Formulation Engineering of Foods
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Edited by

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1 Introduction to Food Formulation Engineering

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1.1 INTRODUCTION

Food products are often structurally complex. This structure, or microstructure, determines the foods flavour (as a result of tastant or aroma release), its texture and mouthfeel, and the eating pleasure derived from its consumption, in addition to the efficiency of uptake during digestion, the bioavailability of active compounds, and the effect it has on appetite and satiety. With the health issues of the modern age, including the prevalence of obesity, food research is often heavily focused on fat reduction, or methods of reducing the uptake of fat or slowing digestion, whilst maintaining sensory appeal and palatability.

Thus, a combination of understanding of material chemistry and material science is needed, together with an understanding of how processing affects food structure, the science behind food consumption, from oral processing through to digestion, and the impact that food formulation engineering can have on liking, sensory perception, digestion, targeted delivery or appetite. This book aims to provide the reader with detailed reviews of the literature in these areas.

The book is separated into three main sections: 1. Designing Structured Foods, 2. Structure–Human Interaction and 3. Food Structure and the Consumer. In the first part of the book we will consider how basic materials can be used to formulate complex food systems, with specific structures, desirable sensory attributes and health benefits. In the second part we will consider structure–human interaction, and how foods can be designed to get the greatest positive impact (in terms of oral processing and/or digestion) when producing healthier, more convenient, and/or more environmentally friendly products. In the third part we will consider psychology, and the impact that food can have both on liking and acceptability, and appetite and satiety.
1.2 THE BOOK

1.2.1 Designing structured foods

In the first part of this book we will consider the design of foods, and the use of complex structures. We will consider how basic materials (i.e. proteins, polysaccharides and hydrocolloids) can be used to structure foods. We will also consider the use of emulsions (the most common use of fats in foods). This section contains four chapters:

Chapter 2 (Harris and Foegeding) considers the use of proteins in foods, by building food structures that provide desirable sensory (e.g. flavour, taste or texture) and health (e.g. nutrition and bioavailability) attributes. Proteins function by providing amino acids for protein synthesis and energy, providing bioactive peptides, and are also essential for the formation and stabilisation of food structures. During food processing, changes in the protein structure can occur, including denaturation or aggregation, racemisation, or covalent modification of amino acids (e.g. Maillard browning). Proteins are important in many colloidal structures, including sols, emulsions, foams and gels, and can contribute to the stability of these systems by adsorbing at the interface. Proteins are essential for health, but also have a positive impact on satiety, as a result of both sensory cues (e.g. thickness/viscosity or savoury taste) and the macronutrient itself. The goal should be to combine food science and nutrition, to produce “nutritious and delicious” protein-rich foods that are highly nutritious and functional, but also highly palatable and satiating, that consumers would choose to eat.

Chapter 3 (Foster) discusses the use of plant cell wall material (PCWM), a material that is not usually utilised, which may have significant and novel use in food products. PCWM could be used as an alternative to refined polymers, which are often accompanied by tight specifications. It can be split into “inner” fibres (that are able to modify texture) and “outer” fibres (which are a source of high insoluble dietary fibre). Understanding of the processing steps (i.e. enzymatic, thermal, mechanical and chemical), and their effect on the polymers within the PCWM can allow for controlled and reproducible food production. This, in turn, requires an understanding of PCWM at a material and molecular level, in order to redesign or optimise processing. The rheological properties of PCWM are similar to hydrocolloid gel networks, where particle–particle interactions and particle size distributions both determine rheological structure. Furthermore, these materials could be used as surface-active materials for emulsion and foam stabilisation (i.e. as “natural” surfactants). Particularly,
β-glucans, are becoming well characterised, and could be used for fat replacement, or as emulsifiers. β-glucans are interesting because of their functionality (ability to decrease serum cholesterol levels). In taking such an approach, the greater availability of molecules retained within natural fibres can be used to provide natural and healthy food ingredients.

