this same concept could be applied to gelatin because gelatin is extracted from the protective and connective tissues. So, naturally, it has more affinity to bond to itself and remains as a bulk phase, rather than mixing with dissimilar macromolecules.

8.4 Effect of temperature change on biopolymer solutions

When the temperature of a biopolymer solution is lowered, the biopolymer molecules start to interact more with each other. If there is no affinity between dissimilar species, it would be more favourable for similar molecules to surround each other, and segregative phase separation occurs.

8.4.1 Phase separation

Change of the temperature or composition in a polymer solution induces a change in Gibbs free energy of mixing. If the Gibbs free energy of the biopolymer solution becomes higher than the sum of the Gibbs free energy of its separate constituents, the biopolymer solution transforms into a phase-separated structure.

8.4.2 Phase diagram: a map for phase separation

The phase diagram is a theoretical tool to illustrate the overall equilibrium behaviour of a system. It shows a graphic depiction of the thermodynamic state of a mixture of components as a function of relevant variable such as pressure, volume, system

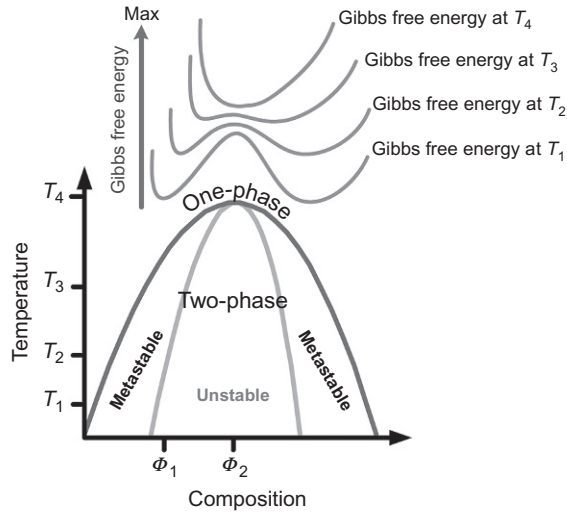

Figure 8.1 Schematic representation of a temperature–composition phase diagram of (bio) polymer solutions with UCST behaviour. Such a (bio)polymer solution undergoes phase separation upon cooling. By decreasing the temperature from T_4 to T_3, T_2 and T_1, the Gibbs free energy curve shows two minima. This indicates that the polymer solution transforms from one-phase to two-phase structure. Composition Φ_1 at temperature T_1 is unstable, at T_2 and T_3 is metastable and at T_4 is stable. Whereas, composition Φ_2 at temperature T_1, T_2 and T_3 is unstable but above T_4 is stable. Adaptation from Firoozmand (2009).

temperature and so on. A phase diagram is constructed based on the change of the Gibbs free energy of the system as a function of mole fraction or temperature.

Figure 8.1 shows the behaviour of a solution blend of two (bio)polymers + solvent. This system exhibits an upper critical solution temperature (UCST), which means that it separates in two phases on cooling. A mixture of starch + gelatin in water also shows an UCST. The Gibbs free energy curves in Figure 8.1 indicate the variation of Gibbs free energy of the system at different temperatures (T_1, T_2, T_3 and T_4) on cooling from high temperature (T_4) to low temperature (T_1). The system at T_4 is stable, and the Gibbs free energy curve shows one minimum, and this indicates that the system at T_4 remains as one phase. On cooling the system to T_3, T_2 and T_1 the Gibbs free energy curve shows two minima, which means that at T_3, T_2 and T_1 there are two compositions in the system whose Gibbs free energy is at a minimum. Therefore it is more favourable for the system to stay in the two-phase state rather than the one-phase state; hence the system phase separates.

8.4.3 Pathway of phase separation

When a solution of biopolymers is in the thermodynamic metastable or unstable states, it moves toward the condition of phase separation. Figure 8.2 shows a simplified cartoon as the first snapshot of thermodynamic events in metastable and unstable regions

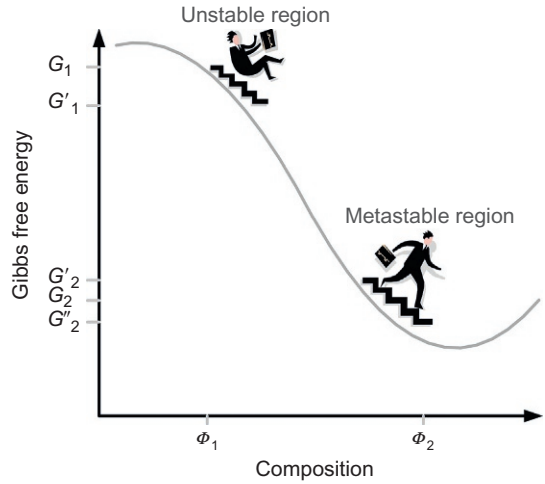

Figure 8.2 A cartoon depicting thermodynamic events during phase separation in metastable and unstable regions at constant temperature. In the unstable region very readily the Gibbs free energy decreases with the starting of phase separation, i.e., falling from the stairs (does not need to consume energy). In metastable region at the very first stage of phase separation, an energy barrier needs to be surpassed, i.e., climbing down the stairs (needs to consume energy) (Firoozmand, 2009).

in a phase diagram before the occurrence of the full phase separation at a constant temperature (above gelation).

This system (Figure 8.2) at composition Φ_1 has a high Gibbs free energy (G_1), and it is unstable, and hence with a small concentration fluctuation it readily phase separates because the Gibbs free energy decreases (G_1'). This phase separation mechanism is called spinodal decomposition. However, at composition Φ_2 a similar small concentration fluctuation leads to an increase of Gibbs free energy from G_2 to G'_2 and a decrease to G''_2. This means that an energy barrier exists that must be overcome before initiation of the phase separation. This energy barrier is associated with the energy cost for the creation of the interface of the newly emerged phase (Wagner et al., 2005). However, a larger concentration fluctuation in the metastable region also induces phase separation, but via nucleation and growth mechanism.

8.4.3.1 Spinodal curve and spinodal decomposition

The spinodal curve lies within the two-phase region (the binodal curve) in the phase diagram (Figure 8.1). It separates the metastable region from the unstable region. It defines the region of composition and temperature where the single-phase mixture is unstable and spontaneously decomposes in two coexisting phases without the presence of an activation energy. Spinodal decomposition has the appearance under the microscope of interconnected and bicontinuous domains as shown in Figure 8.3. One of the distinct morphological characteristics of spinodal decomposition is the particular spacing length scale in the structure, so that the phase-separated domains are roughly evenly spaced, and they have a similar size. In spinodal decomposition, the phase volume does not change with time since its equilibrium is established at early stages, but the concentration of biopolymer in each of the phases does evolve with time (Lundin et al., 2003).

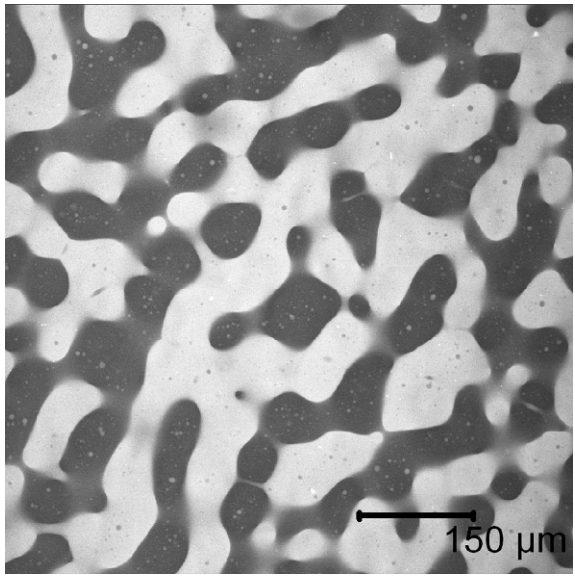

Figure 8.3 Spinodal decomposition in a sample containing 8 wt% starch + 9 wt % gelatin, the bright regions are gelatin-rich whereas the dark regions are starch-rich (Firoozmand, 2009).

8.4.3.2 Binodal curve and nucleation and growth

The binodal curve in the temperature–composition phase diagram (Figure 8.1) separates the one-phase region from the two-phase region. It defines the region of composition and temperature where phase separation is thermodynamically preferred. The mixed (bio)polymer solution may initially exist as an unstable state or a single-phase metastable state. In this latter case phase separation requires the overcoming of an activation energy, and the phase separation mechanism occurs in form of nucleation and growth. Phase separation through the nucleation and growth mechanism results in the formation of spherical domains of one of the phases that grows with time and is surrounded by the other phase in the matrix, as shown in Figure 8.4. The concentration of the dispersed phase does not change with time because the equilibrium concentrations of the two phases are rapidly established, but the phase volumes of the two phases change with time.

8.4.4 Critical point in a phase diagram

The critical point in the phase diagram is the point where the binodal and spinodal curves touch each other. At the critical point the phase stability changes from stable to unstable without passing through a metastable region.

8.4.5 Rectangular coordinate phase diagram

It is often more convenient to use an alternative representation of the phase diagram that is in the form of rectangular coordinates expressing the composition of each polymer component in term of the weight percentage.

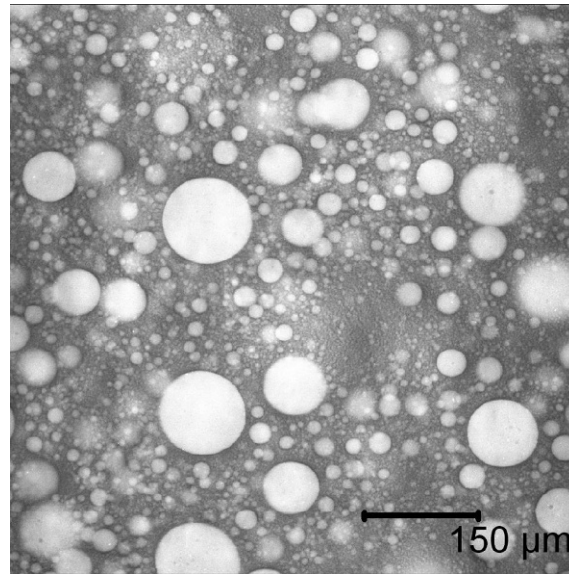

Figure 8.4 Nucleation and growth in a sample containing 8 wt% starch + 9 wt% gelatin, the bright regions are gelatin-rich whereas the dark regions are starch-rich (Firoozmand, 2009).

Figure 8.5 shows a typical representation of a phase diagram for a ternary system such as a mixture of water + protein + polysaccharide that exhibits thermodynamic incompatibility. The binodal curve separates the single-phase region from the two-phase region. The area lying under the binodal curve corresponds to the one-phase region, whereas the area above the bimodal curve represents the two-phase region. A mixture of polysaccharide solution + protein solution with volume proportion AC_1/C_1B_1 whose overall concentration is below binodal curve, remains stable, whereas a mixture of polysaccharide solution + protein solutions with concentration of A and B_2 with volume proportion AC_2/C_2B_2 resulting in an overall concentration above the binodal curve breaks down spontaneously into two phases, E_1 and D_1; likewise the same solutions but with volume proportion AC_3/C_3B_2 produce the two phases E_2 and D_2. These two phases are named the co-existing phases and also mark binodal

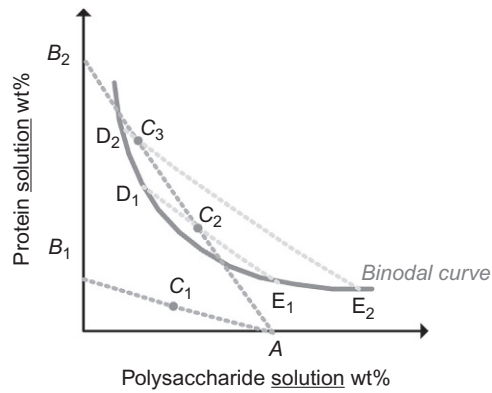

Figure 8.5 Schematic representation of a typical phase diagram of protein + polysaccharide + water. The composition C_1 which is located in single-phase region remains stable. But the composition C_2 and C_3 (above binodal curve) breaks down into two phases $E_1 + D_1$ and $E_2 + D_2$, respectively. The D_1E_1 and D_2E_2 lines are called tie-lines with modification (Firoozmand, 2009).

points. The connecting line of a pair of binodal points is called the tie-line (D_1E_1 and D_2E_2). When the location of the compositions C_2 and C_3 are changed along their tie-line D_1E_1 and D_2E_2, the volume ratio of the co-existing phases D_1 and E_1 and two other co-existing phases D_2 and E_2 are changed, but their compositions remain constant.

8.5 Effect of pH change on biopolymer solutions

8.5.1 pH and biopolymer compatibility

The solubility of biopolymers in water in general is influenced by the pH of the solution. Ostwald and Hertel (1929a) noted that solutions of gelatin + potato starch became a single phase in acid and alkaline pH conditions. It has been found that the miscibility of dextran + gelatin in water is reduced at the p*I* of gelatin, whereas lowering the pH to 0.9 units below the p*I* improved the solubility of both biopolymers in water (Grinberg et al., 1970). In a related study (Grinberg and Tolstoguzov, 1972), it was established that a phase-separated system containing gelatin, and one of the D-glucan biopolymers (amylopectin, glycogen or dextran) could undergo a reversible phase transition from a two-phase to one-phase state by shifting the pH toward acidic or alkaline region in relation to the p*I* of the gelatin. Similar behaviour has been reported in phase-separated compositions of gelatin solutions and glucose syrup on decreasing the pH from 8 and 5.6 to 2 (Vinches et al., 1997). These studies all indicate that the thermodynamic state of the system is very sensitive to pH. That is, a thermodynamically incompatible solution of gelatin + starch, for example, could become thermodynamically compatible by lowering the pH below p*I* of the gelatin, transforming the system from a two-phase state to a one-phase state.

Although lowering the pH may transform the system from a multiple-phase state to a one-phase state, the extent of the correlation between the pH and thermodynamic incompatibility of different types of gelatin is potentially variable. Obviously, incorporating different concentrations of $[H^+]$ into the mix will also influence the gelation behaviour of the gelatin and therefore the rheological behaviour of the mix.

8.5.2 Mechanism

Changing the pH alters the electrical charge on the protein molecules. At a pH below its p*I*, gelatin becomes positively charged, and the magnitude of the positive charges on the molecule increases as the pH is lowered further away from it p*I*. The increased charge density on the gelatin promotes thermodynamic compatibility between gelatin and dextran (Grinberg et al., 1970). The excess charge on gelatin has been shown to prevent phase separation between gelatin and a number of the D-glucans, for example, amylopectin or glycogen by restraining the self-association of the gelatin (Grinberg and Tolstoguzov, 1972) through increased electrostatic repulsion between gelatin molecules. It is known that phase separation is triggered by gelatin self-association in mixed aqueous solutions with polysaccharides such as starch (Khomutov et al., 1995), dextran

(Tromp and Jones, 1996; Anderson and Jones, 2001; Edelman et al., 2001; Butler and Heppenstall-Butler, 2003b; de Hoog and Tromp, 2003) and maltodextrin (Lorén and Hermansson, 2000; Lorén et al., 2001; Williams et al., 2001; Leisner et al., 2002; Butler and Heppenstall-Butler, 2003b). It has also been shown that compositions of glucose syrup + gelatin solutions that are phase-separated at pH 5.6 and pH 8 become compatible at pH 2 (Vinches et al., 1997). Increasing the positive charges on gelatin by decreasing the pH below p*I* also has been shown to inhibit the phase separation in a mixture containing 5 wt% gelatin + 17.5 wt% modified (oxidized) waxy maize starch (Whitehouse et al., 1996).

When gelatin is charged, segregative phase separation is not thermodynamically favourable at low to moderate ionic strengths because phase separation confines the gelatin (polyelectrolyte) and its associated counter ions into a smaller volume (phase). This reduced space represents an improbable entropic state and is therefore an unfavourable electrostatic condition (Piculell and Lindman, 1992; Vinches et al., 1997).

In the case of the negatively charged starch, such as oxidized potato starch, both gelatin and modified starch (potato oxidized starch) have a net repulsive interaction at the natural pH of their mixed aqueous solutions due to the dominant thermodynamic incompatibility. Lowering pH encourages the attraction of the negatively charged starch molecule toward the positively charged gelatin leading to association of the two oppositely charged biopolymers, which modifies the thermodynamic state of the system (Imeson et al., 1977; Stainsby, 1980; Chilvers and Morris, 1987; Antonov et al., 1996; Gilsenan et al., 2003). The complex formed between starch and gelatin apparently remains soluble and does not precipitate. Gilsenan et al. (2003) have reported the same behaviour in a mixture of negatively charged pectin and positively charged gelatin. They ascribed the solubility of the gelatin–pectin complex to the surplus charges on the complexes formed.

In a mixture of gelatin and starch, on changing the pH, the maximum rigidity of the gel is achieved when the gelatin molecules have a specific charge density at which a certain amount of oppositely starch molecules can attach to gelatin via electrostatic attraction, but the gelatin molecules can still connect to each other and form cross-links, that is, form a gel. Increasing the charge density on gelatin by decreasing the pH causes more repulsive interaction between gelatin molecules; it also attracts more starch molecules to the gelatin, so creating thicker steric barriers around the gelatin. If the gelatin molecules repel each other too strongly and are surrounded by starch, they may fail to achieve cross-linking via the triple helix conformation. Hence, the gel will have a lower modulus. On the other hand, at low pH positively charged gelatin molecules repel each other. Therefore they spread out more uniformly throughout in the system, and the tendency toward self-aggregation and phase separation is hindered. Adjusting the pH in a gel containing gelatin/starch/water may be used as a tool to modulate rheology.

It has been shown that the extent of cross-linking in a mixed gel depends on the balance between positive and negative charges in the entire system (Firoozmand et al., 2012). For example, when the system contains a higher concentration of starch, a higher positive charge on the gelatin is required to reach maximum gel rigidity.

However, lowering the pH reduces the gel modulus as accumulated positive charges on the gelatin molecules increase their mutual repulsion and increased attraction with negatively charged starch molecules—this obstructs gelatin cross-linking, with the starch acting as a steric barrier around the gelatin molecules.

8.6 Effect of addition of particles on biopolymer phase separation and rheology

8.6.1 Particles as interface stabilizer

Recently it has been shown that the addition of colloidal particles and micron-sized particles may alter phase separation in thermodynamically incompatible solutions (Poortinga, 2008; Firoozmand et al., 2009a; Nguyen et al., 2013). If the added particles have a similar wetting affinity for both phases (e.g., in a gelatin–starch liquid mixture with water as the solvent), the resulting self-assembly and formation of a viscoelastic layer at the liquid–liquid interface may arrest demixing arising from either spinodal decomposition or nucleation and growth (Clegg et al., 2005; Araki and Tanaka, 2006; Clegg, 2008; Kim et al., 2008). This class of amorphous soft-solid materials termed bijels consists of phase-separated systems in a frozen state (Stratford et al., 2005; Herzig et al., 2007). However the question remains: What type of food-grade particles might be used in biopolymer mixtures (Hanazawa and Murray, 2013)? There currently exist a limited number of naturally occurring materials such as fat crystals (Poortinga, 2008), β-lactoglobulin (Nguyen et al., 2013), quinoa starch (Rayner et al., 2012) and plant spores (Binks et al., 2005) that have been investigated as micron-sized particles to stabilize the liquid–liquid interface in mixed biopolymer solutions. These groups of particles suffer from key limitations. Some of these, such as fat particles, are temperature sensitive and will melt at typical processing temperatures. Others, like starch granules, lose their functionality and might not be able to adsorb to the interface. β-Lactoglobulin, zein protein and soy protein nanoparticles can only be made on a lab scale, whereas hydrophobic cellulose particles have a relatively broad particle size distribution, which makes them less efficient as interface stabilizers.

8.6.2 Using nonviable edible single-celled organisms as particles

Recently, we have shown that nonviable edible single-celled organisms from three groups (yeast, bacteria and algae) could be used as micron-sized particles in gels. The addition of these organisms to phase-separated solutions of gelatin + maltodextrin altered the developing microstructure and rheological properties of the gels (Firoozmand and Rousseau, 2014). Figures 8.7–8.10 show the microstructure of 6 wt% gelatin + 6 wt% maltodextrin solutions containing 2 wt% nonviable edible single-celled organisms. The microstructure of the gelatin–maltodextrin control without cells (Figure 8.6) resembled that of a typical water-in-water emulsion, with the gelatin-rich phase forming a continuous phase containing the droplet-like

maltodextrin-rich phase. Figure 8.7 shows 6 wt% gelatin + 6 wt% maltodextrin containing 2 wt% *Lactobacillus bulgaricus*. The microstructure exhibited a coarsened phase-separated morphology, with the maltodextrin-rich phase appearing as void regions and gelatin-rich phase containing the microbial cells. The microstructure showed signs of secondary phase separation. When *Chlorella* (Figure 8.8) and *Spirulina* (Figure 8.9) were added to the gel (at 2 wt%), phase separation was

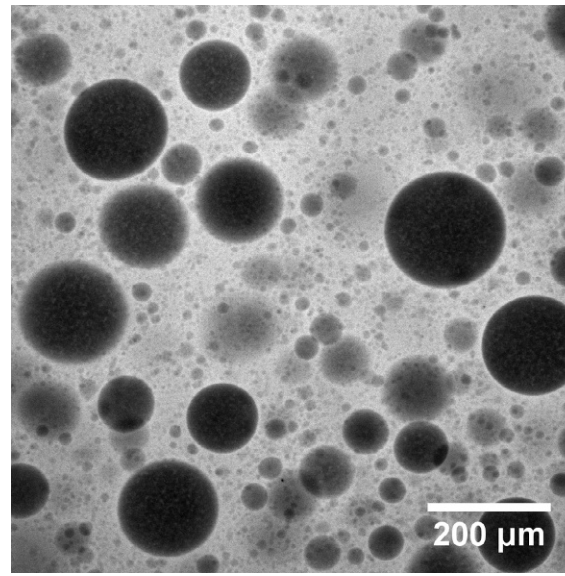

Figure 8.6 Confocal laser scanning microscopy (CLSM) of gels made of 6 wt% gelatin and 6 wt% maltodextrin, the bright regions are gelatin-rich whereas the dark regions are maltodextrin-rich.

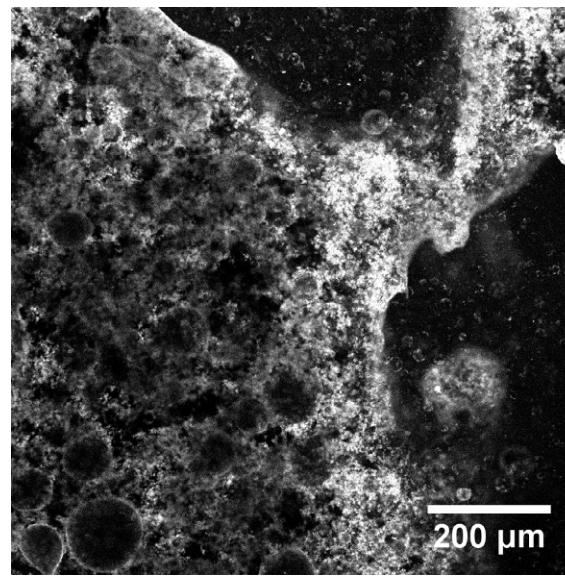

Figure 8.7 CLSM of gels made of 6 wt% gelatin and 6 wt% maltodextrin containing 2 wt% *Lactobacillus bulgaricus*, the bright regions are gelatin-rich associated with cells whereas the dark regions are maltodextrin-rich.

accelerated, and the gelatin-rich and maltodextrin-rich demonstrated bulk phase separation. In both cases, the algae remained associated with the gelatin-rich phase. Addition of 2 wt% yeast changed the microstructure substantially compared to all samples (Figure 8.10) as a primarily bicontinuous phase-separated gel morphology was obtained, one where the cells resided in the gelatin phase and at the interface. Figure 8.11 shows strain sweep tests at constant frequency (1 Hz) and temperature

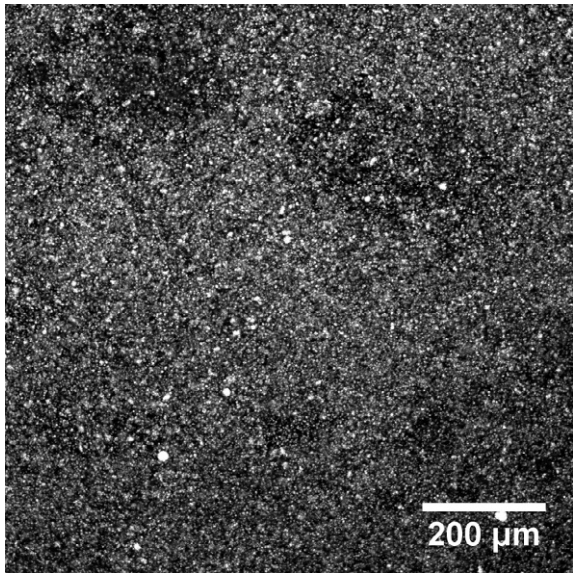

Figure 8.8 CLSM of gels made of 6 wt% gelatin and 6 wt% maltodextrin containing 2 wt% *Chlorella*, the bright regions are gelatin-rich associated with cells whereas the dark regions are maltodextrin-rich.