Chapter 4 (Wolf) details the use of hydrocolloids (water soluble gums) in food structures, to impart specific flow and textural properties, either as water continuous foods, or within the aqueous phase of emulsions. Phase separation in hydrocolloid mixtures can result in water-in-water emulsions, which prior to gelation behave like conventional emulsions (similar in size to droplets within classical food emulsions). By controlling shear and temperature at the time of gelation, sheared gels, or fluid gels, can be produced. Gel suspensions, or filled gels, can also be produced in phase separating hydrocolloid mixtures, where one is gelling, to produce systems where the shape of the particle can be controlled. Similarly, when a gelling hydrocolloid is added to the aqueous phase of water-in-oil emulsions, shaped particles can be produced, which can be used in lipid-based food products. Finally, microfluidics (e.g. rotating membrane processing) has also been used to produce monodispersed gel particles. These phenomena can be utilised to influence food structure, to impart specific flow properties, textures or appearances, in order to produce novel food systems.

The final chapter in this section, Chapter 5 (Pawlik, Fryer and Norton), considers the use of emulsions, either in their simple (oil-in-water, or water-in-oil) or more complex forms (duplex emulsions: water-in-oil-in-water, or oil-in-water-in-oil) in foods. Pickering emulsions are stabilised by particles that are thought to be irreversibly adsorbed to the interface. Surface-active crystalline monoglycerides may also stabilise emulsions in a similar way, and by modifying temperature and inducing melting, molecules from the internal droplet may be release. Nanoeemulsions, that have a droplet size of less than 200nm, have many advantages over conventional emulsions, including being transparent and extremely stable against aggregation and gravitational separation. Duplex, or double, emulsions, which are produced in two emulsification steps, also have many benefits, including the advantage of being able to encapsulate ingredients into the internal droplets, which could then be delivered in a controlled way on consumption. Tri-phasic emulsions are aerated systems that contain both oil and air in an aqueous continuous phase, and water-in-water emulsions, which as mentioned above are a result of the phase separation of incompatible protein or polysaccharide solutions, may both be effective methods for fat reduction in foods.
1.2.2 Structure–human interaction

In the second section of the book we consider the interaction that food has with the people consuming it. This involves understanding of the physics of eating, the perception and manipulation of texture, the release of tastant and aroma compounds, lipid digestion, and the encapsulation and targeted delivery of compounds. This section is split into six chapters:

Chapter 6 (Lillford) considers the physics of eating, particularly related to the human masticatory process. This involves chewing (size reduction via mechanical forces, using teeth), mixing (using the tongue), lubrication and dilution (via the addition of saliva), breakdown and reassembly, and the swallowing of a bolus. The act of eating is complex, because the geometry of the device is complex, there is feedback and feedforward regulation of the actions involved, and there is huge variability between individuals. The foods that we consume (natural or processed), also vary considerably, in terms of structure, mechanical properties, such as work to fracture (and subsequent sound emission), particle size, moisture content, fat content, viscosity, phase volume of air and the presence of ice or fat crystals. These properties affect masticatory processing and food breakdown, and can be related to perceived hardness, juiciness, crispness, moistness, smoothness, creaminess, greasiness and so on, but also enjoyment and pleasure. Understanding the physics of eating is important if we are to appreciate the enjoyment associated with particular foods, and if we are to generate new foods that are pleasurable to eat.

Chapter 7 (Le Révérend, Gouseti and Bakalis) focuses on the interaction between food and the oral “machinery”. It begins by describing the current understanding of oral processing, and its relationship with sensory perception (particularly related to our perception of taste and texture). It also discusses our ability to monitor and model oral processing. Both simulating and modeling oral processing can result in the analysis, and prediction, of food transformations occurring during consumption, which in turn could be related to sensory perception. Simulation could be achieved using rheology (to gain an understanding of bulk viscosity), texture analysis, or tribology (which is the measurement of friction and lubrication), which have been related to thickness, viscosity, hardness, or creaminess, for example. Mouth models have also been investigated, that apply mechanical forces to simulate mastication, in the presence of artificial saliva. The interaction between foods and the oral cavity is discussed, particularly the effect that saliva has on emulsion breakdown, and subsequent sensory perception, in addition to the effect of mucoadhesion on perception. An understanding of the pro-
cesses occurring during consumption could allow food products to be designed that have particular textures or tastes, as a result of breakdown partners and their interaction with the oral cavity.

Chapter 8 (Mills) discusses approaches to salt reduction in foods. Whilst salt is essential for human health, excessive amounts can be detrimental, resulting in hypertension and stroke. Salt is one of the five tastes, which relies on the sodium ion component of sodium chloride. Saltiness perception is affected by factors such as the viscosity of the food matrix (as a result of mixing ability and contact with the oral surfaces), the homogeneity of salt distribution, and the release profile. A number of methods to achieve a significant reduction of salt in foods are discussed, including the gradual reduction of sodium, substitution with other salts or glutamates, enhancement with spices or flavourings, or the use of complex microstructures (such as the inhomogeneous distribution of salt in foods, or the use of water-in-oil-in-water emulsions). These technologies could also be combined, in order to produce food products that maintain the sensory appeal and palatability of the saltier foods that consumers have become accustomed to, but that contain less salt, thus having less of a negative impact on health.

Chapter 9 (Linfirth) highlights the importance of understanding volatile aroma release in foods. Aroma molecules vary according to water and fat solubility and intrinsic volatility, both of which affect the way they partition between different phases of foods, and the efficiency of transfer to the breath, so that they can be detected by the nose. The viscosity of the food can affect the delivery of aroma compounds to the nose, although this is also affected by the type of volatile, and individuals’ eating styles. In gelled systems, gel strength could also affect intensity of aroma perception and release profile. Interestingly, inhomogeneous distribution of aroma compounds did not affect intensity or timing of flavour delivery, as was shown in the case of salt. Instead, flavours could be encapsulated, which can protect flavour compounds and alter the release profile. Different trigger mechanisms could also be utilised, such as hydrolysis by enzymes, mechanical fracture and melting. Understanding flavour perception is important when manipulating food structure (either when simply changing aspects of food itself, or when specifically trying to modify flavour delivery), and should be considered when creating new generations of food products.

Chapter 10 (Golding) considers lipid digestion. The immiscibility of lipids with the aqueous digestive environment means that lipid digestion is achieved by the adsorption of enzymes at the oil–water interface, so is affected by the interfacial area and thus the availability of binding
sites. As such, having a colloidal state during gastrointestinal (GI) transit is necessary for fat digestion. Oral processing is the first step in lipid digestion, ensuring at all ingested fat is delivered to the stomach in an emulsified state, and involves mechanical forces, secretion of mucous (containing surface-active compounds that lower surface tension and provide surface elasticity), production of enzymes and thermal normalisation to 37 °C. The conditions of both the stomach (i.e. acidic pH, release of gastric amylase and lipase, gastric motility and mixing, and temperature) and the intestine (e.g. bile salts) affect emulsion structure and stability. The detection of fat results in the secretion of hormones, which slow the rate of gastric emptying (ensuring full digestion and uptake), and suppress hunger. The structure of fats during digestion can be affected by the presence of proteins, emulsifiers and crystalline fat, so that emulsions could be designed to have specific digestive behaviours, such as reduction in uptake or improved delivery of bioactives.

The final chapter in this section, Chapter 11 (Spyropoulos and Nowak), considers the potential for the use of hydrocolloid formulations in novel foods, specifically designed to impact on the functions in the GI tract. Hydrocolloid-based delivery systems for the encapsulation and targeted delivery of nutrients (e.g. vitamins), microbial supplements (probiotics), dietary fibres (prebiotics), lipids or therapeutic species (e.g. drugs) are discussed. The system can be designed for the protection of encapsulated material, and for the delivery to specific parts of the GI tract (e.g. induced by pH). Hydrocolloids themselves, and/or hydrocolloid-based structures, can also have an effect on physical functions in the GI tract. They can affect gastrointestinal transit time, as a result of increased viscosity or gel formation (as a result of acidic or ionic gelation), and absorption rates (as a result of enzymatic activity). Finally, hydrocolloids may have additional benefits, such as the ability to aid in mucosa healing, reduce post-prandial blood glucose levels, reduce cholesterol absorption, and have the ability to bind mutagens and heavy metals present within the intestine, thus reducing carcinogenic effects. There is clearly a potential for the use of hydrocolloids in the fabrication of novel functional food, which could impart significant health benefits through their action at specific parts of the GI tract.