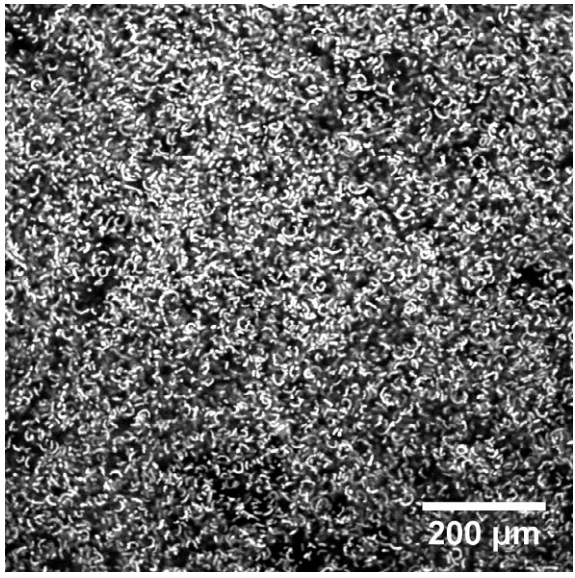

Figure 8.9 CLSM of gels made of 6 wt% gelatin and 6 wt% maltodextrin containing 2 wt% *Spirulina*, the bright regions are gelatin-rich associated with cells whereas the dark regions are maltodextrin-rich.

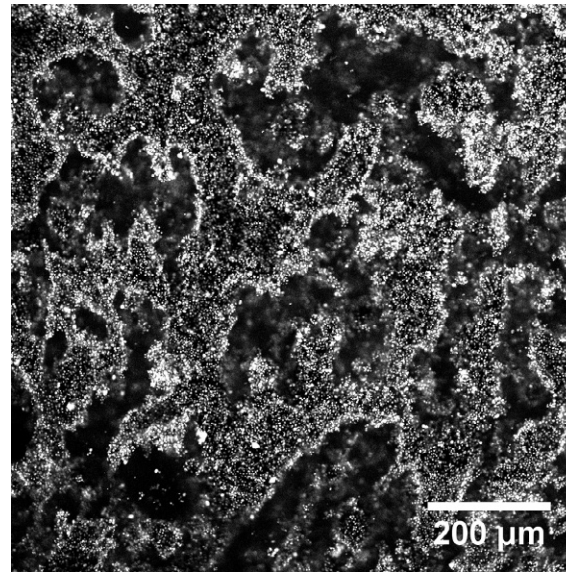

Figure 8.10 CLSM of gels made of 6 wt% gelatin and 6 wt% maltodextrin containing 2 wt% yeast (*Saccharomyces cerevisiae*), the bright regions are gelatin-rich associated with cells whereas the dark regions are maltodextrin-rich.

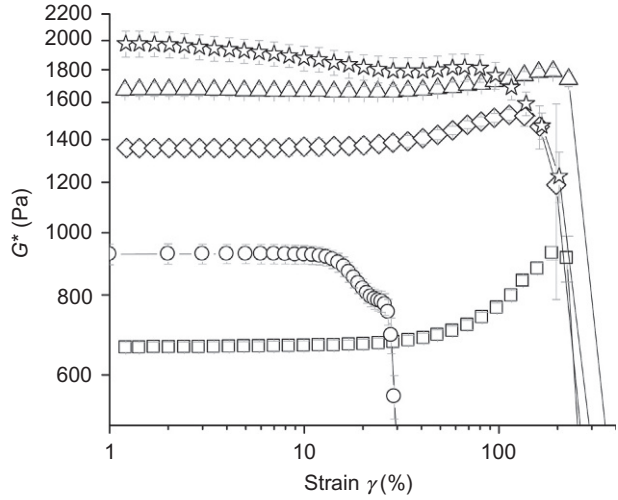

Figure 8.11 Strain-dependence complex modulus (G^*) of gel made with 6 wt% gelatin and 6 wt% maltodextrin subjected to $T_h = 40$ °C and $t_h = 10$ min after 1 h ageing at 25 °C: without added cell □; with 2 wt% *L. bulgaricus* ○; with 2 wt% *Chlorella* ◇; with 2 wt% yeast △; with 2 wt% *Spirulina* ☆.

(25 °C) corresponding to each of the cell-containing compositions mentioned earlier. In this test, the changes in flow behaviour reported as complex shear modulus (G^*) under increased oscillatory strain were recorded. Irrespective of cell type, their presence at 2 wt% in the gelatin + maltodextrin gel increased gel G^* in the following order: *L. bulgaricus* < *Chlorella* < *Saccharomyces cerevisiae* < *Spirulina*. The presence of

cells in the gelatin + maltodextrin gels substantially affected fracture and flow behaviour. The control sample without cells showed strain-hardening, as the value of G^* increased with strain—this clearly implied that the gel became resistant against deformation prior to yielding. At a strain of ~1.8 (180%), it fractured, and G^* fell abruptly. In the presence of 2 wt% *L. bulgaricus*, strain-hardening completely disappeared as the gel lost its elasticity and fractured at a strain of ~0.12 (12%), implying that the gel became very brittle. With *Chlorella* (2 wt%), the gel showed slight strain-hardening and fractured at the strain of ~1.3 (130%). With addition of 2 wt% yeast, the gel became highly strain resistant without any significant increase of G^*. It fractured at a strain 1.9 (190%), meaning that the gel became stiffer with less of an elastic characteristic. Finally with 2 wt% *Spirulina*, the gel became firmer than the other gels, but interestingly it showed slight strain-softening as G^* decreased under increased strain and fractured at a strain of ~0.8 (80%). The various cells electrostatically interacted with the gelatin polypeptide chains and acted as an active filler in the embedded gel, thereby reinforcing the gel matrix. These examples demonstrate the application of single-celled microorganisms as micron-sized particles in thermodynamically incompatible biopolymer solutions to generate varied microstructures with diverse rheological properties. Via addition of different combinations of single-celled microorganisms to phase-separated gels, a variety of gels with a distinct morphology and rheological properties may be obtained.

8.7 Factors influencing the physical properties of the phase-separated gels

8.7.1 Thermodynamic incompatibility

The physical properties of a food gel containing a mixture of two biopolymer components are influenced by various interrelated factors, such as the gelation properties of the individual biopolymers, the interactions between the biopolymers and the processing conditions used to prepare the system from the basic ingredients (Kasapis, 2008). In the absence of any specific associative interactions between the biopolymers, the phenomenon of thermodynamic incompatibility commonly leads to phase separation of a mixed biopolymer solution into two coexisting phases, each rich in one of the biopolymer components (Dickinson and McClements, 1995; Tolstoguzov, 1998).

In the case of gelatin-containing systems, thermodynamic incompatibility has been found to produce an increase in the rate of cross-linking and gelation (Marrs, 1982; Braudo et al., 1986). Concerning the effect of incompatibility on gel rheology, the influence of low-viscosity oxidized starch on the elastic modulus of pectin gels has been investigated (Abdulmola et al., 2000; Evageliou et al., 2000). Thermodynamic incompatibility induces the concentration of like biopolymer molecules into certain domains within the microstructure. This mutual concentration of the macromolecules would be expected to enhance intermolecular cross-linking, with further

consequences for the local microrheology of the domains and possibly the overall macroscopic rheology of the system. As the local concentration of the gelling biopolymer is higher than its average concentration across the entire system, phase separation due to incompatibility might normally be expected to cause an increase in gel strength (Clark et al., 1983).

But it has been demonstrated experimentally that this is not always the case (Braudo et al., 1986). In particular, for instance, the incorporation of oxidized starch in cold-set calcium pectinate gels has been found to cause a large reduction in the elastic modulus (Abdulmola et al., 2000) and also the rheological properties of various phase-separated gelatin-containing mixtures (with polysaccharides) that have been investigated (Kasapis et al., 1993b; Alves et al., 2000; Gilsenan et al., 2003; Ding et al., 2005).

8.7.2 Interaction of components

It is known that the presence of small-molecule sugars can affect the rate of gelatin network formation (Marrs, 1982; Nishinari et al., 1992) and can enhance the stability of gelatin gels against liquefaction (Naftalin and Symons, 1974). Even higher sugar content has been found to raise the melting point of the gelatin gel by increasing the order–disorder transition temperature of the gel (Mitchell, 2000) and also by reinforcing the chain association of the gelatin molecules (Al-Marhoobi and Kasapis, 2005) during ageing (Oakenfull and Scott, 1986; Choi et al., 2004). But beyond a certain concentration of sugar, the gelatin gel weakens again due to lack of water for hydration to sustain gel integrity (Marrs, 1982; Al-Ruqaie et al., 1997; Kasapis et al., 2003).

Gelation of gelatin is triggered by quenching the solution of gelatin below the gelatin coil–helix transition temperature (~30 °C). It is shown that the modulus of the gel containing gelatin and waxy maize starch rises mainly from the increase of gelatin concentration (Abdulmola et al., 1996). Even more, it has been shown that soluble polysaccharides do not contribute to gelatin gel modulus (Harrington and Morris, 2009). When other ingredients are present in a system, the overall rheology is undoubtedly affected indirectly by the combined interactions of the gelatin with the other components in the system.

8.7.3 Microstructure

The most obvious non-rheological manifestation of these molecular interactions is the partially phase-separated character of the gel state. Due to the gelation of one or more of the components, as induced, for instance, by lowering the temperature, this thermodynamically driven demixing may be retarded and kinetically trapped. The resulting event leads to a microstructure that is determined by the balance between gelation and phase separation (Bansil, 1993; Tromp et al., 1995). Such heterogeneous structures can be conveniently observed under the confocal microscope (Tromp et al., 2001).

Phase-separated microstructures induced by spinodal decomposition or nucleation and growth have previously been investigated in detail for systems containing gelatin + starch (Khomutov et al., 1995), gelatin + dextran (Tromp et al., 1995; Butler and

Heppenstall-Butler, 2003a) and gelatin + maltodextrin (Lorén and Hermansson, 2000; Williams et al., 2001; Butler and Heppenstall-Butler, 2003a,b). These studies have given some insight into how the thermal processing conditions and the system composition control the relative kinetics of gelation and phase separation and, consequently, the developing microstructure.

Of particular interest in the formulation of novel textures based on mixed biopolymer ingredients is the extent to which the mechanical properties of the material can be correlated with the microstructure. In addition, various attempts have been made to try to predict the rheology of these mixed biopolymer gel systems using simple physicochemical theories based on models of filled gels or synthetic polymer blending laws (Dickinson and McClements, 1995; Kasapis, 2008). However, these theories have been largely unsuccessful in a quantitative sense, presumably because they fail to take proper account of the complexity of the microstructure and the associated local compositional variations such as redistribution of solvent between the phases, conformational changes due to gelation, different rates of gelation of the biopolymers and the temperature history of the system.

For example, in a phase-separated gelatin–starch gel, the modulus is ultimately determined by the spatially averaged density of cross-linking of the gelatin, which is influenced by the concentration, distribution and domain interconnectivity of the gelatin component within the system. In turn, these factors are related to the detailed characteristics of polymer network formation and the intermolecular annealing processes taking place during the thermal processing. At the same time, the increased local mobility during thermal processing facilitates phase separation into gelatin-rich and starch-rich domains of increasing size, driven by the intrinsic thermodynamic incompatibility of the two biopolymers. The combination of these factors can result in the formation of similar microstructures having different rheological properties or formation of different microstructure having similar rheological properties (Firoozmand et al., 2009b). It can be concluded that the rheology of gelatin–starch–water model systems has no straightforward relationship with the microstructure. One might therefore reasonably conclude that structural features on a scale well below what is accessible to light or confocal microscopes are crucially important in determining the rheological behaviour of mixed biopolymer systems.

8.8 Conclusions

Phase separation is driven by the intrinsic thermodynamic incompatibility of two (or more) mixed biopolymers resulting in a complex microstructure. Different factors, including temperature history, the extent of biopolymer cross-linking, distribution and domain interconnectivity of the biopolymers all influence and control gel rheology.

Phase separation is hindered in a solution containing gelatin/starch by lowering the pH below that of the p*I* of gelatin. By adjusting the pH, the gel formed may have variable rheology. The rigidity of the gels depends on the balance between positive and

negative charges on the biopolymers in the system. When systems contain a higher concentration of starch, a higher positive charge on the gelatin is required to obtain the maximum rigidity. But after reaching the maximum rigidity, the rigidity of the gel formed is reduced by lowering the pH and increasing the positive charge on the gelatin. Because the excess positive charges on gelatin inhibits self-association, the gelatin is less likely to gel.

The addition of single-celled microorganisms as micron-sized colloidal particles may influence the rate of phase separation in a solution of maltodextrin + gelatin. This effect is very sensitive to the nature of the single-celled microorganisms, which exhibit a strong tendency to accumulate at the liquid–liquid interface in the phase-separated microstructure of water-in-water emulsions. The driving force for this phenomenon is most likely the surface tension that exists at the liquid–liquid interface of thermodynamically incompatible biopolymers.

Along with other food-grade (or synthetic) particles, future research efforts should explore the mechanisms of action of such cells to establish a compendium of possible morphologies and rheological properties that may be generated. We are of the opinion that particle (and cell)-stabilized phase-separated gels represent an exciting avenue for the generation of food-grade gels with highly user-defined properties. Such particle-stabilized structures may find practical applications in the fabrication of micro-scale mixed biopolymer systems for use in encapsulation technologies as well as other avenues both in food and non-food applications.

8.9 Sources of further information and advice

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The effect of filler particles on the texture of food gels

9

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9.1 Introduction

Gels play an essential role in a wide range of food products; from jelly desserts and confections to omelettes and tofu, gels are a ubiquitous part of the culinary world. A wide range of gelling agents are available to choose from, in food gels usually proteins or polysaccharides, and this choice can strongly affect the preparation and properties of the gelled product.

Filler particles introduced (either intentionally or unintentionally) into the gel can also strongly affect the gel properties, and the purpose of this chapter is to give an overview of these effects. Depending on the type of filler particles and the interactions between the filler particles and the gel network, properties like gel storage modulus, gelling kinetics and fracture stress can all be desirably modified, and possibly improved.

The first part of this chapter describes some of the most common gelling agents used in food gels and some of the structural and functional/mechanical differences between them. Then the effect of introducing filler particles into gels is examined. The potentially large influence of particle–gel network interactions, or the lack of such interactions, on the texture and properties of filled gels (known as active/inactive filler effects) is an important part of this section. Finally, the last part of the chapter is dedicated to an in-depth look at gelatin-based emulsion gels. Through practical and experimental examples, the previously discussed filler effects and related phenomena are shown and discussed.

9.2 Food gels—types of biopolymers

In the preparation of processed food, gelation/solidification is often used as a means to impart unique sensory attributes and/or improve stability of the raw material. Two main groups of biopolymers are used for structure-forming of such gel systems (Harris, 1990):

- Polysaccharides—usually form transparent fine-stranded gels at low concentrations of polymer. Depending on the polysaccharide used, gels can be formed by heating/cooling, pH adjustments or addition of ions
- Proteins—generally form particulate opaque gels at higher concentrations (5–10%), usually through heating (denaturation), pH alteration or enzymatic interactions

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However, there are exceptions to this classification. Some native starches (e.g., potato starch) and inulin are examples of polysaccharides that can form turbid particulate gels on heating and subsequent cooling at concentrations of 10–15% and above, and proteins can, at certain pH values with sufficient heating, form fine-stranded gels made up of linear chains of interlinked proteins (Damodaran et al., 2008; Meyer, 2009).

In particulate gels, the gelling agent is organised into a three-dimensional network of particles interacting with each other. Each particle is generally an aggregate of smaller molecules, with surface properties enabling interaction with adjacent aggregates. The size of the individual particles is usually large enough to scatter light, turning this type of gel opaque. Due to the large particle size, these gels could also contain large gaps or pores between the particles, causing particulate gels to have a poor water-holding capacity. As mentioned, this type of gel is usually formed by proteins on heating, and common examples are food products based on eggs or soy protein (Mcclements, 2005).

In fine-stranded gels, the gel network is formed through interactions between thin filaments of biopolymer molecules instead of particles. Due to the thin strands, these gels are usually transparent, and the dense network of thin strands gives small pores, leading to a good capacity to hold water. Common examples of gelling agents used in foods giving fine-stranded gels are gelatin and most polysaccharides such as agar or ionically cross-linked alginate (Mcclements, 2005).

The process of gelation of a biopolymer solution is usually accomplished by thermal, acid-induced, salt-induced or enzymatic treatment, and some common biopolymers and their respective gelation mechanisms are listed in Table 9.1.

9.3 Mechanical properties of food gels

The rheological properties of food gels are of great importance, as the functionality of a material is strongly connected to its mechanical and viscous behaviour. This includes the behaviour of the food during processing (cutting, slicing, handling, etc.) and the sensory and textural properties during mastication (Vanvliet and Walstra, 1995). The mechanical properties of food gels can be determined by small and/or large strain deformation studies. This involves deforming a sample and measuring the corresponding response.

For an ideal solid material (Hookean solid), strain and stress are known to be proportional. However, this linear relationship is usually only valid at small strains for ‘true gels’, and the proportionality constant (known as modulus) is hence usually determined by performing small strain deformation studies. The strain can be applied as, for example, shear or compression, in which the shear storage modulus or Young’s modulus can be determined, respectively. Although small strain measurements can provide vital information about the physical properties of biopolymer gels, it has a limited ability to describe functional properties of food gels occurring at larger strains, including the behaviour of the material during chewing. Hence, to obtain an overall impression of the rheological properties of food gels, both small and large strain deformation studies should be performed.

Table 9.1 Examples of biopolymers used in food gels; main source(s) and main mechanisms of gelation (Phillips and Williams, 2009)

Biopolymer	Main source	Gelling mechanism
Polysaccharides		
Agar	Red seaweeds	Cold-set/thermoreversible
Alginate	Brown seaweeds	Multivalent ions (e.g., Ca^{2+}) or acid induced
Carrageenans	Red seaweeds	Cold-set/thermoreversible, gels with ions
Native starches	Cereal grains, tubers	Cold-set after swelling
Modified starches	Cereal grains, tubers	Variable, depending on modifications
Pectin	Citrus, apple, other fruits	Variable, depending on methoxyl content
Proteins		
Gelatin	Hide, skin and bones	Cold-set/thermoreversible
Caseins	Milk	Aggregation
Whey proteins	Milk	Heat-set/denaturation
Ovalbumin	Egg	Heat-set/denaturation
Vegetable protein isolate	Wheat, soybean, maize, barley, pea, etc.	Heat-set/denaturation

Biopolymer-based food gels vary widely with respect to mechanical properties, product performance and applications. As mentioned in the previous section, polysaccharides and proteins are by far the most common biopolymers used to induce gelation. These occur in food gels both as single agents, in protein/protein and polysaccharide/polysaccharide combinations and in polysaccharide/protein mixtures. Polysaccharide gels are usually characterised as being relatively firm with a short texture. This manifests itself with a high elastic modulus and a low strain at failure. These are typical properties of gels made from agar/agarose, alginates, κ-carrageenan, low acyl gellan and low methoxy pectin. Such firm and brittle texture makes, for example, alginates a very good candidate for the restructuring of fruits and vegetables where this also would be the texture of the original foodstuff. Exceptions do, of course, exist, where perhaps the most well-known are gels made from xanthan/LBG and high acyl gellan, which both exhibit soft and flexible texture (Sworn, 2009; Huang et al., 2004).

Mechanical properties of protein gels, on the other hand, show a greater variety compared to that of polysaccharide gels. Because proteins are ampholytes (carrying both negative and positive electrostatic charges at the same time), the strength of a formed gel following the denaturation of, for example, a globular protein will depend on both the pH and the ionic strength. For example, gelling close to the isoelectric point and/or high ionic strength where the overall electrostatic repulsion is at its lowest

often leads to a particulate and coarse network with a high level of plasticity. Such a microstructure gives an easy release of water instead of fail at high deformation. Under conditions of high electrostatic repulsion, on the other hand, a fine-stranded network can be observed. The formed gel will have a high modulus and fracture stress. Random coil type of proteins like gelatin often form a fine-stranded network over a wide pH range and ionic strengths.

Mixed protein and polysaccharide gels exhibit even greater complexity. First, on mixing the two components, thermodynamics will determine if the two components are compatible (miscible) or lead to associative or segregative phase separation under the chosen solvent conditions (see Chapter 8 of this volume). Second, the gels manufactured will differ enormously in mechanical properties depending on this thermodynamic driving force, ranging from an interpenetrating network, a coupled (associated) network and finally a filled (segregated) network. For example of this complexity, gelatin that gels at low pH in the presence of alginate will with time change from a thermoreversible to a thermo-irreversible gel due to electrostatic interactions (Tolstuguzov, 1986).

The use of mixed biopolymer systems can lead to a wide range of direct changes and possibly improvements in the properties of the resulting gel. As an example, several studies have looked at the addition of small amounts of gelatin (e.g., 1%) to milk protein gels. This significantly improves the water-holding capacity of the resulting milk product (e.g., yoghurt), while only inducing small changes in gel firmness, depending on gelatin concentration (Pang et al., 2015; Fiszman et al., 1999). Another example is the addition of galactomannans (such as locust bean gum), which can affect the mechanical properties of gels made from several gelling agents. In the literature, interactions between galactomannans and other biopolymers such as κ-karrageenan (Andrade et al., 2000), pectin (Da Silva et al., 1996) and certain agars (Sousa and Gonçalves, 2014) have been widely described (Phillips and Williams, 2009).

9.4 Particulate-filled gels and emulsion gels

Usually, food gels consist of a large range of ingredients, including solubilised material, aggregated particles, dispersed air bubbles and/or immiscible liquids distributed throughout the finalised product. A discontinuous phase (particles, oil droplets or air bubbles) can be introduced into the gelled matrix to impart improved textural and sensory properties, such as the gelation of heavy cream in combination with sugars (panna cotta) or the fluffy texture of marshmallows. Several aspects of the filler particles may strongly affect the textural and sensoric properties of the final filled gel food product in various ways.

9.4.1 Interaction between filler particles and the gel network—active vs. inactive filler

The interaction between the filler particles and the polymer matrix and the fraction and modulus of the filler particles are all known to influence the mechanical properties of the finalised food gel, as shown in a wide range of studies (Chen and Dickinson, 1999;

Chen et al., 2000; van Vliet, 1988; Dickinson, 2012a; Ring and Stainsby, 1982; Mcclements et al., 1993). The embedded particles can be classified as either active filler or inactive filler, according to their interaction with the gel matrix. Filler particles interacting strongly with the gel matrix provide a connectivity between the continuous and the discontinuous phases, and thus these filler particles are bound to the network and classified as active filler. For these gels, a structural reinforcement is expected if the embedded particles have a higher modulus relative to the polymer matrix. However, for composite gels containing filler particles weakly bound to the structural network, reduced mechanical properties are expected regardless of the filler/matrix modulus (Chen and Dickinson, 1999). These filler particles are classified as inactive fillers. The difference between active and inactive filler particles is illustrated in Figure 9.1.