1.2.3 Food structure and the consumer

In the final section of this book we consider psychology, both in terms of liking and the relationship with health-related technologies, and the
impact that either different macronutrients and/or food structure can have on satiety and appetite. This section is split into two chapters:

**Chapter 12** (Norton) explores consumer acceptability, which encompasses liking, palatability, perceived quality, choice and purchase behaviour, and consumption. The sensory characteristics of the food are incredibly important for acceptability, but situational/environmental (e.g. the physical surroundings, or who we are eating with) and cognitive (e.g. expectations) influences also have an impact on liking and acceptability. The chapter also describes the different direct and indirect methods used by researchers for measuring acceptability, including hedonic measures (e.g. liking questions), experimental auctions, eye-tracking and brain imaging. The chapter also considers some of the current food trends (fat reduction, salt reduction, self-structuring and satiety, and functional or personalised foods), bringing together literature around physical science, sensory science and psychology, in order to understand the impact that these findings have on food engineering, and the design of food structures with specific health benefits. It is important to consider consumer acceptability, as not only does this ultimately determine the success of food products, but food products can only have benefits for health if they are chosen, liked and consumed.

**Chapter 13** (Harrold and Halford) discusses within-meal satiation (that determines meal duration and size, and terminates eating) and post-meal satiety (determines the length of post-meal interval), and the effect that macronutrient composition and food structure have on short-term appetite regulation. The satiety cascade highlights the sensory and cognitive factors that contribute to eating behaviour, and the properties of food that influence appetite control. Gut hormones cholecystokinin (CCK), glucagon-like-peptide-1 (GLP-1), peptide YY (PYY) and ghrelin all influence appetite regulation, as does the central nervous system and the brain. There are a number of methods for measuring appetite, including pre-load designs and ad libitum intake, and measures of subjective appetite sensations. The chapter also discusses the satiating effect that different macronutrients (protein, fibre or lipids) have, and also the impact food structure (viscosity, gelation, encapsulation or emulsification) can have on satiety signals and appetite. However, the authors highlight the impact that a combined approach could have on appetite, whereby food structure could boost the effect of nutritional manipulations and enhance satiety, enabling consumers to restrict their intake, resulting in weight loss and prevention of weight regain.
1.3 **CONCLUSION**

As this book should highlight, a multidisciplinary approach, that utilises information gathered from many disciplines (including material chemistry, chemical engineering, biology, sensory science and psychology), should allow scientists to tackle some of the food-related issues of the modern age. This should allow food products to be produced that use basic materials (e.g. proteins, polysaccharides or hydrocolloids) to structure foods, or the design of food microstructures (e.g. emulsions) in intelligent ways that provide health benefits, such as increased satiety, reduction in the uptake of fats or salt, or the bioavailability of active compounds. These foods should also taste good, delivering flavour and tastants effectively, and having textures that consumers desire (such as creaminess). In order to fully understand how these foods perform, knowledge is required of the physics of eating (including of mastication and food breakdown), the interaction with saliva and the release profiles of both aroma compounds and tastants. The effect that food structure has on digestion, and uptake of both macro- and micronutrients, is also important, in order to produce foods that have limited uptake (e.g. fat-containing foods), or increased uptake (e.g. active compounds). An understanding of consumer acceptability is also required, in order to ensure that foods with health benefits are liked and repeatedly consumed, as is an understanding of within-meal satiation and post-meal satiety, in order to produce foods that can regulate appetite. With extensive understanding of all these areas, scientists can begin to think of creative ways to produce foods that offer all of the above-mentioned benefits.
2 Protein-Based Designs for Healthier Foods of the Future

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2.1 GENERAL CONSIDERATIONS REGARDING PROTEINS IN FOODS

Designing healthy foods is a constant challenge because of the dynamic nature of understanding how diet affects human health. The current conventional wisdom suggests reducing consumption of sugars and sodium, increasing fibre and specific types of lipids (e.g. ω-3 fatty acids) and bioactive phytochemicals, and overall decreasing caloric density (Palzer, 2009; USDA, 2011b). These recommendations are very broad and may change with improved understanding of individual (age, gender, disease or condition-specific) nutrition. What is needed, therefore, is the ability to be flexible in altering food composition to meet health and nutrition goals, while at the same time maintaining quality so that food remains a source of pleasure (Humphries, 2012).