The influence of bound (active) or unbound (inactive) oil droplet filler particles on the overall shear modulus of a gelled emulsion was clearly depicted in a study by Dickinson and Chen (1999). For the gelled emulsions with bound droplets, an increase in overall modulus was obtained as a function of the amount of oil, whereas a decrease in composite modulus was observed for the gelled emulsions with unbound droplets with increasing oil content (see Figure 9.2; Dickinson and Chen, 1999). Besides differences in modulus, connectivity of the oil droplets has also been reported to have a large impact on the release of oil (syneresis) during deformation of gelled emulsions. For droplets bound to the polymer network (active filler), no release of oil was observed, whereas for gelled emulsions with an inactive filler, a large fraction of the dispersed phase was released during shearing (Sala et al., 2007). This may, of

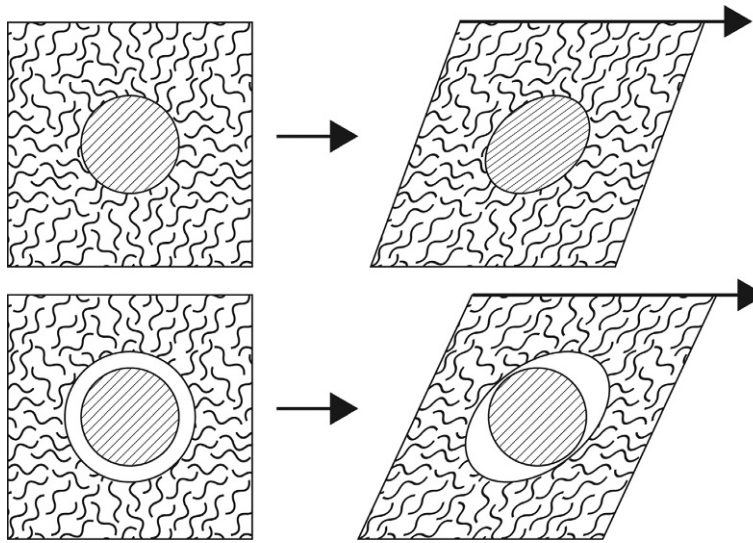

Figure 9.1 Illustrating the difference between active filler (top) and inactive filler (bottom). Adapted from Chen and Dickinson (1999).

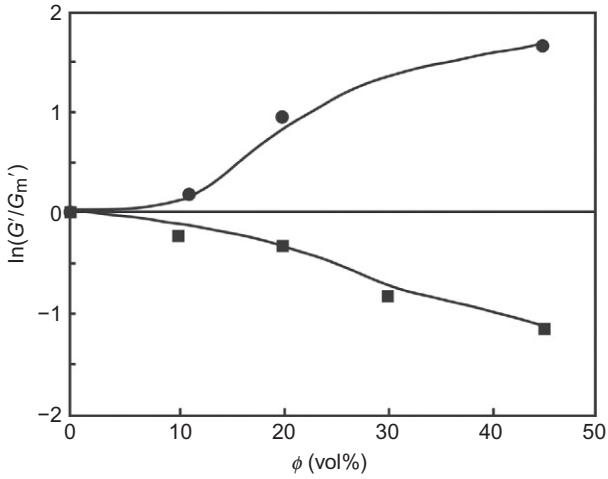

Figure 9.2 The influence of active or inactive fillers on storage modulus (1 Hz, 30 °C) of heat-set whey protein emulsion (85 °C, 35 min, pH 7). The natural logarithm of the composite modulus (G'/G'_{matrix}), plotted as a function of oil content. Filled circle (•), active filler (12 wt% WPI in aqueous phase); filled square (▪), inactive filler (12 wt% WPI+polysorbate 20 in the aqueous phase, surfactant:protein molar ratio 15:1).
Adapted from Dickinson and Chen (1999).

course, influence the sensoric properties, in addition to the stability of the filled gel during handling and/or production.

9.4.2 Filler effect on the modulus of emulsion gels

For filled gels with bound particles, a mathematical derivation of the shear modulus has previously been performed by Van Der Poel (1958). This second-order equation requires some calculations, and a simplified version (Equation (9.1)) of this formula is often used instead (Smith, 1975).

$$\frac{G'}{G'_m} - 1 = \frac{15(1-\nu_m)(M-1)\phi}{(8-10\nu_m)M + 7 - 5\nu_m - (8-10\nu_m)(M-1)\phi} \tag{9.1}$$

In Equation (9.1), M equals the ratio between the shear storage modulus of the filler, the matrix (G_{filler}/G_{matrix}), ν_m equals the Poisson's ratio, and ϕ represents the fraction of oil. The modulus of the filler particles as well as the modulus of the composite material may vary strongly depending on the type of filler particles. Oil droplets are a common type of filler, and their shear modulus has earlier been suggested to be equivalent to the Laplace pressure (Equation (9.2)) of the dispersed phase (van Vliet, 1988).

$$G_{filler} = \frac{2 \times \gamma_{oil-water}}{\text{droplet radius}} \tag{9.2}$$

As observed in Equation (9.1), the shear modulus of the filler is proportional to the interfacial tension ($\gamma_{oil-water}$) and inversely proportional to the droplet size. As the filler modulus has a large impact on the composite modulus, the equation depicted earlier highlights the importance of both droplet size and interfacial tension on the mechanical properties of food gels with oil droplets embedded in the polymer matrix. It should be noted that a theoretical assumption in the derivation of van der Poel's theory is that the filler particles are evenly distributed and nonaggregated. An underestimation in the calculation of the modulus has earlier been reported for flocculated gelled emulsions (Chen and Dickinson, 1998; van Vliet, 1988). This behaviour was attributed to the larger effective total volume of the flocs compared to the total volume of the individual droplets, and a modified equation was suggested to be used for these gels (Chen and Dickinson, 1998).

9.4.3 Stabilisation of food emulsions

As is discussed in Chapter 7, emulsions are inherently thermodynamically unstable. Destabilisation mechanisms such as flocculation, coalescence or creaming can lead to inhomogeneities, phase separation and undesirable textural and sensory properties of the emulsified food product. To avoid this, additives that stabilise the oil droplets are used (Mcclements, 2005).

Proteins and protein derivates, such as soy protein isolate or gelatin, often have an amphipathic structure. When these are used as gelling/emulsifying agents in gelled emulsions, the protein may adsorb at the oil–water interface and rearrange itself around the oil droplets, stabilising the droplets against coalescence. If there are relatively few oil droplets present, and enough protein to create a gel network, the droplets will on gelation be arrested separately in the continuous filled protein gel. As the oil droplets now interact directly with the gel network, the droplets will behave as active fillers, which dictates their influence on the mechanical properties of the composite gel (Dickinson, 2001). However, the addition of a surfactant (often used in processed foods) may lead to a displacement of the adsorbed protein, potentially causing the droplets to behave as inactive fillers. This reduced interaction between the filler and matrix leads to a lowering of the shear modulus as a function of filler content, as would be expected for inactive filler particles.

If the amount of protein available for forming a gel network is too low due to most/all present protein adsorbing at droplet surfaces, the protein-covered droplets may start to aggregate/flocculate together. If there are enough stable droplets and enough interdroplet attractive interaction (from protein adsorbed at the droplet surfaces), a protein-stabilised emulsion gel may be formed by the creation of a particulate network of protein-covered oil droplets sticking together. The mechanical properties of such a gel will be dependent on the interactions between the protein-stabilised oil droplets (Dickinson, 2010).

In practise, protein-stabilised gelled emulsions will often have some oil droplet aggregates/flocculates, and some separate droplets, distributed throughout a gel network. The properties of the resulting gel will then depend on both the protein gel network and the interdroplet interactions (Reiffers-Magnani et al., 1999).

For most efficient interaction between the proteins and the oil droplets, the protein must be properly solubilised. Some proteins show an increased solubility in the presence of salts, which can have an effect in some emulsified food products. An example of this can be seen in the production of ground meat/fat products such as liver pastes or some sausages, where the addition of salt can increase the amount of solubilised protein available for emulsification. Thus, the addition of salt can lead to a final product where the oil droplets/fat particles are more strongly bound to the gel network, giving a more stable emulsion gel with a harder texture, which may or may not be desirable (Matulis et al., 1995; Steen et al., 2014). Similar effects can also be seen with changes in pH, which will affect the amphipathic properties and solubility of proteins through changes in net charge. Differing pH values can either increase or decrease the stability of a protein-based emulsion or change the textural properties and mouthfeel of an emulsified protein food gel. Many proteins, such as casein or whey protein isolate (WPI), display reduced solubility and increased self-aggregation at pH values close to the protein isoelectric point, leading to emulsion destabilisation through flocculation (Mcclements, 2005; Dickinson, 2001).

As opposed to proteins, unmodified polysaccharide-based gelling agents usually lack emulsifying capability, with some exceptions such as high-methoxyl pectins. This property may cause difficulties with obtaining bound particles for such filled gels, as a strong interaction between the emulsifier and the gelling agent is required. However, through chemical, enzymatic or physical modification of the polysaccharide, the emulsifying ability of the gelling agent itself may potentially be improved. As an example, the introduction of lipophilic side chains (through esterification) to increase lipid interactions have been used with success for starches, with a significant increase in the ability to stabilise oil-in-water emulsions, depending on the degree of substitution (Miao et al., 2014; Singh et al., 2007).

There are a large range of studies concerning the influence of filler particles on different polysaccharide- and protein-based gels. This includes gels prepared with proteins, such as whey protein (Rosa et al., 2006), beta-lactoglobulin (Line et al., 2005), sodium caseinate (Dickinson and Casanova, 1999), gelatin (Sala et al., 2009a), soy protein isolate (Tang and Liu, 2013), and bovine serum albumin; Kang et al., 2003), and polysaccharides: high acyl gellan gum (Lorenzo et al., 2013), κ-carrageenan (Sala et al., 2009a,b) and alginate (Sato et al., 2014).

In addition to the use of amphiphilic proteins and polysaccharides, emulsions can also be stabilised by the use of colloidal particles that strongly adsorb and accumulate at the oil droplet interface while remaining intact, providing a steric stabilisation of the droplets. This kind of emulsion is known as a Pickering emulsion, and as long as enough particles are present to fully coat the oil droplets, very stable emulsions may be achieved, even with large oil droplets and at high fractions of oil (Dickinson, 2012b). These have traditionally been made with non-food-grade particles, such as gold–protein conjugates (Rana et al., 2012) or inorganic minerals such as silicates (Kpogbemabou et al., 2014) or iron oxide (Qiao et al., 2012). However, successful Pickering emulsions have also been prepared using food-grade particles, such as starch-granules (Marku et al., 2012), fat crystals (Rousseau, 2013), rod-like chitin nanocrystals (Tzoumaki et al., 2011) and cellulose nanorods (Kalashnikova

et al., 2013). For a good overview on the subject of Pickering emulsions in food, a recent review by Dickinson (2012b) is recommended.

9.4.4 Fracture behaviour of filled gels, the influence of active/inactive filler

As previously mentioned, the behaviour of food gels at large deformation is of large importance, as these properties better reflect the functional properties of the final product (mastication, handling, cutting; van Vliet and Walstra, 1995). The presence of filler particles can significantly influence these properties through changes in gel fracture stress, which strongly affects the chewability and mouthfeel of the product. A reduction in fracture stress can give a product that has a shorter texture and is easier to chew, whereas an increase can give a more chewy/rubbery product.

In general the fracture of gels is suggested to occur by the propagation of cracks. These cracks may be present at the gel surface or be formed at structural defects acting as stress concentrators during deformation. Particles embedded in a polymer matrix will act as structural defects, leading to a stress concentration in the proximity of the particles during deformation (Sala et al., 2009a). From the previous argumentation, a reduction in the fracture stress may be expected with increasing oil content for filled food gels; however, large deformation studies have shown that both increased and decreased fracture stress may occur depending on the type of biopolymer used (Rosa et al., 2006; Sala et al., 2009a). For fine-stranded emulsion gels, with either κ-carrageenan or gelatin as a gelling agent, a lowering in the fracture stress has previously been reported, attributed to the droplets acting as stress concentration nuclei. However, for WPI-based emulsion gels (particulate gels), an increased fracture stress was observed as a function of oil content. This appeared to be caused by the oil droplets filling the empty spaces in the aggregated protein network, reducing the inherent structural defects of the gel (Sala et al., 2009a). In general, it can be reasoned that the complex nature of fracture mechanics makes it difficult to obtain a reasonable explanation by using a simplified model.

As summarised by Sala et al. (2009a), the fracture mechanism of filled gels is dependent on the differences in modulus between the polymer matrix and the embedded particles and whether the filler particles are bound or unbound to the structural network. Different models have been proposed to describe mathematically the influence of bound/unbound droplets on the fracture properties. A model proposed by Nielsen tries to describe the influence of hard filler on the ultimate tensile strain of filled polymer gels (Nielsen, 1966). This model predicts a larger reduction in fracture strain as a function of filler content for bound particles compared to unbound filler. The corresponding fracture stress would, however, not necessarily decrease, as the modulus of the gels containing bound/unbound fillers can be significantly different. A model has also been proposed by Ross-Murphy and Todd trying to describe the influence of filler particles on the fracture stress. This model is based on combining the theory proposed by Nielsen and a theoretical model for the storage modulus (Ross-Murphy and Todd, 1983).

9.5 Gelatin-based emulsion gels

To further illustrate effects of filler particles (in this case oil droplets), gelatin-based emulsion gels are chosen for a closer look. Emulsion gels made with gelatin are used in a large range of food and confection products and even have potential applications for controlled delivery for pharmaceutical and nutraceutical products (Hattrem et al., 2014a; Sato et al., 2014; Haug et al., 2011; Thakur et al., 2012).

Gelatin is a functional and versatile biopolymer, obtained through partial hydrolysis of the parent collagen molecule, a structural protein extracted from various animal by-products (such as skin, connective tissue or bones). This hydrolysis can be performed with either acid or alkali, giving rise to either type A or type B gelatin, respectively.

The ability of gelatin to form thermoreversible gels makes it an important ingredient in a wide range of gel-based food products, such as gelatin desserts, aspic, marshmallows and many other confections. The temperature of its sol–gel transition is close to human physiological temperature, giving gelatin-based foods possibly a unique and often desirable melt-in-the-mouth texture. Gelatin's ease of use and characteristic properties make it a popular choice for the gelling agent in food gels, reflected in its high annual production of more than 300,000 metric tonnes (Haug and Draget, 2009).

Another interesting property of the gelatin molecule is its amphiphilic structure, containing both hydrophilic and hydrophobic amino acids. This enables gelatin to be used as an efficient emulsifier, as it can adsorb at oil–water interfaces and promote stability of the dispersed droplets (Ward and Courts, 1977). Through combining this emulsifying property with gelatin's gel-forming ability, gelatin-based emulsion gels may be produced, where the oil droplets are arrested and stabilised by the solid gel network.

Because gelatin has the ability to adsorb to oil–water interfaces, the oil droplets in a gelatin-based emulsion may interact directly with the gel network and generally behave as active filler particles. As described in Section 9.4.1, the presence of active filler particles can increase or decrease the composite modulus of a gel, based on the modulus of the filler particles themselves. As well as the modulus of the filler particles, many other factors can also affect the texture of emulsion gels, such as total oil content, type of oil, droplet size, type of gelatin and the presence of other surface active compounds.

9.5.1 The effect of oil content and droplet size

As described in Section 9.4.2, the modulus of the oil droplets is suggested to be given by the droplet Laplace pressure. For active filler particles, as in the case of gelatin-based emulsion gels, a droplet modulus higher than the modulus of the continuous phase leads to an increase in composite modulus as a function of oil content, and vice versa. This was confirmed in a study by Dickinson et al. (1985), in which increasing shear modulus with increasing oil content for a gelatin emulsion was observed.

However, a certain amount of gelatin will adsorb at the oil–water interface, potentially making this gelatin unavailable for gel network formation. At low concentrations of gelatin, this effect can be significant, with decreasing shear modulus and even a potential loss of gelling ability at higher oil concentrations (Dickinson et al., 1985).

As can be seen from Equation (9.2), the modulus of the oil droplets is highly dependent on the droplet size. The smaller the droplets are, the higher the droplet modulus, and for active filler particles, higher droplet modulus means higher composite modulus for the emulsion gel. In a study by Sala et al. (2009b), the effect of oil droplet size on Young's modulus and fracture stress for gelatin emulsion gels was examined. As expected, for gels with bound droplets (active filler), an increase in Young's modulus and fracture stress was seen with decreasing droplet size. For example, a 10% gelatin gel with WPI as emulsifier had a Young's modulus of about 25 kPa (4 mm/s deformation) in the absence of oil droplets. After emulsification with 20 wt% oil, the gels showed a clear correlation between decreasing droplet size and increasing Young's modulus, with moduli of 31 kPa, 38 kPa and 45 kPa for emulsions with droplet sizes of 4.22 μm, 1.09 μm and 0.47 μm, respectively. The effect of droplet size on fracture stress was not very large for the gel containing 10% gelatin, but for the gel containing only 4% gelatin an increase in fracture stress with decreasing droplet size was observed.

For unbound droplets, a decrease in Young's modulus was generally seen compared to the oil-free gels. For the system with 4% gelatin, all emulsion gels had a decrease in modulus compared to the oil-free gel. On the other hand, for the system with 10% gelatin, emulsion gels with larger droplets (5.12 μm) showed a decrease in modulus, whereas emulsion gels with smaller droplets (0.90 and 0.45 μm) had similar modulus as the oil-free gel. As for the fracture stress, a significant decrease was seen for all gelatin emulsion gels at all droplet sizes compared to the oil-free gels (Sala et al., 2009b).

As emulsion droplet size to a certain degree can be controlled through processing parameters, this gives a way to influence the modulus of the emulsion gel. However, smaller oil droplets also mean a larger oil–water interface, potentially increasing the gelatin depletion effect mentioned previously.

9.5.2 *Presence of other surface active compounds and differences between type A and B gelatin emulsions*

Food gels often contain other naturally occurring surface active compounds (e.g., lecithins or monoglycerides). In a gelled emulsion, these compounds may displace gelatin at the oil–water interface and reduce the adherence between the oil droplets and the gel network, potentially causing the oil droplets to behave as inactive fillers. This may further lead to a decrease in composite modulus and other textural changes in the gelled emulsions.

An example of this is shown in Figure 9.3, where the storage modulus of gelatin-based emulsion gels (with type A and type B gelatin) with or without the addition (0.5%) of the small surfactant polysorbate 80 (P80) is shown. Without oil, the storage

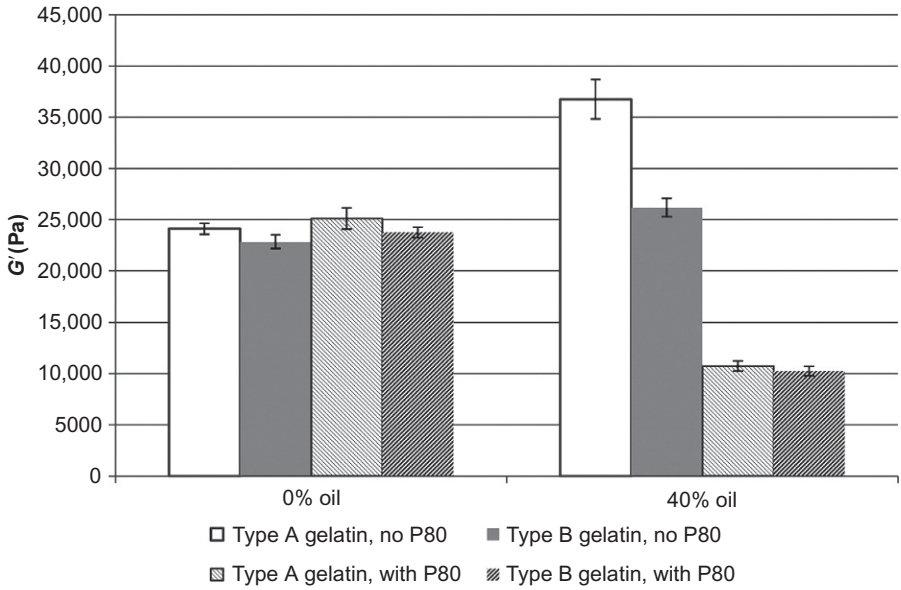

Figure 9.3 Storage modulus (G' (Pa)) measured for gelatin gels (25% gelatin in the water phase, 260 Bloom type A and type B gelatins), without oil and after emulsification with 40 wt% corn oil. Single-colour bars are for gels without P80 added, whereas striped bars are for gels prepared with an addition of P80 (0.5% in the water phase). The storage modulus is measured after first cooling from 60 to 20 °C (−2 °C min) and then 15 min of curing at 20 °C.

moduli of all the gels are similar. However, after emulsification (with 40 wt% corn oil), the emulsion gels without P80 show a slight (type B) or significant (type A) increase in storage modulus, whereas the gelled emulsions with P80 present both show more than a halving in G'. With only gelatin as surface active compound, connectivity between the oil phase and gel network is obtained, and the oil droplets behave as active filler particles, leading to an increase in composite modulus of the gelled emulsion. On the other hand, adding P80 displaces the gelatin from the oil–water interface, making the oil droplets behave as inactive fillers and leading to a decrease in composite modulus.

An interesting observation is the stronger increase in storage modulus for the gelled emulsion with type A gelatin, compared to the one made with type B gelatin, for the systems with no P80 present. This behaviour has previously been attributed to a physical interaction between the oil droplets in type A gelatin emulsions, providing a structural reinforcement of the gelatin network (Hattrem et al., 2014b).

This physical interaction was attributed to the formation of hydrogen bonds between asparagine and/or glutamine and the peptide backbone. During alkali hydrolysis of collagen in the production of type B gelatin, all asparagine and glutamine residues are hydrolysed into aspartic and glutamic acid, respectively. This might explain why this effect is generally not observed for type B gelatins. The droplet flocculation

also affects the gelling kinetics for type A gelatin gelled emulsions. Whereas type B gelatin emulsions keep fairly similar setting and melting temperatures regardless of oil content, type A gelatins show increasing setting and melting temperatures with oil content, with a more gradual change between viscous and elastic texture during heating and cooling. An example of gelling properties and kinetics for a type A gelatin emulsion, in this case a fish gelatin, is shown in the next section. These differences between type A and type B gelatins are important to keep in mind, as choice of gelatin type can significantly affect gel texture, mouthfeel and processing properties for food gel emulsions (Hattrem et al., 2014b).

For the systems with P80 present, no difference is observed between the type A and type B gelatin emulsion gels. As these oil droplets are not bound to the gel network, they behave as inactive fillers, and a reduction in storage modulus is observed for emulsions of both gelatin types.

Adding other emulsifiers that displace gelatin from the droplet surface does not always lead to inactive filler behaviour in gelatin emulsion gels. Studies by Sala et al. (2009a,b) showed active filler behaviour for a whey protein-stabilised emulsion that was embedded in a gelatin gel matrix. This was due to protein–protein interactions between the surface active whey protein at the oil–water interface and the gelatin network, preserving the connectivity between the oil droplets and the gelatin gel matrix (Sala et al., 2009a,b).

For complex emulsified/filled food gel products with a wide range of non-purified ingredients, the presence and exact effects of naturally occurring surface active compounds may be difficult to predict. It is important to keep these potential effects in mind in case of unexpected issues with texture or emulsion stability during the preparation of emulsion gels.

9.5.3 Improving the properties of cold water fish gelatin gels through active filler effects

Collagen from fish sources is easily available through the large amounts of by-products and waste from fish processing, and it would be advantageous to be able to utilise this for gelatin production. Fish-based gelatin would also have other advantages, such as less issues related to religious or cultural practises and a lower risk of carrying certain diseases (e.g., bovine spongiform encephalopathy). Unfortunately, cold water fish gelatins (CWFG, usually type A), have suboptimal gelling properties, in regard to both setting/melting temperature and gel strength, when compared to gelatins from mammalian sources.

However, CWFGs have similar amphiphilic properties as mammalian gelatins, enabling the creation of fish gelatin emulsions where the oil droplets act as active fillers, potentially increasing the composite modulus of the gel. This is shown through an experimental example in Figure 9.4, where the storage modulus of a CWFG solution containing no oil is compared to a CWFG emulsion containing 40 wt% corn oil, through a cooling–heating process from 60 to 10 °C (−2 °C/min) followed by 15 min

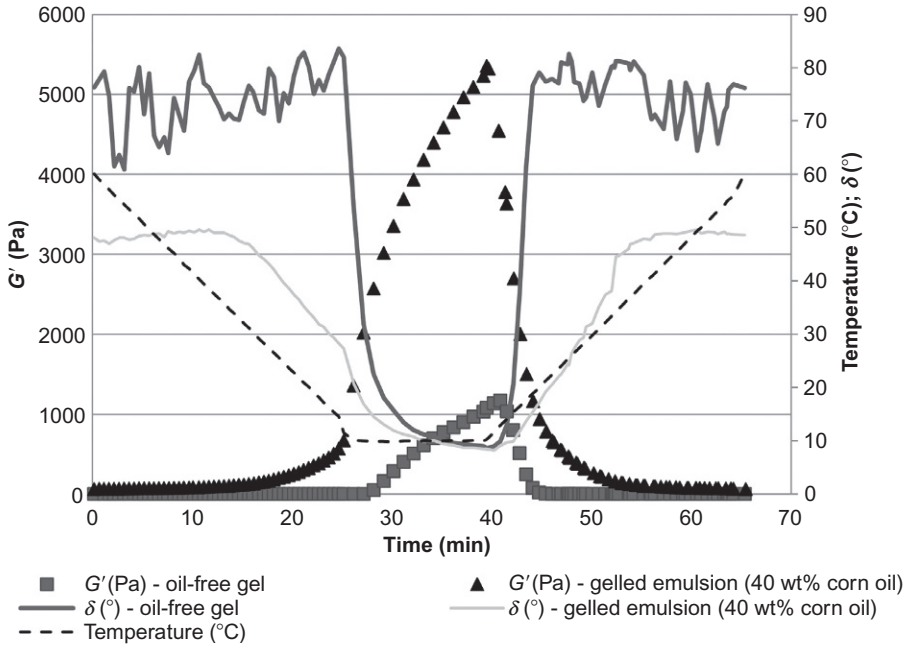

Figure 9.4 Phase angle (δ) and shear storage modulus (Pa) measured for a type A fish gelatin solution (25 wt% gelatin in the water phase), oil-free or emulsified with 40 wt% corn oil. Characterised by performing small strain oscillatory measurements during a cooling–heating process (Hattrem et al., 2014b).

curing at 10 °C, and then heating from 10 to 60 °C again (+2 °C/min). As can be seen in the figure, the maximum storage modulus after 15 min at 10 °C is approximately five times higher for the CWFG emulsion (~5400 Pa) compared to the oil-free CWFG gel (~1100 Pa). A significant difference in the gelling kinetics can also be observed from the figure, as shown by the phase angle (δ). Although the oil-free CWFG gel has setting and melting temperatures below 15 °C, the CWFG emulsion shows increased setting and melting temperatures, with a much more gradual change in δ during heating and cooling.

As CWFG usually is prepared by acid pretreatment (type A), oil droplet flocculation may occur, as for the type A mammalian gelatin described in Section 9.5.2. This previously discussed effect can also help explain the very large increase in storage modulus and the significant changes in gelling kinetics for the CWFG emulsion compared to the oil-free CWFG solution.

These results show that significant changes in gel texture and gelation kinetics can be achieved for the CWFG gels by the introduction of active filler particles (oil droplets), although the storage modulus is still quite low compared to gels made with mammalian gelatin, even at 10 °C. This potential of tailoring CWFG gelling properties might open the door for utilisation of CWFG in a wider range of products.

9.6 Future trends

A growing awareness of the source and properties of food ingredients has increased the demand for new types of biopolymers and gelling agents for use in the preparation of food gels. Gelatin is one of the most commonly used gelling agents and is usually produced from mammalian sources, which is encumbered with religious and ideological limitations. This has created a demand for gelatin produced from alternative sources, such as fish. As previously discussed, CWFG suffers from suboptimal gelling properties; however, gelatin prepared from warm water fish species has gelling properties close to existing mammalian gelatins. As such, the production of gelatin from these sources would be expected to rise (Hattrem and Draget, 2014).

Besides fish gelatin, an increased demand for alternative gelling agents from vegetarian sources, such as agar and alginate, may be expected. Gels prepared from these alternative gelling agents, however, usually lack the melt-in-the-mouth texture obtained from gelatin. By using mixed gel systems, enhanced rheological and textural properties can be obtained for these biopolymers, which might enable their use as gelatin substitutes.

Chemical modification of existing biopolymers is also a viable approach to improve their functional properties. Polysaccharide-based gelling agents usually lack emulsifying capability, potentially causing the filler particles to behave as inactive fillers, giving rise to undesirable sensory and textural properties. By introducing hydrophobic moieties on the polysaccharide molecules, they may act as both emulsifying and gelling agent, potentially providing connectivity in the prepared filled gel. The introduction of functional groups may also directly improve the gelling properties of the biopolymer.

Modification of the properties of the filler particles may also be used as a means to provide changes in the rheological properties of filled gels. For example, by adjusting the shear forces or other parameters during emulsion preparation, the final droplet size of the gelled emulsion may be controlled. This would influence the overall firmness of the gels, as modulus of the composite gel is strongly connected to the droplet size of the oil phase. Besides inducing changes in the droplet size or interfacial tension, phase transition from, for example, a liquid to a crystalline state can be used to provide changes in the modulus of the filler, influencing the overall textural properties of the gel.

9.7 Sources of further information and advice

For further information on the subjects discussed in this chapter, the following books and articles are recommended:

A comprehensive review on the topic of gelled emulsions is given in the following article: Emulsion gels: The structuring of soft solids with protein-stabilised oil droplets, review article (2012), written by Eric Dickinson

An excellent overview about the subject of food emulsions and ingredients: Food Emulsions: Principles, Practices, and Techniques (second edition, 2005), written by David Julian McClements

On the topic of biopolymers, the following books are recommended:

Food gels (1990), edited by Peter Harris—An overview of the most common gelling agents used in food gels

Handbook of Hydrocolloids (second edition, 2009), edited by Glyn O. Phillips, Peter A. Williams—A substantive reference guide for the various hydrocolloids used in the food industry

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Part Four

Modifying the texture of specific food commodities

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Texture of breakfast cereals and extruded products

10

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10.1 Introduction

The worldwide pasta and breakfast cereals have a turnover of US$20bn and US$30bn, respectively, and both businesses have been growing during the past few years. These two product categories share many similarities such as the raw materials and the extrusion technology applied for their production.

Texture of breakfast cereals and pasta is influenced by the type of cereals used for their manufacturing. Therefore, food developers and technologists must have deep insight concerning composition and properties of each cereal quality to select the right raw materials and to design optimal recipes. A description of the constituents of the main cereal grains, focusing on wheat, is provided in Section 10.2. Extrusion technologies applied to these products allow the full range of properties of cereal flours to be exploited. For instance, cold extrusion of pasta occurs under gentle conditions and high moisture content and delivers dense structures. Such a process enables creation of a protein network around uncooked starch granules. The opposite process of extrusion-cooking, which is used to generate breakfast cereals, aims at cooking starch under increasing temperature and pressure, providing textures that are ready to eat. These technologies lead to significant changes in starch and protein properties. Section 10.3 describes these physicochemical modifications. For both product categories, a wide range of textures can be generated depending on the processing parameters. A review of these parameters is given in Section 10.4.

Pasta and breakfast cereal businesses are considered to be mature (the first reference to pasta was in the twelfth century). Nevertheless, they show strong innovative potential linked to improved nutrition, on-the-go consumption and sensory variety that will be discussed in the last section of this chapter.

10.2 Type and composition of major grains used in extruded cereals

10.2.1 Cereal grains and their major molecular fractions

Together with taste and appearance, texture is a key parameter influencing consumer preference. In pastas or noodles, firmness and stickiness are key preference drivers. In breakfast cereals crunchiness and/or crispiness are liked by consumers, whereas soggy

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textures are rejected. The texture of these products is mostly impacted by their mechanical properties during the first bites and the physicochemical changes occurring during mastication and hydration by saliva. These mechanical and acoustic parameters are determined by the product's supramolecular, micro- and macrostructures. The supramolecular, micro- and macrostructures are themselves affected by the raw material properties, their modifications during processing and the viscoelastic properties of the resulting hydrated dough.

The source and type of cereal grain have a major effect on the properties of processed cereals. In 2012, the main crop produced on Earth was maize, followed by rice, wheat, barley and oats (FAOSTAT, 2014). In Europe, wheat was the most cultivated crop, whereas it was maize in North and Latin America. Maize is also largely produced in African regions, whereas in Asia rice is the most cultivated grain. The macronutrient composition of the major crops used in the industry is displayed in Table 10.1. Their composition exhibits similar carbohydrate levels, whereas they differ significantly in their protein and dietary fibre content.

The type of grain used for producing extruded products differs according to the application, geography and consumer habits. Pasta, primarily consumed in Europe, is mostly made of wheat, whereas rice is the base cereal for noodles, consumed in Asian countries. Rolled oats are mostly consumed in porridges and muesli. Combination of grains is also very common. Rice and/or maize are, for instance, combined with wheat in extruded breakfast cereals as they improve their size impression and crispiness (Moraru and Kokini, 2003).

Wheat is the cereal of choice for dough-based products such as pasta. Indeed, gluten from wheat provides unique viscoelastic properties. These properties allow the production of pasta with appropriate firmness and a low disintegration rate during cooking. They are so unique that commercial gluten-free pasta, targeting consumers with intolerance to gluten (coeliac disease), often fail in reproducing similar properties compared to pasta based on wheat (e.g., Gallaghera et al., 2004; Zannini et al., 2013). Wheat can be classified as hard or soft wheat, depending on whether it contains high- or low-protein/gluten content. Durum wheat (often also referred as macaroni wheat) is preferred for pasta making due to its high-protein content and viscoelastic

Table 10.1 General composition of unprocessed whole-grain flour

	Moisture (%)	Carbohydrate (%)	Protein (%)	Lipid (%)	Fibre (%)	Energy (kcal)
Rice (brown)	12.0	76.5	7.2	2.8	4.6	363
Maize (whole, white)	10.9	76.9	6.9	3.9	7.3	422
Wheat (whole)	10.7	72.0	13.2	2.5	10.7	408
Barley (dehulled)	12.0	74.5	10.5	1.6	10.1	345

Data obtained from USDA National Nutrient Database for Standard Reference.

properties of gluten. Cooked pasta from durum wheat maintains acceptable texture for consumers, resists surface disintegration during cooking and retains a firm structure or 'al dente' consistency. On the contrary, the type of wheat traditionally used for bread making shows a gluten strength considered to be too strong. It results in doughs that are 'too tough' for pasta (Liu et al., 1996).

Breakfast cereals and pasta made of whole-grain flours often show textures that are much less preferred that those obtained from refined flours (Camire, 2004; Chassagne-Berces et al., 2011). Whole-grain cereals are composed of three parts: the endosperm, bran and germ fractions. The endosperm mostly contains starch granules surrounded by a protein matrix. The structure of a wheat grain is displayed in Figure 10.1. The level of the different fractions as well as their composition depends on the type and variety of cereals. The endosperm is the largest fraction of the wheat grain (about 80%). It contains mostly starch (65–70%) and gluten proteins (Cornell and Hoveling, 1998). The bran fraction represents about 12–15% of the wheat grain and consists of its outer shells (Cho and Clark, 2001). It is composed of about 45–50% of dietary fibre, of which about 80% are insoluble (Rallet et al., 1990). The wheat germ is rich in vitamins, proteins and lipids and accounts for nearly 3–5% of the weight of the grain (Cornell and Hoveling, 1998). By analogy, the maize kernel is also composed of three fractions, which are the germ, representing 10–12% of the maize kernel, the pericarp (6–8% of weight) and the endosperm, which represents 82% of the grain weight (on dry basis; Rooney et al., 2004). In brown rice the content of these three fractions differs from maize or wheat as it is richer in endosperm. The rice kernel is composed of

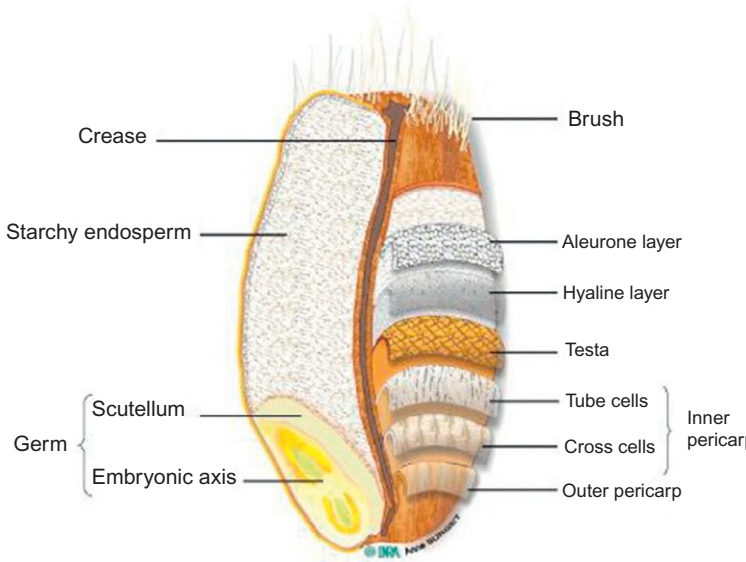

Figure 10.1 Structure and components of a wheat grain.
Modified from Saulnier et al. (2007).

4–6% pericarp, 2–3% germ and 89–94% endosperm (NIIR, 2006). During the refining process the bran and germ fractions, are removed. The resulting refined flour is mostly composed of starch, and it is therefore rich in calories. The commercial cereal fractions are not homogeneous with respect to tissue structure and biochemical compositions. Some of the fractions remain in the produced flour because of the difficulty of performing a sharp separation by sieving/sifting. This difficulty to obtain a sharp separation of the biological fractions is illustrated in Table 10.2. The table displays the composition of commercial whole-wheat flour, wheat bran and refined wheat flours. Based on the composition of the commercial fractions, reconstitution of whole-wheat flour can be achieved by combining 75% of refined flour, 20% of bran flour and 5% of germ flour.

For a given source and type of wheat grain, the degree of refinement, and therefore the purity of the endosperm, varies depending on the food application. The ash content is often used as a grading factor: the higher the level of ash, the lower the purity of starch. For instance, a 550 grade (German grading system) indicates a level of ash comprised between 0.490% and 0.580% on dry bases (Papegeorgou and Skendi, 2014). Such a type of flour is mostly used for biscuits, extruded breakfast cereals or mainstream bread varieties. Flours with a higher level of starch purity such as those with a grade of 450 are mostly used for pastries. The particle size of refined flours also plays a major role in the final properties of the product. Several studies revealed that finer particle size of flours provides better quality noodles due to their higher water uptake (e.g., Hatcher et al., 2002). The distribution of the flour particle size may also influence the water uptake during dough preparation and therefore the dough's viscoelastic properties. Particle size distributions that are too wide result in an uneven distribution of water. The smaller particles take up a large amount of water, whereas the largest ones are hydrated to a lower degree. Both over- and underhydration would affect dough development and lead to a poor quality of pasta (Manthey and Twongly, 2006). The particle size of starch flours also significantly influences starch transformations during extrusion-cooking of breakfast cereals manufactured by direct expansion. For instance, Garber et al. (1997) reported a reduced starch gelatinization

Table 10.2 General composition of unprocessed whole wheat and the grain fractions generated during milling

	Moisture (%)	Carbohydrate (%)	Protein (%)	Lipid (%)	Fibre (%)	Ash (%)
Whole-wheat flour	11.9	62	11.8	1.8	10.9	1.6
Wheat bran	11.6	20.1	13.8	3.5	45.5	5.6
Wheat germ	11.4	33.6	28.4	8.8	13.3	4.5
Refined wheat flour 550	13.5	71.2	11.3	0.9	2.5	0.7

Data from Robin (2011e).

during extrusion when reducing the particle size of corn flour from 1600 to 400 μm. In cold pasta and hot breakfast cereal extrusion, finer starch granules may act as filler particles and increase the resistance to mixing as well as the resulting dough viscosity.

10.2.2 Physicochemical properties of starch

Starch is the dominant macromolecule in cereals. Native starch is a semi-crystalline granule composed of two polymers: amylose and amylopectin (Figure 10.2). Amylose is essentially a linear molecule of α(1–4)-linked D-glucopyranose with a polymerization degree in the order of 1500–6000 (Zobel, 1988). Amylopectin is characterized by branch chains of α(1–4)-linked D-glucose with different chain length. The glucose molecules are connected by α(1–6)-linkages. Its polymerization degree ranges from 3.10^5 to 3.10^6 (Zobel, 1988).

The granular structure, molecular weight of amylose and amylopectin, protein and lipid content vary according to the botanical origin of starch. Starch is composed of different levels of structural organization, which are (i) molecular structure meaning the ratio between amylopectin/amylose, (ii) supramolecular structure determined by the arrangement of both polymers within single granules and (iii) the granule structure and the granule size distribution. The granules can occur in different sizes and shapes:

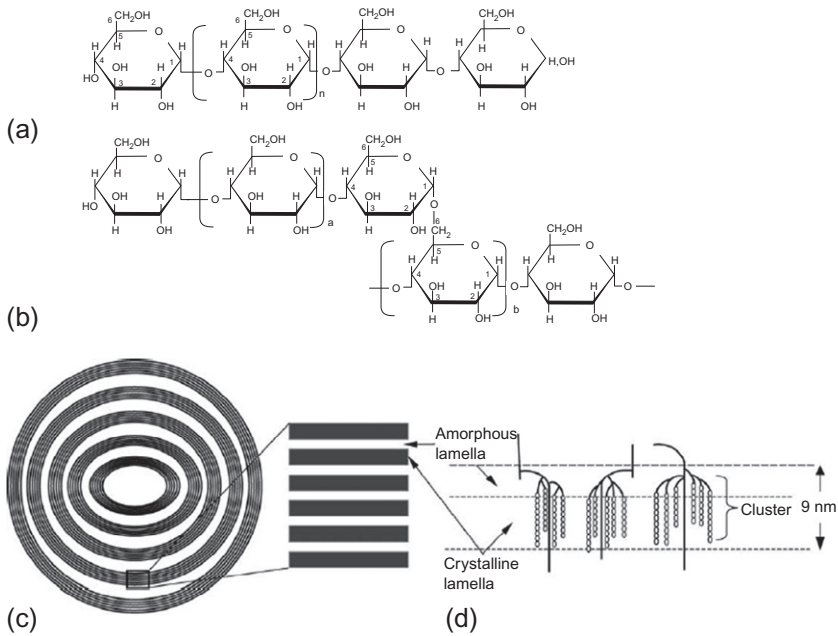

Figure 10.2 Chemical structure of amylose/amylopectin (a, b), organization of the amorphous and crystalline regions in the starch granules (c) and double helices structure (d). Adapted from Tester et al. (2003).

spheres, polygons, ellipsoids, platelets or irregular tubules. Depending on the botanical origin of the starch, the dimensions of the granules range from 0.1 μm to at least 200 μm in their length (Gallant et al., 1992). Scanning electron microscopical images of typical starch granules from rice, wheat and maize are shown in Figure 10.3.

Starch granules have a complex and highly ordered semi-crystalline structure. A representation of the starch granule structure is given in Figure 10.2. Their crystallinity varies between 15% and 45% according to the source of starch (Zobel, 1988). Within the granule, the crystalline and amorphous regions alternate in the form of concentric rings, exhibiting a Maltese cross when observed under polarized light. The crystalline structure of starch originates from the organization of amylose and amylopectin in the granule and the capacity of the linear double helix branches to align in parallel and thus form highly organized bands. Based on the widely accepted 'cluster model', the branching points in the amylopectin molecules are randomly distributed, while being clustered. Among these clusters, those of short linear chains may create more crystalline regions than the regions composed of the branching points (Pérez et al., 2009). This crystalline organization depends on the origin of starch and can be classified depending on its pattern into A, B or C types. Most cereal starch exhibits A-type crystallites, whereas tubers (e.g., potatoes, roots) and amylose-rich cereals exhibit a B-type pattern. Starch in legumes shows a C-type pattern, which is a combination of A- and B-type crystallites. Wheat starch may show A- and/or B-type crystallites. A-type and B-type crystallites differ in their packing of double helices and water

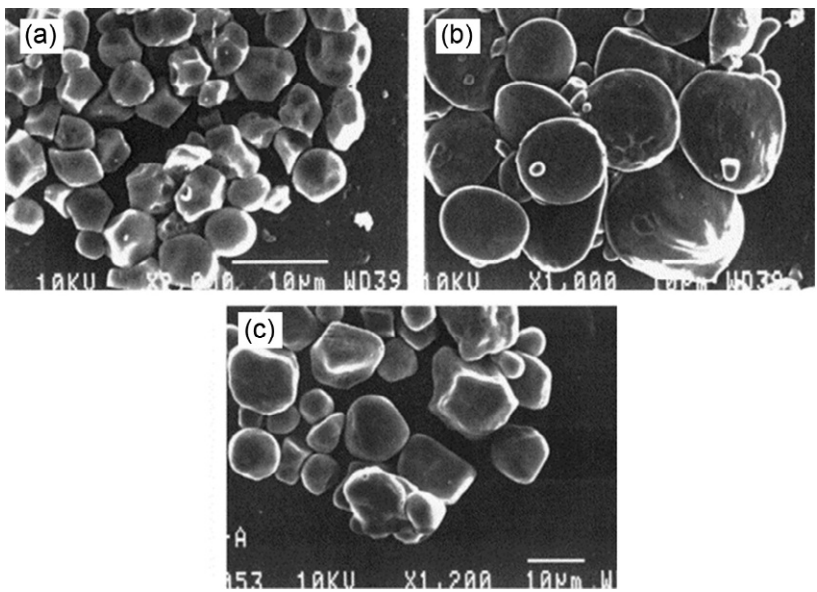

Figure 10.3 Scanning electron micrographs of starch granules from (a) rice, (b) wheat and (c) maize.
Modified from Singh et al. (2003).

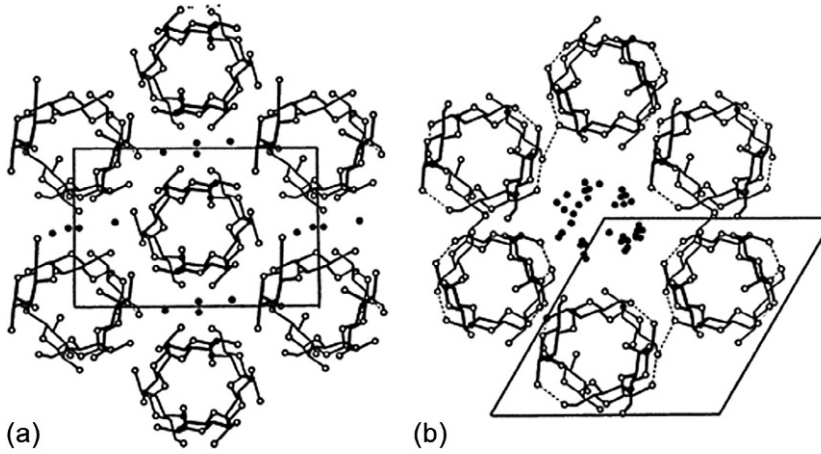

Figure 10.4 Arrangement of double helices in A- (a) and B-type (b) crystallites in starch granules.
Reproduced from Wu and Sarko (1978).

content. Representations of the double helices and water arrangement in the A- and B-type starch crystallites are shown in Figure 10.4. Starch granules with B-type have smaller particle size (average diameter of 3–5 μm) than A-type granules (average diameter of 13–16 μm). Due to their higher surface-to-volume ratio, B-type granules hydrate faster, swell quickly and bind more water that A-type granules. Although evidences are not clear, an optimal ratio between wheat starch A- and B-type granules may be beneficial to the dough and pasta quality (Soh et al., 2006).