Proteins are biopolymers that are designed for specific biological functions. They are a diverse group of molecules that do everything from catalyzing reactions (enzymes) to providing a structural framework for muscles (collagen). Foods are consumed to provide the molecules needed to sustain life, and proteins provide amino acids which are used to create new proteins or energy. Moreover, they are the source of bioactive peptides with diverse effects, including the regulation of blood pressure, cholesterol levels, vascular function, immunomodulation and the correction of inborn errors of protein metabolism (Gilani et al., 2008; Madureira et al., 2010; Ballard et al., 2012; Udenigwe and Aluko, 2012). They have been shown to enhance satiety and fat loss (Gilbert et al., 2011). While the ultimate goal is to provide molecules for nutrition and health (eat to live), food scientists also see proteins as building blocks, which produce food structures that are associated with enjoyment (live to eat). For example, milk is converted to cheese by
linking casein micelles into a continuous gel network that is surrounded by a solution of water and dissolved molecules. Fat particles are trapped within the porous structure (Fox, 1987). This food “structure” contributes to the sensory quality and health/nutritional properties of the food.

Assuring the availability and affordability of high-quality protein in a form that is not only acceptable, but desirable to the diversity of world cultures, is a challenge. In the short term, there are many developed nations that are experiencing an obesity epidemic, and they could benefit from foods that are less dense in calories and also more satiating. However, looking several decades ahead, there is a fear that world population will surpass food production or that food prices will rise to a point where the poor cannot afford them (Swinnen and Pasquamaria, 2012). This food security concern should fuel research into foods that are sustainable, energy dense and efficiently digested. Both obesity and food security challenges warrant a critical evaluation of our food supply to determine how we can improve it to match ever-changing societal goals. Current goals of reduced caloric density (especially fat) and sodium content are based on health considerations, but present challenges when designing foods that meet the compositional requirements and remain desirable choices (Palzer, 2009). A food that has the preferred composition based on health and nutrition considerations, but falls short on flavour, texture and affordability will not be successful (Childs and Drake, 2009). This begs the question, “How do we have it all in terms of quality, health/nutrition and affordability?” The answer could be found with an understanding of how to design elements of food quality, health/nutrition and affordability into food structures.

The concept of “food structure” and “food structuring” has been emerging as a way to view how foods deliver, and can be designed to deliver, desirable sensory and health attributes (Tolstoguzov and Braudo, 1983; Aguilera, 2005, 2006; Chen et al., 2006; Day et al., 2009; Purwanti et al., 2010; Turgeon and Rioux, 2011). Food structure design builds on concepts that were classically assigned to colloidal systems (Dickinson, 1992, 2006, 2011; Norton and Norton, 2010) and are currently under a more general umbrella of soft-matter physics (Donald, 1994; Mezzenga et al., 2005; Ubbink et al., 2008; van der Sman and van der Goot, 2009; van der Sman, 2012). One common aspect of colloidal and soft-matter approaches is the importance of mesoscale structures in the micrometer range that are between molecular (nanometer) and macroscopic structures. Examples are oil or gas droplets in respective emulsions and foams. As stated by van der Sman (2012), “It happens that this size is similar to the length scale that humans can sense with the tongue, and thus often sets the scale for structured food.” Another key element to the soft-matter physics approach is that structures are considered to contain all essential infor-
mation and chemical properties are not necessary to describe behaviour. This allows us to formulate some general hypotheses regarding food structure and delivery of desirable sensory and health properties.