The level of amylose in starch varies according to the botanical origin. Wheat and maize starch contains roughly 25–30% of amylose. Nevertheless from a same botanical origin the level of amylose can significantly vary in starch due to weather and soil conditions. This is the case for waxy starches, which contain up to 99% of amylopectin. High-amylose maize starches contain up to 85% of amylose. The ratio between amylose and amylopectin in processed cereals influences the final product properties. For instance, the expansion volumes of extruded maize (made by direct expansion) show a bell-shaped curve with the amylopectin content in starch. Maximum expansion volume is achieved at 50% of amylose content (Chinnaswamy and Hanna, 1988). Increasing amylose content leads to entanglement of the linear amylose molecular and high viscosity melts, which hinder expansion. On the other hand, at high amylopectin ratios, lower melt viscosities are generated that lead to high expansion. This also leads to higher collapse during expansion (Della Valle et al., 1996). Similar studies were performed on durum wheat in spaghettis. An optimum of 32–44% of amylose in starch was reported to significantly increase dough elasticity and firmness. Such results may be explained with the granule structure of high-amylose starch. Granules are more tightly packed and, on swelling, provide more resistance to rupture (Soh et al., 2006).

Waxy durum wheat was reported to deliver shorter cooking time, similar weight loss on cooking and lower firmness values compared to standard durum wheat used in spaghetti (Grant et al., 2004). The crystalline pattern (A, B or C type) and degree of crystallinity affect the melting temperature of the starch and therefore the thermal energy necessary to 'cook' starch. Starches with A-type crystallites tend to melt at higher temperatures than B-type starches. In addition, starches with A-type crystal are characterized by a gelatinization temperature that increases with the overall crystallinity of the starch granules, whereas the opposite is generally true for starches with a B-type crystalline pattern (Pérez et al., 2009).

Starch gelatinization is an endothermic process that corresponds to the dissociation temperature of starch molecules. The crystals disintegrate into an amorphous structure. This phenomenon is reversible until the temperature reaches the gelatinization temperature. Above this temperature, irreversible processes such as swelling, loss of birefringence and starch solubilization occur (Srichuwong et al., 2005). Gelatinization is also associated with a change in viscosity, which can be captured by viscosimetric measurements performed such as Rapid Visco Analyzers (RVA). RVA measurements are performed at excess of water and increasing temperature. Typical pasting profile of starch obtained by Rapid Visco Analysis is shown in Figure 10.5. Upon heating, the viscosity of the starch paste is first increasing due to granular swelling and leaching of starch components from the granule. Further increasing the temperature, a maximum viscosity is reached. Full swelling of the granule and eventually total disruption of the granule structure leads to a decrease in the viscosity of the paste. Amylopectin is primarily responsible for granular swelling and viscosity. During granular swelling, the hydrogen bonds between the starch chains are eliminated

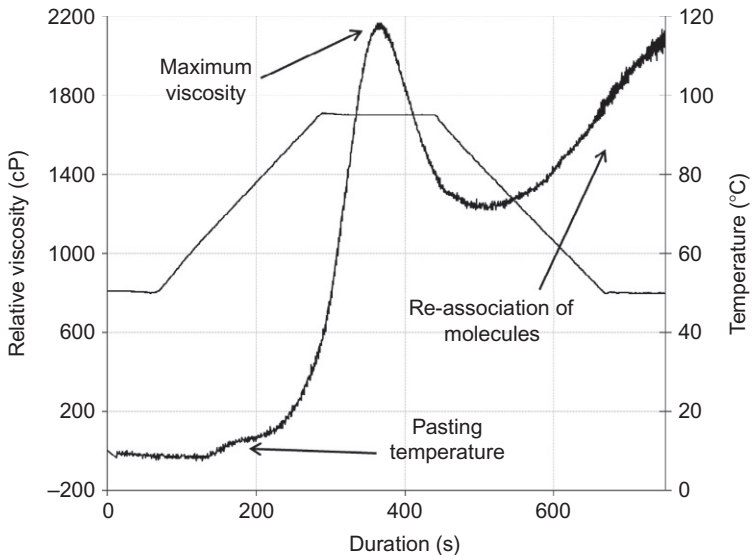

Figure 10.5 Typical pasting profile of wheat starch granules.

Table 10.3 Typical amylose content, crystalline pattern and gelatinization temperature of unprocessed cereal starches

	Amylose content (% in starch)	Main crystalline pattern	Typical gelatinization temperature (DSC peak temperature) (°C)
Wheat starch	25	Type-A	58
Maize starch	26	Type-A	67
Rice starch	17	Type-A	72
Waxy maize starch	1	Type-A	68

Data extracted from Zobel and Stephen (2006).

and replaced with hydrogen bonds between starch and water molecules. The increase in the hydrodynamic volume of starch molecules results in an increase in viscosity. Amylose is the main component of starch leaching out from the granules. During cooling of the hot paste, amylose molecules rapidly aggregate and lead to an increase in the paste viscosity. This process is called retrogradation. Gelatinization can also be assessed using differential scanning calorimeter (DSC). DSC has the advantage over RVA in that it provides information on starch gelatinization at lower water content and higher temperature. Typical gelatinization temperatures of maize, wheat and rice are displayed in Table 10.3 and vary between 60 and 70 °C.

10.2.3 Quality and functionality of cereal proteins

Proteins are commonly the second-largest component in cereal grains. Proteins exhibit varying effects on the final product properties depending on the type of process and the applied processing conditions. For instance, in high shear and high-temperature processes such as extrusion-cooking, the role of wheat and maize proteins on the melting properties and final product texture is relatively small. In pasta products, the protein type and content is influencing significantly the viscoelastic properties of the dough and of the resulting textural properties. Proteins change also the rheology of the dough. Indeed, pastes of native wheat starches show a shear-thickening behaviour. Mainly amylopectin is responsible for this rheological behaviour (Dintiz et al., 1996). On the contrary, gluten causes a shear-thinning behaviour of starch pastes. Once gelatinized, wheat starch also displays a shear-thinning behaviour.

In dough based on wheat flour, gluten proteins are known to be the predominant factor responsible for the viscoelastic properties (Faubion and Hoseney, 1989). Other cereals do not display the unique viscoelastic properties of gluten containing wheat and therefore do not offer similar properties to wheat. As previously mentioned, wheat flours are classified according to their hardness, gluten and protein content. Generally, wheat flours with high-protein contents also contain higher levels of gluten. Soft wheat with lower protein content (e.g., 7–9%) and lower gluten strength are used

in products for which the development of a gluten network is not desired or is even detrimental to the product quality. This includes cakes, cookies, crackers, quick breads and some types of pastry. Common wheat (*Triticum aestivum*) is preferred for white breads, Asian noodle, cakes and pastries. Dough made with common wheat exhibits a good balance between elasticity and extensibility, thus delivering a good expansion while holding the gas during baking (Dexter, 1993). For making pasta such as spaghetti, vermicelli, macaroni and lasagna noodles, a hard wheat such as durum wheat (*Triticum durum*) is preferred. It is characterized by a high content of protein and high gluten strength.

Protein content is the primary factor influencing pasta quality, whereas gluten strength is an important secondary factor, especially in pasta, which is dried at high temperatures. The level of protein in commercial wheat flours varies generally between 7% and 14% in refined flour. Wheat proteins are mostly composed of gluten proteins. Gluten, extracted with water, contains 75–86% of proteins, the rest being starch, lipids and hemicellulose, which are embedded into the gluten–protein matrix (Bloksma and Bushuk, 1998). Gluten proteins are composed of two fractions: glutenin and gliadin, present in almost equal proportions. Glutenin is a high-molecular-weight molecule (3×10^6 Da) producing a rubbery mass when fully hydrated. On the contrary, gliadin is a relatively small molecule (30,000–100,000 Da) and generates a viscous, fluid mass on hydration (Stauffer, 2006). Nevertheless, gliadin and glutenin significantly differ in molecular structure and properties depending on the wheat species. The combination of these two fractions exhibits cohesive, elastic and viscous properties.

10.2.4 Dietary fibre in cereals

10.2.4.1 Arabinoxylans and β-glucans

The dietary fibre content of cereals generally varies between 4% and 12%, depending on the cereal type. Dietary fibre is mostly present in the outer layer of the grain (bran; see Table 10.4). Functional and nutritional properties of dietary fibres are determined by their solubility. The majority of whole-grain cereal flours contain insoluble fibre. The exception is oat for which the level of soluble and insoluble fibre is almost equal. Arabinoxylans and β-glucans are the major types of dietary fibre found in cereals. They can be soluble or insoluble. Arabinoxylans are present in wheat, barley, oat, rice or sorghum, and their chemical structure varies depending on the botanical origin. Some arabinoxylans fractions found in wheat flour are soluble (0.5% on average), whereas others are insoluble (1.7% on average) in water (Ordaz-Ortiz and Saulnier, 2005). This fibre type is, as the name suggests, mostly composed of arabinose and xylose. A large part of arabinoxylans is removed during the refining process, resulting in a decrease of the arabinoxylans content from 5.8% (native grains) to 2.2% (in refined wheat flour; Saulnier et al., 2007). Durum wheat arabinoxylans contain a higher proportion of arabinose than common wheat (*T. aestivum*). Arabinoxylans appear to have a limited influence on the rheological properties of dough and pasta (Ingelbrecht et al., 2001; Turner et al., 2008).

Table 10.4 Content of total, insoluble and soluble dietary fibre in unprocessed whole-grain flours, refined cereal flours and cereal bran

Cereal flour	Total dietary fibre	Insoluble dietary fibre	Soluble dietary fibre	References
Wheat flour	3	1.9	1.1	Dreher (2001)
Maize flour	2.9	2.9	0	Lue et al. (1991)
White rice flour	2.7	–	–	Dreher (2001)
Barley flour	4.8	2.4	2.4	Vasanthan et al. (2001)
Whole-wheat flour	14.2	11.5	2.6	Dreher (2001)
Whole maize flour	12.8	11.7	1.1	Serna-Saldivar (2010)
Whole oat flour	10.4	–	–	Dreher (2001)
Whole white rice flour	1.3	0.9	0.4	Serna-Saldivar (2010)
Whole barley flour	15.4	11.5	3.9	Serna-Saldivar (2010)
Whole sorghum flour	11.8	10.8	1	Serna-Saldivar (2010)
Wheat bran	48	45.6	2.4	Dreher (2001)
Maize bran	88.1	86	2.1	Dreher (2001)
Oat bran	24.7	13	11.7	Dreher (2001)
Rice bran	27	24.5	2.5	Kahlon et al. (1998)
Barley bran	72.5	69.4	3.1	Dreher (2001)

Modified from Robin et al. (2012b).

Cereal (1,3–1,4)-β-D-glucans are cell wall polysaccharides situated in the cereal endosperm and in the aleurone cells. Cereal β-D-glucans are linear homopolymers of D-glucopyranose arranged in (1,4)-linked β-D-glucose residues separated by single (1,3)-linkages. β-Glucans represent 1% of the wheat grain, 3–7% of the oat grain and 5–11% of the barley grain (Skendi et al., 2003). These fibre types are characterized by a degree of high solubility. There are also variations in the structure of β-glucans found in different cereals (Cui et al., 2000). Because of their relatively low amount found in refined wheat and in whole wheat, their effect on the dough properties and pasta/breakfast cereals quality is very limited.

10.2.4.2 Cereal brans

Cereal brans are rich in cell wall dietary fibre. The content of fibre in brans depends on the source of cereals and varies between 25% in oat and rice up to 88% of the bran mass in maize (Table 10.4). Fibre in maize, wheat, rice and barley brans are at 90% soluble. An exception is oat bran, in which the proportion between soluble and insoluble fibre is more or less equal. Brans are generally detrimental to the quality of processed cereals. This becomes obvious when comparing the extrusion-related properties of processed whole-grain flours to those of refined flours. For instance, Manthey and Schorno (2002) reported poor mechanical strength of whole-wheat spaghetti compared to spaghetti produced with refined flour. Cooking losses were also higher for whole-grain spaghetti due to the disrupting effect that insoluble bran/germ particles exhibit on the gluten network. This leads to an increasing degree of disintegration. This disruption of the gluten matrix in dried spaghetti may also promote water absorption and accelerates swelling and rupture of starch granules. In addition bran particles will leak out into the cooking water (Manthey and Schorno, 2002). The presence of water-soluble components within the bran and aleurone layers further increases the observed cooking losses due to partial solubilization of dry matter.

These negative effects of insoluble fibre fractions on pasta quality were further demonstrated by increasing wheat bran content in durum pasta to 30%. This increase in bran negatively impacted major sensory properties such as firmness and stickiness and led to a decrease in pasta firmness (Kordonowy and Youngs, 1985; Aravind et al., 2012). To overcome the detrimental impact of cereal brans, many studies focused on the enrichment of pastas with oat or barley brans because of their high level in soluble β-glucans. Incorporation of barley β-glucans at various levels in durum pasta while maintaining acceptable sensory properties was reported by Marconi et al. (2000) and Dexter et al. (2005). However a darker colour of the product was not avoidable. Other soluble dietary fibre, such as inulin, were also used to fortify pasta. A comprehensive review of the effect of fibre on pasta quality is provided by Foschia et al. (2013).

The addition of cereal bran to extruded-cooked cereals produced through direct expansion at low moisture content also significantly influences the texture of cereal products. A systematic decrease in sectional expansion (which determines size impression) and increase in product density was reported when replacing refined by whole-grain flours or supplementing refined flours with cereal bran. Figure 10.6 shows typical cellular structures of extruded refined and whole-grain wheat flour as observed through X-ray tomography. Extruded whole-wheat flour shows increasing number of small cells (Robin et al., 2011a,b). This leads to harder textures (Robin et al., 2012a). The impact of cereal bran can be explained by an interplay of several factors. On one hand the increasing degree of nucleation caused by bran particles leads in the die to a higher density of nucleated cells. On the other hand, bran is acting as a filler in the extruded continuous starch matrix and will affect its viscoelastic properties because it will hardly change its elastic and plastic moduli due to water absorption (Robin et al., 2011c). Furthermore, bran is also more hydrophobic than starch, and it will absorb only

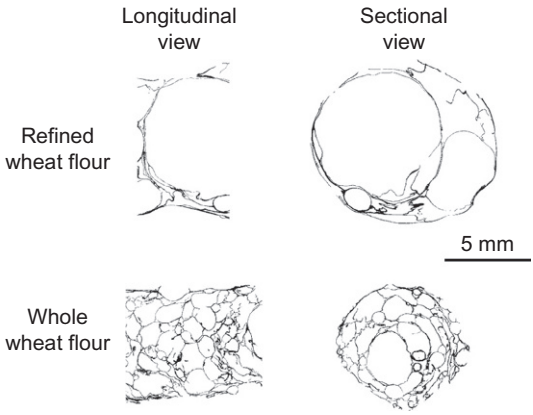

Figure 10.6 X-ray tomography cross-sectional images of extruded cereals made with refined and whole-wheat flours made by direct expansion.

a very limited amount of water. This means that at the same water content and filling level in the extruder, more water molecules are available for hydration of the starch if a significant amount of bran is added. The increasing water content of the starch will impact its physicochemical transformation during extrusion (Robin et al., 2011d). Finally, an increased bursting rate of bubbles due to bran particles disrupting the continuous starch layer surrounding bubbles is observed. Figure 10.7a shows bran particles distributed within an extruded wheat flour matrix. This image highlights the low compatibility of bran particles with the continuous starch matrix. On the opposite, incorporation of soluble fibre in extruded cereals provides higher expansion ratios, lower densities and larger cell sizes, resulting in softer textures compared to extruded cereals enriched in insoluble fibre (Robin et al., 2012b).

Processing of cereals may also modify the characteristics of dietary fibre, especially extrusion-cooking at low moisture content changes the fibre fractions significantly. An increased solubility of dietary fibre in oat, wheat or maize bran was reported as a result of exposure of the matrix to significant mechanical stress at a high bran concentration (Robin et al., 2012b). The particle size of wheat bran was also reduced when increasing the specific mechanical energy input (see Figure 10.7b).

10.2.5 Lipids

Although lipids account for only a low percentage of the grain weight, they significantly influence the physical properties of processed cereals. The content, and composition, of lipids varies according to the cereal type and is generally accounting for 2% and 4% of the grain weight (see Table 10.1). Lipids can be bound to starch-forming amylose–lipid complexes. These complexes can be built during biosynthesis in the plant or may be formed during processing. Lipids that are not associated to starch molecules can be either dissipated in the molecular matrix or they are bound to other molecules. The content of free lipids ranges from 1.5% to 2.0% of the kernel

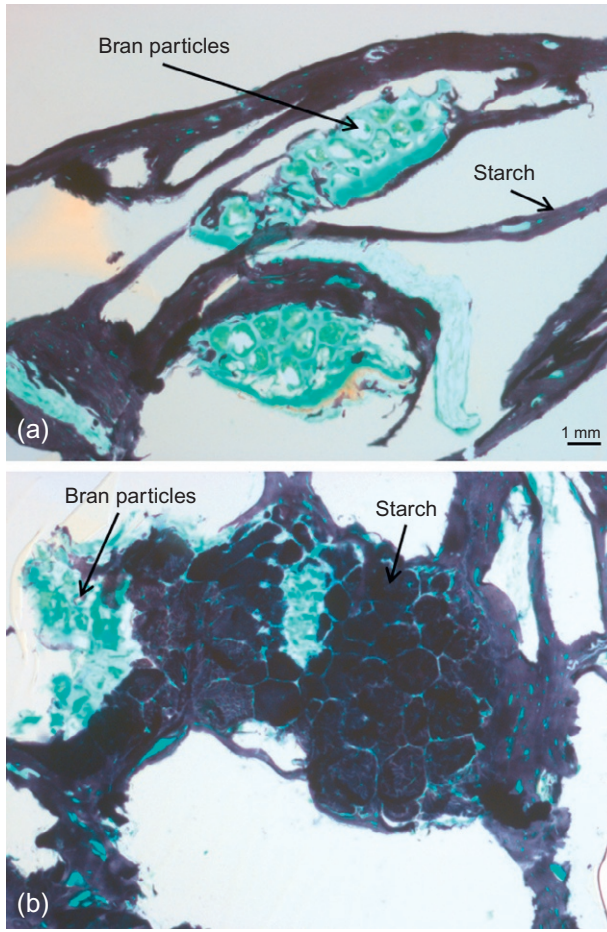

Figure 10.7 Light microscopy image of bubbles/cell walls of whole-wheat flour extruded by direct expansion. Exposure of the cereal melts in the extruder barrel to different degree of shear stress in the extruder barrel. (a) Low shear stress leading to almost intact bran particles and (b) high level of shear stress showing bran particles with reduced particle size.
Adapted from Robin et al. (2012a).

weight for cereals like barley, rice, rye and wheat. In oats, millet, maize and sorghum this substance class accounts for 3–7% of the grain weight (Chung and Ohm, 2000). Lin et al. (1974) reported that lipids do not affect quality of spaghetti. In contrast, Matsuo et al. (1986) observed that monoglycerides influenced greatly spaghetti quality by decreasing stickiness and improving tolerance to overcooking. This was attributed to the formation of water-insoluble amylose–lipid complexes, which reduce the amount of free amylose causing stickiness. Aravind et al. (2012) observed that the addition of durum wheat germ to durum wheat in spaghetti had no impact on quality up to 10% substitution. The addition of lipids in extruded cereals made by extrusion with direct expansion is generally reported to have a negative effect on the expansion

and hence on the resulting texture of the final product. Their addition during extrusion lubricates the extruded melt and decreases significantly the degree of starch gelatinization. Reduced conversion/gelatinization of starch ultimately results in a lower viscosity of the extruded melt, decreasing expansion volume. Nonetheless, these effects of lipids may still depend on their melting profile, which is given by the solid fat content curve. Lipids with higher melting temperature are reported to have a beneficial effect on the expansion of the melt (Moraru and Kokini, 2003).

10.3 Physicochemical modifications of starch and proteins during cereal extrusion

The structure of processed cereal products can be described as a continuous carbohydrate phase in which minor ingredients are dispersed. In the case of extruded breakfast cereals, the continuous phase is composed of amorphous starch, whereas air bubbles form the discontinuous phase. Minor ingredients such as proteins are distributed within the amorphous starch phase. On the contrary, in pastas or noodles, starch granules are distributed within a continuous protein network formed during kneading. Here starch represents the discontinuous phase. The dominant components, starch and proteins therefore play a major role in the final properties of pasta and the behaviour of the product during processing and preparation by cooking. The physicochemical transformations of starch and proteins during processing is impacted by several parameters such as the source of cereal, moisture level, distribution of these molecules within the different ingredients, processing temperature, mechanical stress and residence time.

10.3.1 Damaged starch

The first physical modification of native starches happens during milling. Indeed, milling leads to significant disruption of the starch granules, reducing their particle size and making its internal structure more available to water. Damaged starch can absorb up to five times more water than intact native starch (Milatovic and Mondelli, 1991). Therefore, a high degree of starch damage strongly increases water absorption of the flour and may be in the meantime detrimental to the development of a gluten network. This might decrease the quality of the pasta and, for instance, lead to pasta stickiness (Grant et al., 1993; Manthey and Schorno, 2002). In noodles, an increasing starch damage reduces both internal and surface firmness due to a higher degree of swelling and softening linked to plastification caused by water uptake (Oh et al., 1985). Higher starch damage in extruded breakfast cereals manufactured by direct expansion was also associated with smaller pores, a softer texture and an increasing degree of adhesiveness (Launay and Lisch, 1983). Starch damage is impacted by the type of cereal, milling conditions and the resulting particle size. Flours from hard wheat tend to contain a greater proportion of damaged starch compared to those made of soft wheat. This may be explained by the tight chemical and physical connections between starch and proteins observed for hard wheat. When undergoing milling, these connections tend to be disrupted to a greater extent in hard wheat than in soft wheat. Care should therefore be taken when selecting the type of cereal to minimize starch damage.

10.3.2 Starch hydration, swelling and melting

Starch is a semi-crystalline material. It displays two major physical phase transitions when submitted to heat at constant water content. Upon heating, amorphous starch structures exceed the glass transition temperature (T_g) forming a rubbery amorphous phase. At this stage, starch molecules are increasingly mobile and exhibit a larger hydrodynamic volume. The glass transition temperature of carbohydrates is function of the moisture content; water molecules act as plasticizers by increasing the molecular mobility. It is also affected by the molecular weight of starch molecules as shown in Figure 10.8; the lower the molecular weight, the lower the glass transition temperature. When further increasing the temperature (T_m), the highly ordered crystalline parts are melted into an amorphous viscous melt. Variations in glass transition and melting temperatures as a function of the water content are illustrated in so-called state diagrams. The state diagram, which can be established for each material, illustrates the state of the material as a function of moisture content (or water activity) and temperature. These states are separated by the glass transition, melting and stickiness lines/curves. For both pasta and breakfast cereals, the conditions of temperature and moisture at each step of the process (a summary of the extrusion conditions applied to pasta and breakfast cereals manufacturing is provided in Table 10.5) can be displayed in these state diagrams and used to explain and illustrate physicochemical transformations of starch.

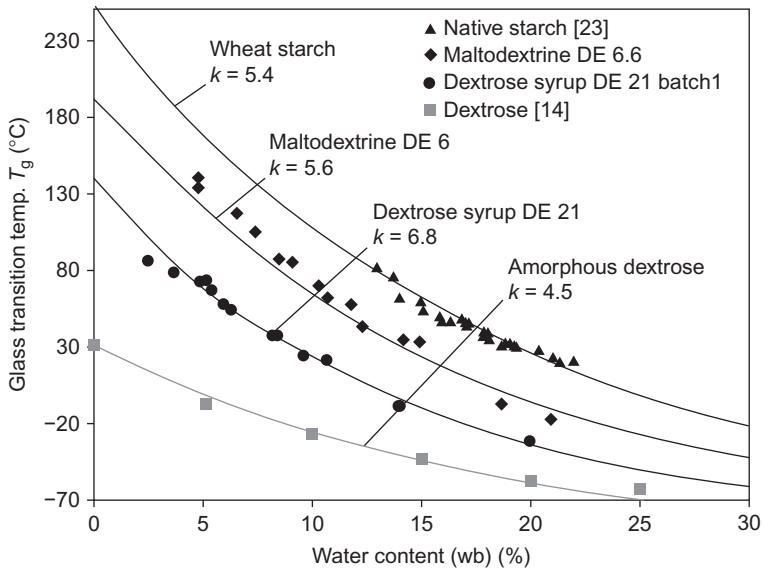

Figure 10.8 Glass transition temperature of starch, maltodextrin, dextrose syrup and dextrose as a function of their water content.
Reproduced from Palzer (2009).

Table 10.5 Temperature and moisture content applied in extrusion of pasta and breakfast cereals

Extrusion technologies	Temperature of dough/melt during processing (°C)	Moisture content (%)
Pasta cold extrusion	55	30–35
RTE breakfast cereals by cooking-extrusion and flaking (pellet to flaking)	<100	25–30
RTE breakfast cereals by cooking-extrusion with direct expansion	140–180	15–20

Data compiled from Guy and Horne (1988) and Dexter (2004).

In the pasta- or noodle-making process, cereals flours are blended, hydrated with water and formed through sheeting or cold extrusion into different shapes. These shapes are then dried. The aim of these processing steps is to develop a protein network around the starch granules. These shapes and the starch they contain are finally cooked shortly prior to consumption. For instance, noodle cereals are blended, formed into thin sheets through roller pairs, cut into strands, occasionally formed to square cakes and steamed/fried to remove water. During this final heating step starch gelatinizes. During pasta making by cold extrusion, starch is first hydrated from about 10% to 12% water content to about 30% water content before subjecting the dough to a maximum of about 50 °C in the extruder. After successive drying of the pasta at 50–90 °C, which is usually performed in cycles, a final moisture content of 12.5% is reached. It can be observed in Figure 10.9 that the glass transition of starch is reached already during the hydration phase. At the end of the hydration process, the amorphous material of the starch granules is in its amorphous rubbery state. In this state, molecules show increased mobility and physical reordering, and chemical reactions are promoted. Upon drying and further cooling, amorphous starch structures are transformed into their glassy state. Selected drying conditions of temperature and time must enable to generate an even moisture content distribution, and therefore a uniform glass transition temperature, across the pasta structure to avoid local stresses and crack formation and propagation. The diffusion coefficient of water is enhanced when the material is in a rubbery state and therefore drying kinetics is accelerated (Zweifel et al., 2000, 2003). The temperature of starch never reaches its gelatinization temperature during processing. Nevertheless, it was reported in several studies that cold extrusion of pasta leads to a moderate damage of starch. A very limited gelatinization may also occur locally due to mechanical energy dissipated, for example, as heat at interfaces between the dough and the extruder die. Drying at higher temperature (>60 °C) was reported to induce molecular rearrangement of starch molecules in the granules, similar to annealing, and the formation of amylose–lipid complexes (Zweifel et al., 2000; Petitot et al., 2009). During cooking of pasta in water, prior to consumption, water is in excess, and the temperature reaches the gelatinization point.

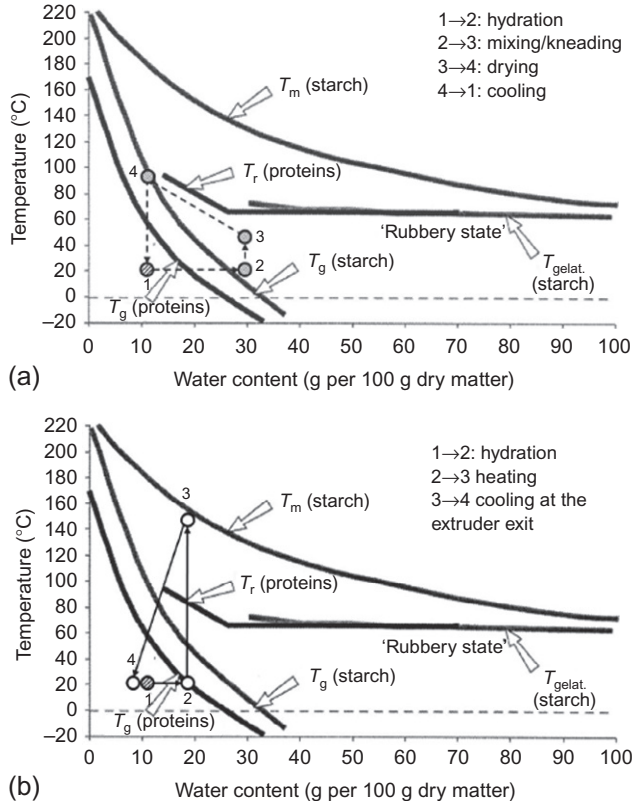

Figure 10.9 State diagram of wheat starch and gluten including the manufacturing process for cold extrusion of pasta (a) and extrusion-cooking of breakfast cereal with direct expansion (b). Modified from Cuq et al. (2003).

Starch granules embedded in the tight gluten network are swelling, and a significant increase in product volume is observed.

During cooking-extrusion of breakfast cereals and unlike cold extrusion in pasta making, starch is usually fully cooked. The level of moisture in the process differs depending on the technology used. Breakfast cereals produced by batch-cooking are usually processed at a high level of moisture (∼30%) so that the starch is fully gelatinized. Pellet extrusion is an alternative extrusion technology, which generates semi-finished cooked breakfast cereals that are later expanded during toasting/drying. During pellet extrusion, starch is also cooked at a high moisture content and at temperatures above the gelatinization temperature. Nevertheless, temperatures are maintained below 100 °C to avoid puffing at the die exit. On the contrary, extrusion-cooking with direct expansion occurs at lower moisture content and temperatures that are significantly higher than 100 °C. In this process, the level of required water to avoid blockage of the equipment depends on the presence of other plasticizers

such as small sugar molecules and polyols. It varies between 15% and 20%. The conditions of temperature and moisture during extrusion-cooking with direct expansion can be illustrated in the state diagram (Figure 10.9). In the first zone of the extruder, starch is mixed with water. While hydration occurs, the temperature along the extruder barrel increases and finally reaches about 150 °C. As the material leaves the die, moisture flashes off, while the expanded material is cooling down quickly. The level of moisture in the extruded product at the die exit is generally 5–10%. Further drying, combined with toasting, occurs prior to optional coating with a syrup. The coated breakfast cereals are then further dried to reach about 2–3% of water and cooled down to 30–40 °C. At these conditions, the amorphous starch matrix is in its glassy state. The final water content of breakfast cereals is key in driving acceptable textural properties over shelf life. For instance, crispiness, which is associated with the glassy state of the continuous starch matrix, was reported to be significantly reduced from a threshold water activity of 0.50 (7% water content; Sauvagoet and Blond, 1991).

It can be observed on the state diagram in Figure 10.9 the level of water in the extruder during extrusion-cooking with direct expansion (from 15% to 20%) does not allow complete swelling and bursting of starch granules. Starch granules are therefore melted under high temperature (ranging from 140 to 180 °C) provided by local dissipation of mechanical energy and further disrupted under the applied mechanical shear stress. The resulting cohesive melt is in its rubbery state when it leaves the die and exhibits viscoelastic properties. Under cooling and drying at the die exit, the mobility of starch molecules reduces as the temperature of the expanded product reaches the glass transition temperature. While further cooling down, the glassy state is reached, and a crispy product structure is obtained. The degree of starch transformation of extruded breakfast cereals generated by direct expansion depends on the processing conditions. It significantly influences the final volume and texture of extruded cereals. Higher degrees of starch gelatinization in wheat/maize flour in the extruder are generally obtained when decreasing water content of the feed or increasing the screw speed or barrel temperature (Doublier et al., 1986; Robin et al., 2011d). Although, the resulting expanded product is characterized by an amorphous starch matrix, some starch may retain its granular shapes and some crystalline amount under gentle extrusion conditions. Such materials display a viscosity peak during rapid visco analysis as reported for extruded wheat flour by Robin et al. (2011d).

Unlike cold extrusion of pasta, extrusion-cooking involves high shear forces and leads to a reduction in molecular weight of starch molecules. Starch amylopectin branches were reported to be more sensitive to degradation compared to amylose (e.g., Brümmer et al., 2002). Other studies observed that the molecular weight of both amylose and amylopectin are reduced on extrusion (Colonna et al., 1984). The decrease in the molecular weight of starch molecules increases their water-absorption capacity, leading to an increased sogginess of breakfast cereals in milk. Other ingredients may interact with starch or water and modulate the degree of starch gelatinization and degradation during extrusion-cooking. For instance, sugars were reported to reduce the physicochemical transformations of starch. They act as a plasticizer, reducing the viscosity of the melt (Fan et al., 1996a,b). Wheat bran and other hydrophobic

ingredients may affect starch transformation by leaving more free water available for plasticization of the starch (Robin et al., 2011d).

10.3.3 Proteins

Gluten proteins, the major protein fraction in many cereals, are also undergoing physicochemical changes during processing. In the pasta-making process, a three-dimensional network of gluten proteins is formed during mixing and kneading. This protein network surrounds the starch granules and provides elastic properties to the dough. Figure 10.10 shows a typical gluten network in the different areas of cooked pasta. This network is the result of the aggregation of gluten proteins through the formation of intra- and interprotein bonds. In this context a higher level of glutenin proteins and especially low-molecular-weight glutenin subunits were shown to significantly improve the dough quality (Edwards et al., 2003). As for starch, the hydrothermal path of gluten can be mapped in the state diagram as illustrated in Figure 10.9. Upon hydration and kneading gluten molecules are distributed within the matrix. During kneading and later during cold extrusion, these molecules are transformed into their rubbery state and accordingly they experience an increased molecular mobility. Proteins can then interact and aggregate together to form a viscoelastic network, mostly driven by entanglement of glutenin subunits and thiol-disulphide reactions. During drying further interactions between the gluten molecules occur, while water evaporates and finally stops the aggregation due to a reduced mobility of the gluten proteins. At high-temperature drying (>60 °C), large protein aggregates are formed, and disulphide/sulphydryl reactions between glutenin and gliadin occur. High-temperature drying also increases protein denaturation compared to drying at lower temperatures, which promotes further cross-linking between the glutenin and gliadin and increases the rigidity of the protein network (Zweifel et al., 2003). Pasta cooking in boiling water further increases the protein network interactions, which limits the swelling of starch granules and retains integrity of the pasta during cooking (Wagner et al., 2011).

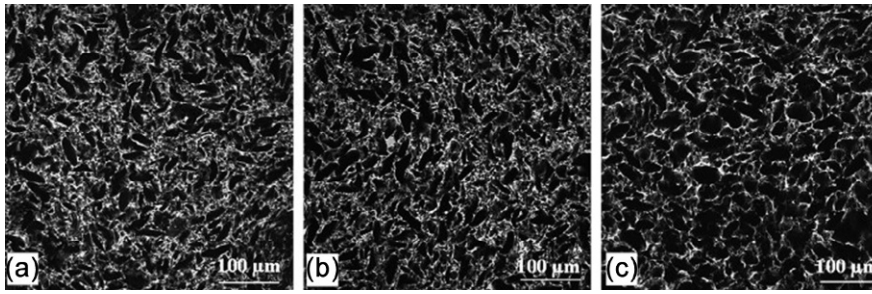

Figure 10.10 Confocal laser scanning microscopy of high temperature-dried cooked pasta in the central (a), intermediate (b) and outer (c) regions of pasta strand. White areas correspond to proteins and dark areas to starch granules.

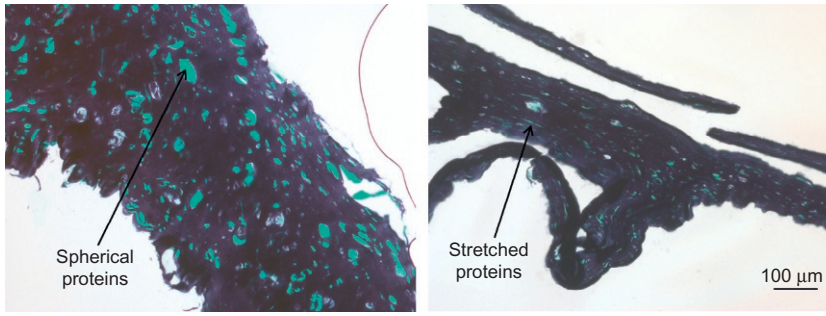

Figure 10.11 Microscopy pictures of extruded wheat flour extruded while applying varying degrees of mechanical stress: (a) low mechanical stress, and (b) high mechanical stress. Adapted from Robin et al. (2011b).

Cooking-extrusion is performed at high shear stress, high temperature and with short residence time. Unlike in pasta or bread, the high shear forces combined with the short residence time do not allow the development of a dough texture. Figure 10.11 shows the distribution of wheat gluten within extruded wheat flour. In extruded wheat flour processed by direct expansion, wheat proteins are randomly distributed within the starch matrix and do not form a three-dimensional network. Therefore the continuous phase is composed of starch, with gluten acting as a discontinuous filler phase. Nevertheless, some studies indicate that glutenins and gliadins may interact through hydrophobic interactions and disulphide bonds forming networks during extrusion-cooking (Li and Lee, 1996). Despite the formation of only a discontinuous protein network, Faubion and Hoseney (1982) reported that upon an increase of gluten up to 11%, a decrease in expansion volume of wheat starch was observed. Only a few studies evaluated the influence of cereal proteins on the textural properties of extruded flours. Only the impact of wheat proteins seems to be significantly investigated. Batterman-Azcona and co-workers reported that during extrusion-cooking of breakfast cereals made from maize, protein bodies containing zeins were destroyed and dispersed within the continuous starch phase. On the contrary, protein bodies were mostly intact and were still in contact with each other in breakfast cereals produced in batch cookers (Batterman-Azcona and Hamaker, 1998; Batterman-Azcona et al., 1999). Grits from harder maize were reported to deliver better expansion volumes, and therefore textures, than do grits from softer maize (Robutti et al., 2002).

10.4 Extrusion technologies applied to cereal texturization

10.4.1 Cold extrusion of pasta

Pasta can be manufactured either by extrusion, lamination or shaping on a belt, followed by drying. Lamination is used for making linguini, pappardelle, tagliatelle, lasagna sheets and square spaghetti. The shapes can remain flat, or nested forms

can be provided through a nesting machine. Shaping on a belt allows producing special shapes that are traditionally twisted or dragged by hand (or by machines). The dough is twisted and dragged on a conveyor belt to obtain the desired shape. Such technology is used to make trofie and cavatelli. Although these technologies are used to some extent, extrusion is the most applied process for pasta making, as it is versatile and can generate a wider range of shapes. Extrusion is traditionally used to produce pasta with shapes such as spaghetti, macaroni, fusilli, penne and more complicated shapes like 'radiators' or 'wheels'.

Pasta extrusion starts by mixing the flour and added ingredients in an airtight mixer in order to limit the incorporation of air. The presence of small air bubbles may weaken the structure of pasta and activate enzymes (Hosenay, 1986). The dough is then extruded. Extrusion occurs at low temperature (also named as cold extrusion) and enables kneading and shaping the dough in a single piece of equipment. The dough in pushed through a die under pressure at high moisture content (about 30%). Temperatures are kept low (35–50 °C) to avoid swelling of starch granules and degradation of gluten proteins. Throughputs are usually around 2.5 tonnes/h. The extruder is generally equipped with a water-cooling system, enabling to maintain the dough at the adequate temperature. A low temperature also avoids expansion/puffing of the dough, which is not desired in pasta making, as pores would decrease pasta cohesiveness. The design of the die enables us to produce a wide range of shapes. A typical process diagram of pasta extrusion is shown in Figure 10.12. It is generally

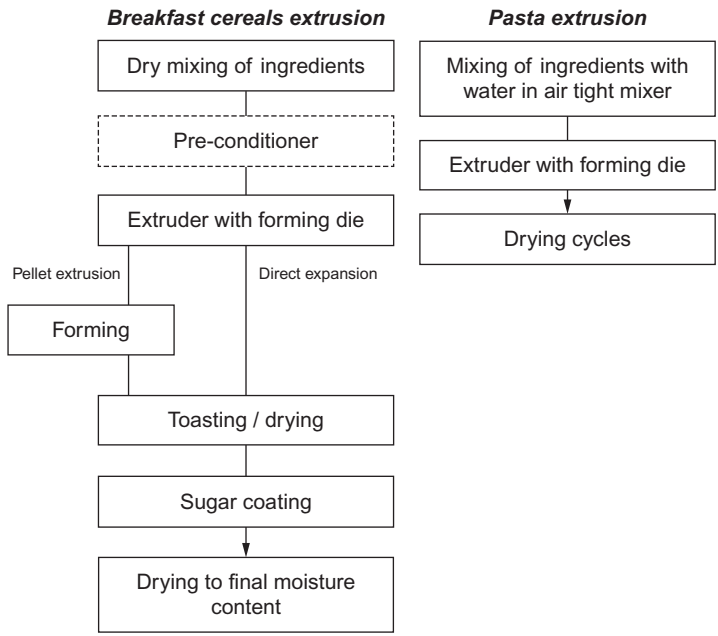

Figure 10.12 Flow diagram of extruded breakfast cereals and extruded pasta including all key unit operations.

accepted that pasta obtained from cold extrusion are of lower quality compared to those obtained by lamination. This may be explained by the formation of a protein matrix during extrusion that has some discontinuities (Pagani et al., 1989). Extruded pasta also tends to absorb more water during cooking, leading to higher cooking losses. An increased degree in starch degradation, due to shear stresses, in cooked pasta may explain this difference (Zardetto and Dalla Rosa, 2009). The types of mixer and extrusion conditions significantly affect pasta quality (Carini et al., 2010). For instance, Abecassis et al. (1994) reported that extrusion performed at low temperatures leads to better pasta qualities with lower cooking losses, lower deterioration of the surface and increase of the dough's viscoelastic properties. The same authors also observed that higher hydration of semolina and higher screw speed improved pasta quality.

Drying of pasta reduced the water content below the legal limit of 12.5% of water. It also enables reducing the water activity below 0.6 to reduce enzyme activity and prevent proliferation of undesirable bacteria. During drying, generally occurring in drying cycles, pasta also acquires its final colour and consistency. Pasta must be dried uniformly and homogeneously to avoid local stresses and maintain cohesiveness. These local stresses may be the result of non-homogeneous distribution of water between the surface and the inside of the pasta shape. Differences in water affinity between starch, gluten and the other added ingredients may also affect the distribution of water within pasta. Inhomogeneous water distribution leads to stresses and finally cracks. The pasta drying cycle is divided in two steps: during the pre-drying phase, water is evaporated quickly. The second drying phase is made of several drying cycles with resting periods in high humidity environment (during resting the water can migrate and distribute more evenly). Drying in cycles thus reduces mechanical stress and cracking resulting from water gradients (low water leads to contraction/lower volume; this causes the stresses). At the end of this phase, the pasta shapes are cooled down. Alternatively, high-temperature drying is performed at 60–84 °C. It enables to reduce the drying time to 8–11 h, whereas very high drying temperature above 84 °C enables to further reduce the drying time to 2–5 h (Petitot et al., 2009). Nevertheless, as previously mentioned, drying at high temperature leads to changes in starch and gluten properties that are detrimental to the quality of pasta.

10.4.2 Extrusion-cooking of breakfast cereals

Ready-to-eat (RTE) breakfast cereals are mostly characterized by their crispiness and/or crunchiness. Crispiness is a key sensory attribute that must be maintained throughout product shelf life and during consumption in cold milk. In this context, the material properties and cellular structure play a major role in the kinetics of water and milk uptake during consumption and storage.

The first technology used in the early twentieth century to produce breakfast cereals was flaking. This technology involves steam cooking of flours and mixing ingredients in a batch cooker at about 120 °C and moisture contents of 28–34% for 1–2 h. This step is followed by forming the flakes between counter-rotating laminating rolls applying a pressure of 150–200 bars. In the meantime, the formed flakes are

also toasted to reduce moisture content and generate flavours. During these different steps, starch is gelatinized, enzymes are deactivated and colour development occurs as a result of the Maillard reaction and caramelization of sugars (Caldwell et al., 1990). Similar to steam cookers, which include no direct expansion, extruder-cookers can also be used to cook starch. They enable to shape pellets at the die exit and drying/toasting effects are observed while the mass passes the extruder barrel. Compared to steam cookers, pellet extrusion is a continuous process that exposes the cereal mass to higher shear and elongational stresses. The moisture content in the extruder inlet is about 30% to enable a complete gelatinization of starch in the extruder, whereas temperature is maintained in the barrel below 100 °C to avoid direct expansion. At the die, the moisture content is about 20–22%. The pellets are then transferred in tempering drums (40–60 °C) for cooling. The drum rotation helps to reduce sticking of material to the walls. The pellets are then flattened between two rolls before being dried by exposing them to air with a temperature of 220–270 °C. During drying also puffing of the flakes is obtained. The next step is a toasting at 160–200 °C to generate colours and flavours while further reducing moisture content, which provides the final crispiness (Bouvier, 2001). Obviously the water content in the pellets prior to drying and toasting will have direct influence on the dough viscosity and thus on the generated expanded product. Too-low moisture content will generate low puffing degree and hard textures.

Extrusion-cooking with direct expansion offers a wider range of product dimensions, shapes and textures compared to batch-cooking or pellet extrusion. One major advantage of extrusion-cooking is that it enables mixing, kneading, cooking, shaping and texturation in the same equipment. It also allows high production capacities with throughputs up to 1 mT/h at affordable production costs. Single or twin-screw extruders with co-rotating screws can be used for extrusion-cooking with direct expansion. The latter generates higher shear stresses in the cereal melt. The profile of the screw elements varies along the length of the shaft. At the beginning of the screw, the distance between screw turns is long. This ensures a rapid transport of the initial dry powder. After introduction of water into the extruder, the distance between two turns is reduced, allowing appropriate mixing between water and powder. Moving toward the die/extruder outlet, the distance between turns is then further reduced along the screw. Such screw profiles enable to further mix and knead the resulting melt toward the extruder outlet. Pressure builds up near the end of the extruder due to the conveying action of the screw and the reduced free cross-sectional area of the die. This leads as well to an increasing shear stress between the barrel walls and the screw turns. In the final part of the screw, close to the die, reverse elements may be added to further increase pressure, which would increase the degree of expansion at the die exit. Designs of screw configurations are thus considered by manufacturers and users of extruders to be a key competitive advantage.

A typical process diagram for manufacturing of breakfast cereals by extrusion-cooking with direct expansion is displayed in Figure 10.12. The first step is generally including the introduction of a dry mix of flours and minor ingredients in the extruder. In the next barrel section water is added. The use of plasticizing ingredients such as sugars enables to reduce the melts viscosity and therefore to operate at a lower water

content. Temperature gradually increases along the extruder to reach 140–180 °C at the end of the barrel. While the mass moves along the barrel, starch is hydrated and gelatinized and the wet powder is transformed into a continuous viscoelastic melt. A pre-conditioner may be installed in front of the extruder to temper and hydrate the dry mix and increase its homogeneity (Bouvier, 2001). Steam may also be injected in the barrel to further increase the temperature during the process while limiting the addition of water. Indeed, viscosity of amorphous materials is a function of both water content and temperature. The rheological properties of the melt determine its capacity to expand. As the melt is leaving the die, moisture flashes off. This leads to the formation of bubbles and pores in the final product. After leaving the die a part of the expanded melt collapses due to bubble bursting. This phenomenon is illustrated in Figure 10.13 and is favoured by a too-low viscosity of the melt. The properties of these pores (porosity/bubble density, size distribution and shape) partially drive the final texture of breakfast cereals. This relationship can be described through a power-law; the higher the porosity, the lower the hardness. At a given porosity, differences in porous structure may significantly change the tensile strength of the extruded body. For instance a finer cellular structure is harder than a coarser one (Robin et al., 2010). Bursting of bubbles also occurs at the surface of the expanding melt, as illustrated in Figure 10.14 and may influence its final mechanical properties and hence texture. The density of breakfast cereals is negatively correlated with hydration and hydration rate. The temperatures at which breakfast cereals are produced appear to modify the hydration rate of breakfast cereals (Sacchetti et al., 2003).