**Hypothesis 1.** Molecules are assembled into food structures that, through a series of cognitive processes, including oral processing, determine human liking or disliking.

**Hypothesis 2.** Similar food structures, in terms of oral perception of desirability, can be generated by various combinations of molecules (e.g. different proteins may serve the same function).

**Hypothesis 3.** Food structures impact delivery and utilisation of bioactive molecules and can be designed for specific health/nutrition effects.

The first two hypotheses are essential to making foods with altered composition, for example, reduced fat or varied protein sources, while producing a similar level of liking. If they are proven valid, then the key to producing successful products is determining which structure(s) and structural transformation during consumption are essential to a level of liking. Hypothesis 3 is essential to translating information gained from single-molecule mechanistic investigations into a functional food.

### 2.2 PROTEIN REACTIONS IMPORTANT TO FOOD STRUCTURE AND HEALTHY FOODS

Our understanding of the science of proteins is eloquently unfolded in the book titled *Nature's Robots, A History of Proteins* (Tanford and Reynolds, 2001). A robot is a fitting metaphor for teaching the roles of proteins in biological systems because proteins produce locomotion and automate biological functions such as energy production. The mantra in protein chemistry has been “sequence determines structure, and structure determines function.” The word “function”, from a biochemical perspective, is describing the role of a particular protein in a biological system, for example, myosin functions in muscle contraction. However, the concept of “function” is equally applicable in foods and “food protein functionality” is a commonly used concept (Cherry, 1981). From a general food perspective, proteins function by: (1) providing amino acids for protein synthesis and energy, (2) providing bioactive peptides and (3) being the main molecules forming and stabilising a variety of food structures (Foegeding and Davis, 2011).

The common starting point for proteins is a description of the properties of amino acids, followed by depictions of the various levels of
structure (e.g. primary, secondary, tertiary and quaternary) (Creighton, 1993). For biological applications, this is usually sufficient because the inherent structure of the protein, that is, the structure found in its natural biological environment, is what determines function. In foods, that structure is more often the starting point rather than the final state. Converting raw biological materials into foods involves a variety of unit operations that can cause changes in protein structure. These include denaturation/aggregation, alteration of the stereochemistry of the amino acids (racemisation) or covalently modifying amino acids (Damodaran, 2008). In addition, protein ingredients are seldom 100% single proteins, and other compounds may alter their biological activity or ability to form food structures. The key reactions occurring in food processing are outlined below.

### 2.2.1 Denaturation/aggregation

The simplest definition of protein denaturation is the change of inherent structure. For some proteins, such as enzymes and others which have clear biological activity assays, this is an easy reaction to follow. Experiments are designed to measure the loss of catalytic or biological activity as some extrinsic factor, i.e. heating, is applied and the coinciding changes in secondary, tertiary or quaternary structure are determined. This allows for an assessment of the level of structural change needed to decrease biological activity. In foods, denaturation is more often the reaction that is associated with producing, rather than diminishing, the desired function. Moreover, with a few exceptions, denaturation is linked with aggregation in foods.

Denaturation/aggregation of proteins at an air–water or oil–water interface determines the topological and structural elements of the interfacial protein film that will, in turn, contribute to foam and emulsion stability, respectively (Murray et al., 2011). Thermal processing is required for food safety in producing protein-containing beverages and this will cause protein denaturation and aggregation. In beverages, the goal is to minimise aggregation in order to produce small aggregates that remain stable over the desired shelf life. In contrast, when making soft-solid foods by protein gelation (e.g. cheeses, cooked egg white and processed meats), the goal is to direct the aggregation process so that a continuous gel network is formed. In both cases, the objective is to control aggregation to produce a specific final structure.