Textural properties of breakfast cereals can be modified by varying the extrusion conditions while maintaining the shape. The main parameters that can be varied are feed rate of the dry mix, water content in the melt, barrel length to diameter ratio, screw configuration, speed, barrel temperature and diameter/length of the die at the extruder exit. These variables primarily affect the degree to which starch is transformed and thus the resulting viscoelastic properties. A too-low viscosity of the melt would lead to a collapse of the extruded structure during expansion, leading to a reduced final volume. On the other hand, a too high viscosity would hinder the growth of bubbles, also leading to reduced volumes and hard textures. For instance, it is generally accepted that, within the standard conditions applied in extrusion, increasing moisture content leads to lower final volumes, coarser cellular structure and harder textures (Moraru and Kokini, 2003). Design of the die at the extruder exit is also a key part of the process enabling texturization of breakfast cereals. The die diameter

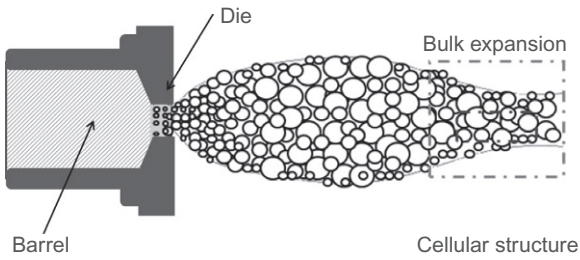

Figure 10.13 Schematic representation of bubble nucleation at the die, bubble growth and bubble collapse during expansion at the die exit.

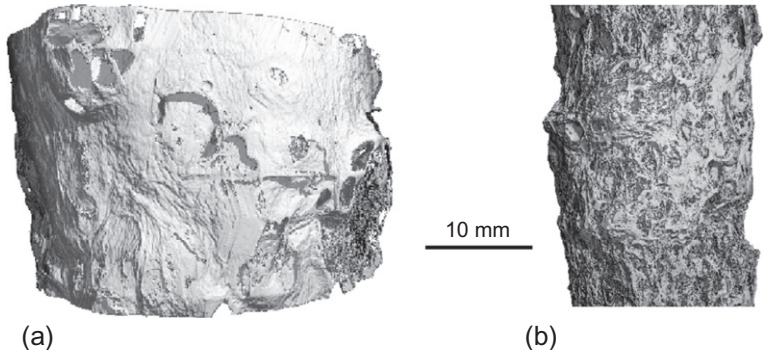

Figure 10.14 X-ray tomography images of the surface of extruded refined wheat flour (a) and whole-wheat flour (b).

and length aim at restricting the flow at the end of the extruder barrel, increasing pressure and leading to the melt expansion once leaving the die. The final shape is defined by the design of the die, swelling of the melt at the die exit due to elastic forces and a sudden release of pressure.

Co-extrusion is the technology of introducing a filling, usually a fat-based cream, into a cereal shell during extrusion. It enables to deliver multisensorial experiences combining soft and hard textures. Co-extrusion was a long time ago introduced in other industries such as the cable or polymer industry. It is also applied to breakfast cereals, confectionery and pet food to generate pillow-like shapes or tubes. Fat-based fillings are most often used as they do not show migration of water from the filling to the cereal shell, which would cause sogginess. Fat-based fillings are nevertheless energy dense. Chocolate fillings are usually tempered and pumped into the injector nozzle at the centre of the die, while the cereal shell is expanding around. Delivering the right amount of filling into the cereal shell during the continuous extrusion process and in the meantime limiting its influence on the degree of expansion is challenging. In this matter, the viscosity of the tempered filling is key to avoid leakage of the filling out of the extruded cereal shell at the die exit and later on during cutting/crimping.

Direct expansion of breakfast cereals is governed by the pressure difference between the melt at the extruder die exit and the atmospheric pressure, leading to a sudden decrease in pressure at the die exit. Operating at low moisture contents and at high temperatures (generally around 150 °C) are necessary conditions to enable puffing of the melt. Nevertheless, these high temperatures and strong shear forces generated in the extruder limit the use of sensitive health-providing ingredients such as vitamins, polyphenols, milk powder or vegetables, which may be degraded or may lead to burnt flavours in the finished product. The use of vacuum extrusion enables to achieve acceptable expansion volumes while operating at temperatures closer to 100 °C and thus can be used for incorporation of ingredients sensitive to temperature/shear. The use of vacuum extrusion implies that the die exit area and all the downstream equipment must be maintained under vacuum. Similarly, the injection

of inert gases such as carbon dioxide or nitrogen (gases may be in supercritical state) may enable to generate expanded product while maintaining a lower viscosity of the melt in the extruder and lower temperatures than 100 °C (Rizvi et al., 1995).

10.5 Future trends

The pasta and breakfast cereals businesses are often considered to be matured. Nevertheless, both categories show strong innovation potential. First of all, the increasing awareness of consumers concerning the nutritional composition of their food, pressure from key opinion leaders and nutritional programs from governments encourage the industry to provide healthier products. In terms of nutrition, pasta and breakfast cereals differ greatly. Pasta, when consumed on their own, are generally regarded as healthy. They provide complex carbohydrates, which are slowly digested. On the contrary, breakfast cereals often contain a relatively high amount of sucrose. As a response to these public health concerns, the food industry is increasing, year after year, the whole-grain and/or dietary fibre content in breakfast cereals. The majority of breakfast cereals contains today 30–40% of whole grain. A few products even consist up to 80% of whole grain. It is likely that the increase in whole-grain and/or dietary fibre content will continue in the future. The ultimate goal would be to reach levels for which health claims may be permitted. As of today, the European Food Safety Authority (EFSA) or the U.S. Food and Drug Administration (FDA) permit health claims only on a few cereal fibres (such as wheat arabinoxylans, barley or oat fibre). Whole-grain claims are also only permitted in a few countries such as the USA, UK or Sweden. Nevertheless, research continues to correlate diets rich in these ingredients with tangible health benefits. Increase in whole-grain and/or dietary fibre content is linked to major technological challenges related to texture that must be addressed to reach higher levels without losing consumer preference. In this context the selection of the right blend of cereals, addition of minor ingredients significantly impacting extrusion and innovative technologies such as co-extrusion with fillings rich in nutritious ingredients or vacuum extrusion are key to further improve the nutritional profile of extruded cereal-based products. Physical modifications of whole grain or its constituents may also be an option to limit its negative impact on the sensory experience, providing the resulting solution is affordable.

But also the pasta category is facing increasing changes related to nutrition. Intolerance to gluten and the common belief that gluten-free diets are healthier forces the industry to explore other sources of cereals than wheat and even so-called pseudocereals. Maize and rice are generally used as replacers delivering similar taste and texture to wheat-based pasta. The most recent striking revolution in the pasta world is the switch in consumption habits from in-home to out-of-home and on the go. Similar to ‘wine bars’, pasta can now be consumed in dedicated ‘pasta bars’. As part of the same trend, ‘pasta or lunch boxes’ containing pre-cooked pasta can be found in retail. Ensuring pre-cooked pasta with a texture that is close to that of freshly cooked pasta is a challenge that the industry is successfully overcoming.

10.6 Sources of further information and advice

This chapter aims at provided essential insight on the texturization of breakfast cereals and extruded products. Further information concerning the composition of the wheat grain can be found in Cornell and Hoveling (1998) and in Kulp and Ponte (2000). An extensive review of starch properties and its applications is given by BeMiller and Whistler (2009). More information on the chemistry and physicochemical properties of wheat gluten can be found in Cornell and Hoveling (1998). The review of Belton (1999) also provides valuable insight concerning the viscoelastic properties of gluten in dough-based products. Further data on pasta technologies are included in Manthey and Twongly (2006) and Dexter (2004). More information related to the formulation of gluten-free products can be obtained from Arendt and Dal Bello (2008). Guy and Horne (1988) and Guy (2001) provide fundamental knowledge regarding extrusion-cooking of cereal products. A comprehensive review of expansion mechanisms and impact of food ingredients on extruded products is further given in the review of Chinnaswamy (1993) and Moraru and Kokini (2003). More information on whole-grain foods and dietary fibres' health claims can be found on the websites of the European Food and Safety Authority (EFSA) and Food and Drug Administration (FDA). Project HEALTHGRAIN is part of the EU 6th Framework Programme and is aiming at exploiting the bioactivity of European cereal grains for improving nutrition and health benefits. An extensive set of information on whole-grain properties, definition and nutrition can be found on the website related to this project (www.healthgrain.org). The American Association of Cereal Chemists is a non-profit organization providing research, education, superior technical service and advocacy in the area of cereal grain science. Further information can be found on their website (http://www.aaccnet.org). Among the non-exhaustive list of top research institutes, the Institute of Process Engineering in Life Sciences at the Karlsruhe Institute of Technology (Germany) and the Texas A&M University (USA) are publishing extensive scientific work on food extrusion. The Whistler Center for Carbohydrate Research in West Lafayette (Indiana, USA) focuses research on the physicochemical properties of starch and cereals. INRA Montpelier (Institute of Agropolymer Engineering and Emerging Technologies, France) is a research institute focusing on properties of cereal grain components.

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Texture modification of soy-based products

11

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11.1 Introduction

Soybeans (*Glycine max* (L.) Merrill), an annual plant native to East Asia, have now become one of the most globally important crops. As the potential applications in food, health benefits, and economic value of soybeans were gradually realized in the last century, soybeans were cultivated worldwide, and their production kept increasing. At present, the main producers, including the United States, Brazil, Argentina, China, and India, provide nearly 90% of the world's soybeans (Singh et al., 2008). Besides being crushed into soy meal and edible oil, soybeans have been consumed as a staple food since ancient times in East Asia, particularly China. Across the ages, soy-based products, including soymilk, soy sauce, miso, tofu, and texturized vegetable protein, have been developed in the Oriental diet. The nutritional value and texture that are delivered to people by these foods are responsible for their long history and wide influence.

Food can be recognized as the vehicle of nutrients, which is assembled by biopolymers such as proteins, polysaccharides, and oils in different length scales. High protein (40% dry weight) and high oil content (20% dry weight) is a distinguishing feature of soybeans among the major stable foods (Kinsella, 1979). Of the two main components in soybeans, soy proteins, which exhibit excellent emulsifying, gelation, and foaming properties during processing, closely correlate with the construction of the microstructure and the resulting texture in soy-based products (Hermansson, 1986; Molina et al., 2001; Were et al., 1997). Heat treatments for the sake of sterilization, inactivating the antinutritional factors, and improving the digestibility and the functional properties of the proteins are inevitably involved in the processing of fermented and nonfermented, liquid and semisolid soy-based foods (Wilgus et al., 1936). Meanwhile, soy proteins are sensitive to the change of temperature. Once beyond the denature temperature, they tend to unfold and consequently aggregate along different pathways according to the applied processing conditions such as temperature, heating time, pH, and ionic strength. The outcome of the aggregation, including the size and shape of the resulted protein particles and the microstructure of the obtained protein network, greatly affects the attributes and quality of the soy-based products. In the past, soybean products were prepared by means of the experience that had been accumulated for years. As research has continued in recent years, food scientists try to find out the connection between the aggregation behavior of soy proteins and the texture of the soy-based products. This gives us an opportunity to get an insight into the production of the soy-based products and improve their quality and extend the application of soybean foods.

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11.2 Soy-based products and compositions in soybeans

11.2.1 Soy-based products

Soy-based products refer to the foods that are made from soybeans via various processing methods. With thousand years of development, a great variety of soy-based products can be found in the Eastern daily diet. Screen cleaning, soaking, milling, and cooking are the essential procedures for converting beans into different traditional soy-based products. Depending on whether microbes are introduced during processing, soy-based products can be classified into two categories: fermented and nonfermented products. Among them, liquid and semisolid foods are more common. Traditionally produced soy-based products used to be prepared with primitive facilities and heavy labor intensity. Since mechanization and automation, the level of the equipment was drastically improved, and soy-based foods are now manufactured in much more efficient ways, and the quality of the products is able to be controlled more precisely.

11.2.2 Compositions in soybean

Besides the improvement of the facilities, the enhancement of the product quality is also due to better understanding of the components in soybean and their physicochemical properties. Prior to being the material for food processing, soybeans are the seeds that are made up of seed coat and embryo (Pratap et al., 2012). The chemical components stored in soybeans are prepared for the germination. As a result, a variety of nutrients, proteins, oil, carbohydrates, minerals, and vitamins are present in soybeans. Soybeans contain about 20% oil (dry weight), which makes them an important oilseed crop (Osborne and Mendel, 1917). After they are subjected to crushers and solvent-extraction, soybean oil, which is regularly used in cooking, food processing, and industrial applications, is separated from the meals. Substantial unsaturated fatty acids (about 60%) that are a benefit for health were found in the soybean oil (Deckelbaum and Torrejon, 2012). Phospholipids (about 1.5%), which is a valuable component in soybean oil, play an important role in the emulsifying properties of soy-based products. The lipophilic components affect the flavor of the soy-based products to a considerable extent. Carbohydrates that account for about 30% of the dry weight are another major constituent in soybeans. The insoluble fractions that belong to dietary fibers constitute the seed coat. The rest of the soluble fractions act as the source of carbon, which can be utilized during fermentation (Chen et al., 2012). The phytochemicals in soybeans are involved in the health issue, which is brought by the consumption of soybeans and their products (Setchell, 1998). Isoflavones and saponins have been proven to be associated with health benefits for reducing the prevalence of various chronic diseases. Trypsin inhibitors, phytic acid, and lectins, which are classified as antinutritional factors, are the targets to be inactivated and minimized during the processing of soybean-based foods (Liener, 1994).

11.2.3 Soy proteins

Among all the ingredients in soybeans mentioned earlier, soy proteins are recognized to play the leading role in the nutritional value and the texture of the soy-based products. Protein accounts for about 40% of dry weight of soybeans (Osborne and

Mendel, 1917). Moreover, soy proteins are able to provide a great deal of essential amino acids to human beings and possess a high Protein Digestibility Corrected Amino Acid Score, which is very close to the animal proteins such as meat, milk, and eggs (Schaafsma, 2000). This makes soy proteins a good substituent for protein products derived from animals. Due to the quantity and quality of soy proteins, soy-based foods have been an important source of protein intake for human beings, particularly the people in East Asia and vegetarians.

About 90% of the proteins in soybeans exist as storage protein (Kinsella, 1979). Early in the 1960s, Wolf et al. (1961) attempted to separate soy proteins via ultracentrifuge for the first time. According to the ultracentrifugal pattern, four fractions of globulins with different sedimentation constants of about 2, 7, 11, and 15S were present. Each fraction consists of proteins with similar molecular weight. The globulins in 7S and 11S fractions, the two typical representatives of the legume proteins (vicilin-like and legumin-like storage protein), are the major constituent of soy proteins (Doyle et al., 1986).

β-Conglycinin, hemagglutinin, lipoxygenase, and β-amylase can be tracked in the 7S fraction. β-Conglycinin with a molecular mass of about 180–210 kDa, which is a trimeric glycoprotein consisting of three subunits, α (67 kDa), α′ (71 kDa), and β (50 kDa), comprises most of the 7S globulin (Thanh and Shibasaki, 1978a,b). These subunits are all N-glycosylated and contain core regions (Maruyama et al., 1998). The extension regions that play a key role in preventing protein aggregation were only found in α and α′ subunits (Maruyama et al., 2002). Seven types of β-conglycinin that were assembled by different subunit combinations via hydrophobic interactions have been identified, including αα′β, α′ββ, αββ, ααβ, ααα′, ααα, and βββ. It has been demonstrated that the consumption of soy proteins together with isoflavones is able to efficiently reduce the concentration of cholesterol, which is beneficial for prevention of cardiovascular disease. Consequently, the U.S. FDA has approved a health claim for soy proteins. And β-conglycinin dominated this effect in soy proteins (Duranti et al., 2004; Lovati et al., 1996).

Unlike the 7S fraction, glycinin with a molecular mass of about 360 kDa is the single component in the 11S fraction. Glycinin is a hexamer composed of acidic (35–45 kDa) and basic polypeptides (22 kDa) linked by disulfide bonds (Badley et al., 1975; Moreira et al., 1981; Staswick et al., 1981). Five specific acidic–basic subunit pairs ($A_{1b}B_2$, A_2B_{1a}, $A_{1a}B_{1b}$, $A_5A_4B_3$, and A_3B_4) that assembled into glycinin are composed of six acidic polypeptides (A_{1a}, A_{1b}, A_2, A_3, A_4, and A_5) and five basic polypeptides (B_{1a}, B_{1b}, B_2, B_3, and B_4; Maruyama et al., 2003).

11.2.4 Thermal aggregation behavior of soy proteins

As the building blocks of protein, the diversity of the amino acid residues renders proteins amphiphilicity. The arrangement of the hydrophilic and hydrophobic groups on the surface of the molecules has a direct impact on the conformation, assembly pathway, and interfacial properties of the protein molecules. The forces that stabilize the conformation of the protein molecule are disturbed under the actions of heat, pressure, shearing, the change of pH, and ionic strength during processing. Once the actions are removed, the rearrangement of the groups leads to the growth of the protein particles,

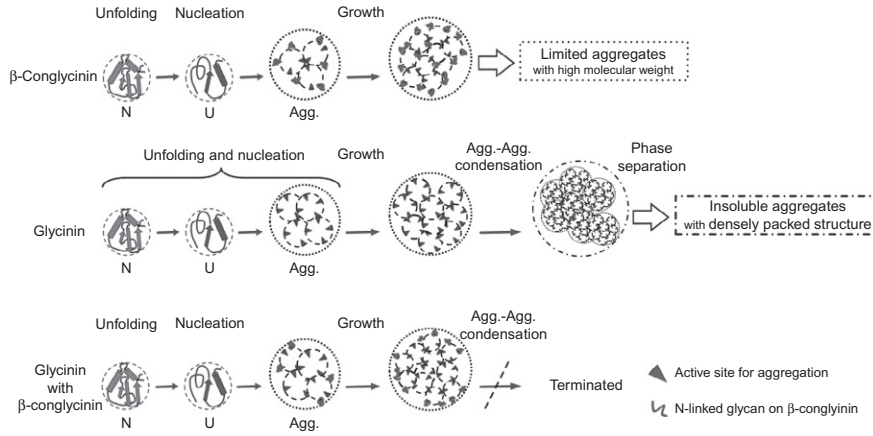

Figure 11.1 Diagrammatic depiction of β-conglycinin and glycinin thermal aggregation behavior at pH 7.0. N, native state; U, unfolded state; Agg., aggregates (Guo et al., 2012a).

gelation, emulsifying, and foaming properties. Therefore, understanding the kinetics of soy protein aggregation and the structure of the aggregates during heating is of importance for manipulating the quality of the products (Figure 11.1).

Different performances were observed when β-conglycinin and glycinin were heated individually (Damodaran and Kinsella, 1982; Wolf and Tamura, 1969). At neutral pH, the solubility of β-conglycinin was not altered by thermal treatments. The opposite situation occurred in the case of glycinin. It was often thought to be responsible for the poor thermal stability of soy proteins. However, it was found that the solubility of glycinin could be improved when it was heated with β-conglycinin. These phenomena were attributed to the aggregation behaviors of the two globulins. Guo et al. (2012a) had tried to elucidate these behaviors, applying the model of Lumry–Eyring nucleated polymerization. As indicated in this model, protein aggregation experienced five stages including conformational change, prenucleation, nucleation, polymerization, and condensation. The N-linked glycans and extension regions in β-conglycinin effectively limited the aggregation degree of β-conglycinin. The growth of β-conglycinin aggregates, which was by means of consuming "monomers," was stopped at the stage of polymerization. The aggregation of glycinin had gone through all the stages, particularly condensation. In this advanced stage, the association between the aggregates that eventually led to the formation of insoluble materials was involved. By contrast, β-conglycinin aggregates that were assembled by less monomers possessed a limited size and a less compact structure. Accordingly, β-conglycinin remained in the supernatant after it was subjected to heat treatment. Without the help of hydrophilic groups that could provide sufficient repulsive barriers among the molecules, the aggregation degree of glycinin was much higher than that of β-conglycinin. Significant increase of the particle size and density in the core made glycinin aggregates appear as precipitate after heated. When heated together, β-conglycinin/glycinin complex was formed via the hydrophobic interaction between them. As the active sites for further aggregation were covered

by β-conglycinin, the aggregation of glycinin was restricted and terminated at the polymerization stage. Therefore, proteins in common soy-based products that contain both of the two globulins often exhibit good thermal stability when the pH was away from their isoelectric point (p*I*).

11.3 Texture and viscosity of soymilk

11.3.1 Soymilk

Soymilk is a traditional vegetable protein beverage, which is made from whole soybeans. It is originated in China and is now becoming more and more popular in the diets around the world. As it is named "soymilk," its protein content is close to that of cow's milk. Meanwhile, some phytochemicals in soybeans that provide health benefits are brought into soymilk. Therefore, it is regarded as a natural substituent of milk for adults who are intolerant of cow's milk.

Inactivating the antinutritional factors, removal of beany flavor derived from the action of lipoxygenase, and providing fine and smooth mouthfeel are the three main challenges for soymilk manufacture. The first two aspects are associated with the nutritional value and aroma of soymilk. The latter one is relevant to the size distribution of the particles that exist in soymilk and the rheological properties of the dispersion. A series of procedures are employed to make dry beans into this healthy and tasty drink. Soybeans are first soaked in water to allow a complete hydration of the protein and other components. After this mixture is ground into a slurry, filtration or centrifugation is conducted to separate soymilk and okara (solid residue). Thermal treatment with the purpose of destroying the antinutritional factors and sterilizing for some microbial in soymilk is carried out during processing. Homogenization helps to minimize the size of the particles and narrow their size distribution in soymilk, improving the mouthfeel of the drink and making the particles stable in suspension during storage.

In these operations, heat treatment receives most attention in modifying the quality of soymilk, and it is the most practical way to achieve this goal. The transition of the conformation and the aggregation behavior of the soy proteins during heating determine the texture. In the heating process, the arrangement of the soy protein subunits occurs. The α and α′ subunits in β-conglycinin and the acidic polypeptides in glycinin, which possess abundant hydrophilic groups and provide sufficient repulsive barriers, are disassociated and located outside the polymerized basic polypeptides (Ren et al., 2009). Due to the formation of this β-conglycinin/glycinin complex, soymilk remains a stable suspension even though it is comprised of denatured proteins. The procedure and equipment for heating was thought to affect the viscosity of soymilk (Liu and Chang, 2007). Heat treatment can be thought to be a procedure for protein particle growing, which is not favorable for the particle stability and producing smooth texture. However, homogenization focuses on reducing the particle size via mechanical forces. It not only decreases the size of the protein aggregates, but also minimizes the oil droplet size (Cruz et al., 2007).

As several procedures are involved during soymilk processing, some of other methods and parameters also influence the viscosity of the resulted products. The

work of Vishwanathana et al. (2011) revealed that the application of various grinders such as mixer grinder, stone grinder, and colloid mill and different temperatures and durations of soaking had a profound effect on the particle size, protein recovery, and viscosity of soymilk. Furthermore, the formula of soymilk also affects its texture. The viscosity of soymilk is sensitive to the change of protein concentration in soymilk (Liu and Chang, 2007). When the protein concentration exceeded a critical value, a significant increase of the viscosity was observed (Ringgenberg et al., 2012). Moreover, a modest addition of vegetable oil might increase the viscosity of soymilk and offer a smoother mouthfeel to the customers.

11.3.2 Fermented soymilk

To form varying types of texture, increase the aroma, improve the flavor, and improve nourishment utilization efficiency of food, microorganisms are often employed in food processing. Yogurt is a fermented dairy product obtained via the bacterial fermentation of milk. When milk is substituted by soymilk, soy yogurt is produced. The operations of microbials mask the beany flavor in soymilk (Hou et al., 2000) and convert isoflavones into aglycones, which are able to offer more health benefits to human beings (Lee et al., 2013; Tsangalis et al., 2002). More important, the texture of soymilk is modified after the fermentation. With a similar situation to milk, the components in soymilk encouraged the growth of specific bacterials, including *Lactobacillus*, *Streptococcus thermophilus*, and *Bifidobacterium* (Leblanc et al., 2004; Tsangalis et al., 2003). The micro-organisms consume the carbohydrates and some flatulence factors producing acids such as acetic acid and lactic acid (Hou et al., 2000). The resulting acidification and decrease of pH lead to the aggregation of soy proteins and their hydrolyzates. The consequent microstructure determines the texture of the resulting products. A smoothie-like consistency would be an expected sensory experience for soy yogurt. It is considered a joint result of the rheological, particle size, and tribological characteristics (Sonne et al., 2014). Modification to soymilk and the addition of stabilizer had been conducted to improve the texture of soy yogurt. Cruz et al. (2009) revealed that homogenization with ultrahigh pressure conducted to soymilk helped to improve the firmness, deformable behavior, and water-holding capacity of the resulted soy yogurt. Some hydrophilic gums such as xanthan gum, locust bean gum, guar gum, and carboxymethylcellulose usually act as thickeners or stabilizers in liquid and semisolid foods. El-Sayed et al. (2002) reported that the presence of these gums did not disturb the growth of the bacterial in soymilk. Adding gums either singly or in combination markedly increased the viscosity of the fermented product. The soy yogurt with xanthan gum gained higher sensory scores than the soy yogurts treated with other gums in this work.

However, as a fermented product, the starter culture is the soul of soy yogurt. The growth and metabolism of the microbial during fermentation are the dominating factors for the protein aggregation behavior and the texture of resulted soy yogurt. Not all the yogurt starter cultures are able to make full use of the components in soymilk. The mismatch of the substrate and the microbial certainly leads to the slow growth of the microbial and lower acidification rate. As a result, longer fermentation time, weaker

gel hardness, the growth of undesired microbials, and unfavorable aroma and flavor greatly reduce customers' acceptance. Screening suitable microorganism for the fermentation of soymilk is of great important. Perfect match of substrate and the starter culture is the prerequisite for producing soy yogurt with desired quality. The fermentation that was employed mixed cultures, including the use of kefir, could be completed in a shorter time, and it produced soy yogurts with higher gel hardness and better quality when compared to the products with a single culture (Liu and Lin, 2000; Mishra and Mishra, 2013). As protein is the major component in soybean, the type and amount of carbohydrates in soymilk might not meet the requirements for fermentation. Carbohydrate supplements such as glucose, inulin, lactose, sucrose, and fructooligosaccharide were found to be effective in enhancing the acidification rate during fermentation, and the resulting soy yogurt showed more viscous and pseudoplastic properties than the control soy yogurts (Donkor et al., 2007; Liu and Lin, 2000; Mishra and Mishra, 2013; Tsangalis et al., 2003).

As mentioned earlier, some polysaccharides that are often used as thickeners or stabilizers can modify the flow characteristics of fluids and stabilize the particles in suspensions. However, not only the supplementary polysaccharides but also the polysaccharides produced during fermentation were able to modify the texture of soy yogurt. Feng et al. (2012) isolated a high exopolysaccharide (EPS) producing lactic acid bacteria (LAB) strain, and Li et al. (2014) used it as a starter culture for soy yogurt producing. It was found that the water-holding capacity and the apparent viscosity of the resulted soy yogurt were significantly higher than those of the products with the cultures that produced less EPS. The application of biomacromolecule producing starter culture is another effective strategy for modifying the consistency and rheology of soy yogurt.

11.4 Texture of tofu

11.4.1 Tofu

Tofu, a transliteration from Chinese, refers to a traditional soy-based product bean curd. Originated in China, tofu has been prevailing in the cuisine based on the Oriental culture for centuries. It is a food made by coagulating cooked soymilk and then pressing it into soft curds. Retaining most of the nutrients in soymilk, tofu has a bland flavor and white color and provides a texture that is smooth, firm, and coherent, but not hard or rubbery (Poysa and Woodrow, 2002). People in East Asia and vegetarians use various cooking methods and recipes to turn tofu into delicious dishes.

The preparation of tofu is an old industry. Although the technology and equipment have gained considerable development, it still follows the traditional production principle. Whole soybean is usually used as the starting material. Soaking, grinding, and boiling are conducted in sequence to prepare soymilk. Then, the coagulant is added, and the soymilk is left to stabilize. After this, the resulting curds are subjected to pressing, and the product of tofu is obtained.

When we look on this traditional food with current knowledge, tofu can be regarded as a hydrogel, which is supported by soy proteins. The coagulating of soymilk, the key step in tofu making, is thought to be the gelation of soy proteins. Hydrogels are water-swollen polymeric materials with a three-dimensional network structure that depends on covalent or noncovalent interactions between macromolecules (Clark and Ross-Murphy, 2009). While preparing soymilk, proteins in soybean are first extracted into the aqueous medium. The subsequent boiling procedure unfolds the conformation of the proteins and makes them the building blocks for the formation of gel network. As the interactions among these blocks are strengthened or produced by the coagulants, the blocks assemble into a three-dimensional network. As a result, the water in soymilk is fixed in this structure.

The texture of tofu depends largely on the network structure that is sustained by soy proteins. A variety of factors are involved in the assembly of the gel network. The addition of different coagulant results in tofu with distinctive texture. Two types of coagulants, calcium ion and acidifier, are now used commercially. For centuries, calcium coagulants such as bittern (calcium chloride type) and gypsum (calcium sulfate type) have been used to prepare Chinese-style tofu, which is made up of a relatively firmer network. As we know, soy proteins are sensitive to the divalent metal ions, especially the calcium ion. Small amounts of calcium ion can lead to the formation of protein cluster in soymilk. When the calcium ion appears in soymilk, the electric barriers that are used to prevent the approaching of the unfolded protein particles are weakened. The calcium ions act as bridges between the adjacent charged carboxylic groups on neighboring protein molecules and cause them to aggregate (Kao et al., 2003). As sufficient soy proteins that serve as building blocks and proper amounts of calcium ions that are able to produce enough bridges are present, a homogenous gel network rather than clusters is formed.

Glucono-δ-lactone (GDL), which is a kind of acidifier, is often employed to produce Japanese-style tofu with relatively more tender and smooth texture. When compared to the calcium coagulant, the application of GDL possesses a much shorter history. Once GDL is added to soymilk, the gradual release of hydrogen ions makes the pH of soymilk approach the p*I* of soy proteins. As the negative charges of the denatured soy proteins are neutralized, the attractive interactions that are attributed to hydrophobic and hydrogen bonding take the place of electrostatic repulsion. Under this situation, the protein particles get close together, and aggregation occurs. Eventually the three-dimensional network is formed (Liu et al., 2004; Ringgenberg et al., 2013).

When hydrochloric acid is used as an acidifier, protein precipitates rather than continuous gel is obtained in soymilk. In this case, the aggregation of soy proteins also occurs. The building blocks assemble into aggregates with irregular structure along various path ways, which is unable to form a homogeneous gel network. This implies that the rate of acidification plays an important role in the formation of the gel network. Aggregation of the denatured proteins definitely happens during acidification. The addition of hydrochloric acid brings an acidification with excessive speed to soymilk. There is not enough time and chance for the soy proteins to rearrange and build up the gel network. For GDL, different additions in relation to the acidification rate result in tofu with different microstructures and texture (Kohyama et al., 1992). Harder tofu was obtained when more GDL was involved during preparation. Softer tofu with better

water-holding capacity was prepared with a lesser amount of GDL. A similar situation occurred to tofu with calcium coagulants. It has been revealed that different calcium salts also have the difference coagulation process (Zhang et al., 2013). Tofu coagulated by calcium sulfate, which is less soluble in water, possessed a much finer and uniform honeycomb-like structure than the tofu prepared with calcium chloride (deMan et al., 1986; Kao et al., 2003; Tsai et al., 1981).

Calcium ions and GDL are the most widely used coagulants in the preparation of tofu. There are still other types of methods and coagulants for inducing the gelation of soy proteins. Heat-induced gelation is a very important property for preparing soy-based products (Kang et al., 1991; Puppo and Añón, 1998). Heating is the prerequisite for tofu making. Aggregation of soy proteins occurs inevitably in this procedure (Guo et al., 2012a), and blocks for gelation are prepared. When the heated soymilk is cooled, the rearrangements induced by the attractive interactions among the blocks occur (Puppo and Añón, 1998; Renkema and van Vliet, 2002). As the concentration of soy proteins and the heating temperature meet certain requirements, the structure that is able to immobilize free water is produced at this time (Liu et al., 2004; Nagano et al., 1992). Furthermore, microbial transglutaminase (MTGase), which catalyzes an acyl transfer reaction between the γ-carbonyl group of a glutamine residue and the ε-amino group of a lysine residue and results in the covalent cross-linking of proteins (Motoki and Seguro, 1998), had been shown to effectively induce soymilk with a certain level of solid content to form tofu (Tang et al., 2007). Because the denatured protein blocks are stuck together and form a gel network by means of this covalent interaction, the resulting gel exhibits better deformation capacity and toughness than the soy protein gels induced by calcium ion and GDL.

A series of procedures are employed in the preparation of tofu, and its texture depends on the complex interrelationship of many variables (Shih et al., 1997). It had been concluded that they were classified into four categories: soybean characteristics, soymilk processing variables, coagulant characteristics, and coagulating conditions (Liu and Chang, 2004). It was thought that soybean cultivar played an important role in the quality of tofu (Kim and Wicker, 2005; Stanojevic et al., 2011). Tofu made from different soybeans might give people different texture perceptions. In fact, this is mainly due to the content of 11S globulin and the ratio of 11S to 7S globulins (Cai and Chang, 1999). These two major components in soy proteins have different gelation characteristics. Possessing considerable amounts of hydrophilic groups such as glycan and extension regions that do not appear in 11S globulin, aggregation of 7S globulin is limited to a great extent when compared to 11S globulin. It has been reported that the gels of 11S globulin induced by different coagulants including calcium ion, GDL, heating, and MTGase are significantly firmer than those corresponding gels made from 7S globulin (Guo and Ono, 2005; Kohyama and Nishinari, 1993; Renkema et al., 2001; Tang et al., 2007). Inappropriate storage conditions such as high temperature and high humidity decrease the solubility of the proteins in soybean. Less proteins are extracted into soymilk and participate the formation of gel network, leading to the decline of the yield and quality of tofu (Hou and Chang, 2003, 2004; Kong et al., 2008). The pressing procedure applied to soybean curd during processing into tofu has a profound effect on the moisture content, yield, and some textural parameters of tofu, especially the Chinese-style tofu

(Gandhi and Bourne, 1988). With proper pressure, desired texture of tofu could be made. Furthermore, coagulating conditions that refer to the temperature, pH, and ionic strength during coagulating also affect profoundly the yield and textural properties of the resulting tofu (Puppo and Añón, 1998).

11.4.2 Novel soy protein-based gels

To extend the texture range of edible gels and build up new models to study the relationship between food structure and texture perception, soy proteins, including soy protein isolate (SPI) and 11S globulin, isolated from soy meal a by-product of soy oil processing, are used as the starting material to fabricate edible gels with polysaccharides using novel strategies.

Proteins and polysaccharides, which are the two most commonly found hydrocolloids in food, are often used to manipulate food texture. The coexistence of the two biomacromolecules is bound to the occurrence of phase separation (Tolstoguzov, 1991). The texture of the resulting gel depends more on the biopolymer, which plays the dominant role in the phase-separated microstructure. Changing the ratio of soy proteins to polysaccharides is a simple strategy to obtain various microstructures and prepare soy protein-based gels with specified texture (Zhu et al., 2008). Protein/polysaccharide mixed gels are often fabricated by the gelation of either the protein or the polysaccharide constituent. Food gels with more than one gel network have been seldom reported. Guo et al. (2014) applied a sequential ionic–covalent gelation method to fabricate edible gels with double network structure. In this work, gellan gum and SPI were cross-linked by potassium chloride and MTGase one after another. This double network system combined the structural and mechanical characteristics of the gellan gum and SPI networks. Gels with distinctive mechanical and oral processing properties were prepared by regulating the composition ratios of gellan gum to SPI.

Protein gels can be divided roughly into two types, fine-stranded and coarse aggregated gels (Hermansson, 1986). The former network that may be transparent is formed by association of molecules into strands in a more ordered way, whereas the latter that is nontransparent is formed by "random" aggregation. The difference between these two gels is not only the extent of transparency, but also the aggregation degree. Increasing the extent of protein aggregation results in a firmer gel network, but the grown aggregates and strands inevitably decreases the transmittance of the gel. Therefore, enhancing the transparency and firmness of the protein gel simultaneously seems to be contradictory. Guo et al. (2012b) used a two-step strategy for preparing soy protein-based hydrogel with transparent appearance and firm network. Soy 11S globulin and dextran sulfate (DS), an anionic polysaccharide, were subjected to heating to unfold the protein molecules and form highly changed protein/polysaccharide complexes. As these blocks were separated by the electrostatic barrier provided by DS, MTGase was introduced to cross-link the complexes and create a covalent gel network. The transparency and firmness of the resulted gel increased with the increase of DS addition (Figure 11.2).

Gels with pore sizes in the micron range have been widely used in tissue engineering, drug delivery, and superabsorbent products. Synthetic polymers, chemical cross-linking

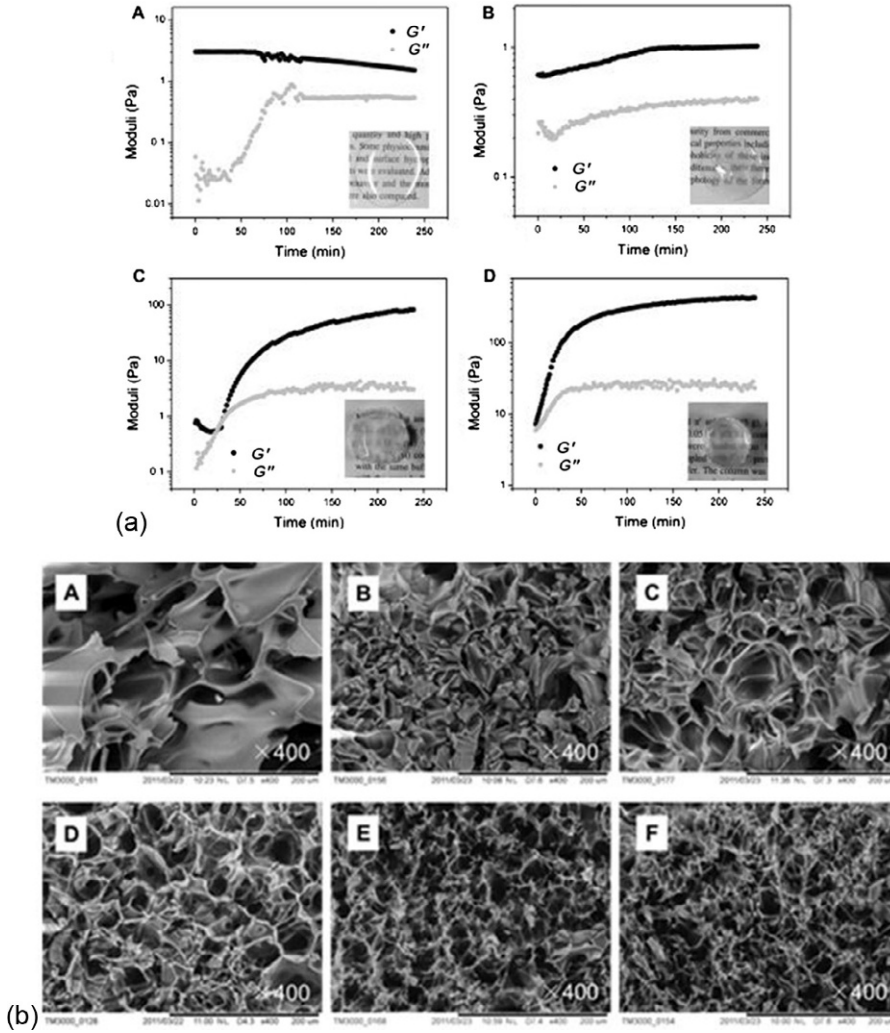

Figure 11.2 Time sweep rheological profiles (a) of glycinin hydrogels prepared in different ways (A, glycinin; B, preheated glycinin; C, glycinin with DS; D, preheated glycinin with DS) and SEM images (b) of glycinin hydrogels cross-linked by MTGase with various amounts of DS (weight ratio of DS:glycinin: A, 0; B, 1/100; C, 1/75; D, 1/50; E, 1/30; F, 1/10) (Guo et al., 2012b).

reagents, and complicated and time-consuming procedures are required in the fabrications of these porous gels. These might not be suitable for producing similar structures in the food industry. Guo et al. (2013) developed a fast and simple strategy for preparing controllable porous architecture in soy protein-based gel. It was based on the foaming and gelation characteristics of soy proteins. The preheated SPI dispersion with MTGase was subjected to high-speed homogenization. Plenty of foams that acted as porogen templates were produced during homogenizing. Meanwhile, the substrates were

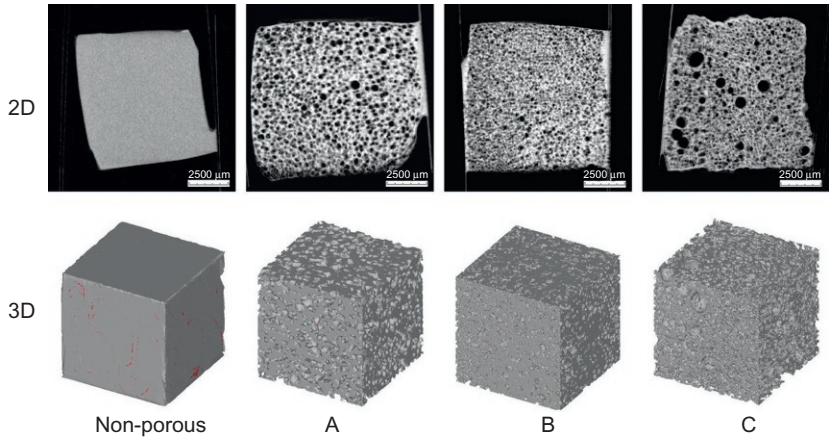

Figure 11.3 Two-dimensional (2D) X-ray microtomography section images and three-dimensional (3D) reconstructions of the porous soy protein hydrogels prepared by different additions of MTGase. A, 5.33 U MTGase/g SPI; B, 6.67 U MTGase/g SPI; C, 8.00 U MTGase/g SPI (Guo et al., 2013).

thoroughly exposed to the enzyme, and the gelation of soy protein was quickly accomplished. As a result, the porous structure was obtained. With this strategy, soy protein gels with controllable porous architecture are able to be prepared in a fast and simple way (Figure 11.3).

11.5 Fermented soy-based flavorings

11.5.1 Soy sauce

Soy sauce, which is a fermented soy product, has long been an indispensable condiment in the kitchen or on the table of the families and restaurants in East Asian countries. With a deep reddish-brown and liquid-like appearance, soy sauce is used for enhancing the umami taste and adding color to the foods in the cuisines derived from various cultures. Soybeans together with wheat and brine are the starting materials for this food seasoning. Four major stages, including soybean meal steaming, koji-making, brine fermentation, and sauce extraction with the help of the microflora which consists of *Aspergillus oryzae*, salt-tolerant yeast and bacteria, converted the ingredients in the starting materials into the flavor and aroma compounds (Cui et al., 2014; Qi et al., 2014; Yan et al., 2013). The fabrication of soy sauce is greatly influenced by the local food culture and food ingredients. Despite similar appearances, soy sauces from different regions exhibit various tastes and aromas. However, the producers and customers are focused more on the flavor brought by soy sauce. For the ones that are more often used during cooking, such as dark soy sauce, starch is added to thicken the brewed soy sauce.

11.5.2 Fermented soybean paste

Fermented soybean paste is another category of flavorings that has acted as an essential assistant in kitchen cooking in Oriental countries for hundreds of years. The raw material and procedures for preparing soy paste are similar to those for soy sauce. Soaked and steam cooked soybean seeds together with various supplementary cereals are used to produce different soy pastes (Zhao et al, 2011). The pastes in China are mostly made from soybeans and wheat flour. Rice or barley is often involved in the production of the Japanese soy paste miso, which is mainly used as the base for soups (Fukushima, 1979). Two fermentation processes are employed during the production of these soy pastes (Peng et al., 2014). *A. oryzae* is inoculated to the cooked grains to make koji. The prepared koji is mixed with soybeans and salt, leading to the second fermentation stage. With the activity of yeast and LAB, proteins and carbohydrates in soybeans are hydrolyzed. As a result, the amino acids that are regarded as umami enhancer are released, and the mixture becomes a semisolid paste that is smooth in texture (Kumazawa et al., 2013; Ogasawara et al., 2006).

11.5.3 Natto

Natto is a traditional fermented soy-based product from Japan. The health benefits and palatability provided by natto have been enjoyed by the local people for centuries (Villares et al., 2011). Unlike soy sauce and soy paste, the characteristic odor, flavor, and texture of natto make it more than a taste enhancer. Also, the preparation of natto is more convenient and takes less time when compared to the other two seasonings mentioned earlier (Fukushima, 1979). Prior to fermentation, the whole bean is subjected to dehulling, soaking, and cooking, which are essential in the preparation of soy products, turning its components into a state that can be utilized by the microbial more efficiently. It is then inoculated with *Bacillus subtilis*, which comes from rice straw (Murooka and Yamshita, 2008), and followed by fermentation for up to 20 h. Afterward, the mixture is cooled and aged in a refrigerator. In the natto products, the intact soybeans with palatably soft texture retain their original shape. They are covered with a white-colored mucous substance and have a slimy appearance (Hu et al., 2010). The silky and sticky mass is the result of the formation of poly-γ-glutamic acid during fermentation (Ho et al., 2006). For a natto product with good quality, its texture should be neither too firm nor too soft, and the beans are able to cling well to the chopsticks when using them to stir the natto product (Hu et al., 2010; Yoshikawa et al., 2014). It has been revealed that the natto sensory preference was positively correlated with sucrose and oil content in soybean, but negatively correlated with seed hardness, protein, calcium, manganese, and boron contents (Yoshikawa et al., 2014). Furthermore, a prolonged fermentation could lead to firmer texture, higher concentration of ammonia, and lower quantity of mucilage, which decreases the quality of natto (Wei et al., 2001). It has been shown that extending the steam cooking time can reduce fermentation time and obtain a good-quality natto product (Wei et al., 2001).

11.6 Future trends

Food is responsible for providing nutrients, energy, and pleasure to humans. As a necessity in our life, food receives more attention due to its responsibility of sustaining our lives. The texture, aroma, and flavor of food is a source of pleasure. Although it is a pursuit on a higher level, the demand for producing tasty food that is able to bring more joy to people keeps growing all around the world.

As mentioned earlier in this chapter, food texture is related to the assembly of the components in food and the resulted microstructure. Recent studies have revealed that the aroma and flavor perception of food were also affected by the architecture in food (Koliandris et al., 2008; Holm et al., 2009; Sala and Stieger, 2013; Sala et al., 2010; Stieger and van de Velde, 2013). Healthy diet is one of the most important issues in food today. How to decrease the consumption of salt, sugar, fat, and flavors in food is a concern by both to customers and manufacturers. It had been proven that fabricating specific food structures can increase the release of salt and sugar during oral processing (Koliandris et al., 2008; Holm et al., 2009; Sala and Stieger, 2013; Sala et al., 2010; Stieger and van de Velde, 2013). This implies that similar flavor perception can be achieved by modifying food microstructure rather than the addition of substituents when less salt and sugar are used.

Soy proteins are the main component that affects the health benefits, structure, and texture in soy-based products. Although they are used to be only responsible for providing nutrients during seed germination, they still exhibit various functionalities after they are subjected to different processing. Modifying the blocks for building network structure, managing the aggregation behavior of proteins, and making use of the interaction between proteins and polysaccharides are practical methods for extending the types of structure and texture in soy-based products. With these strategies, soy-based products with less flavors, including salt and sugar, but similar qualities are able to be prepared.

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