Chiti and Dobson (2006) proposed a model that accounts for protein folding, unfolding and aggregation (see Fig. 2.1). Starting with the nascent chain coming off the ribosome, the unfolded protein forms an intermediate structure that folds into the native structure. The native structure can be assembled with other polypeptides into functional
Fig. 2.1 Model for protein folding, unfolding and aggregation proposed by Chiti and Dobson (2006). Reproduced with permission from Annual Review of biochemistry by Richardson, Charles C. Reproduced with permission of Annual Reviews in the format Republish in a book via Copyright Clearance Center.
quaternary structures (functional oligomers or fibres). This ordered pathway is what should occur under normal protein synthesis. Off-pathway aggregates are also depicted in the model. Formation of disordered aggregates as the terminal structure is shown in the upper pathway. Alternatively, disordered aggregates can be an intermediate before forming ordered β-structure aggregates, amyloid or amyloid-like fibrils. Food processing operations start with proteins in the native, functional oligomer or functional fibre state and move backwards through the denaturation/aggregation pathways.

2.2.2 Racemisation

The predominant stereoisomer of amino acids is “L”, although there are reports of naturally occurring D-amino acids (Friedman, 2010). Since L-amino acids are used for protein synthesis, conversion of L- to D-amino acids during processing is generally viewed as undesirable (Friedman, 2010).

2.2.3 Covalent modification

Proteins and amino acids contain functional groups that are susceptible to covalent modification during food processing. One of the most reactive groups is the primary amine found as the ε-amino group on lysine or the amino terminus of a protein or peptide. It readily reacts with reducing sugars (i.e. sugars with an antomeric carbon in a hemiacetyl or hemiketal ring) and starts the Maillard reaction that produces brown colour and many of the highly desirable flavours in heated foods (e.g. breads, meats, coffee and many more) (Friedman, 1996; Purlis, 2010). While it is true that covalent modification prevents the ability of that amino acid to be used in protein synthesis, the loss of amino acids needs to be evaluated in perspective with the amount of intact amino acids that remain (O’Brian et al., 1989). Covalent modification only becomes a problem when it results in a lowering of the nutritional value of the food or creates some anti-nutritional factors.

2.3 USING PROTEINS TO FORM AND STABILISE STRUCTURES

The transformation from protein-rich agricultural crops and livestock to food products is shown in Fig. 2.2. A bean field, a chicken and a dairy cow (see Fig. 2.2a) are used to illustrate the process. The raw materials produced are beans, eggs, meat and milk (see Fig. 2.2b).
Minimal processing of these materials would involve heating to produce a safe product with desirable sensory qualities (see Fig. 2.2c). Protein reactions involved are heat denaturation/aggregation and possibly covalent modification via Maillard browning (note the brown stripes on the cooked chicken breast). A more extensive transformation occurs when the raw materials are converted to food products. That generally involves several processing steps and the addition of other ingredients (see Fig. 2.2d). Formation of tofu (beans), flan (eggs), hot dogs (meat) and cheese (milk) requires the loss of recognisable biological structures (most evident in beans and meat) and the creation of colloidal structures. Therefore, the formation, stability and desirability of these and similarly formed foods (e.g. breads, ice cream and many more) depend on the creation of colloidal structures.

Proteins are key components of colloidal structures found in foods. The simplest system is skimmed milk, where the colloidal particles of casein micelles and whey proteins are dispersed in an aqueous solution.
of sugar (lactose) and salt (Walstra et al., 1999). However, foods that consist of single colloidal structures are the exception, as most foods are a combination of several colloidal structures. For example, whole milk adds another degree of complexity in adding milk fat globules such that the system is a sol and emulsion mixture. In the following section, different types of colloidal structures will be defined based on basic elements in formation and stabilisation. This will be followed by describing some protein-based foods that are composites of colloidal structures. It should be noted that this is not intended to be a comprehensive description of colloidal aspects of foods, as this subject has been addressed by books (Dickinson, 1992; McClements, 1999) and excellent review articles (e.g. see Dickinson, 2006, 2011; Rodríguez Patino et al., 2008; Ikeda and Zhong, 2012).

2.3.1 Colloidal structures

2.3.1.1 Sols

Dickinson (1992) describes colloidal materials as those that “contain structural entities with at least one linear dimension in the size range of 1 nm to ∼1 μm.” A sol is a solid particle dispersed in a liquid medium. This fits food protein dispersions containing globular proteins from milk and egg, which are typically on the order of a few nm, to casein micelles that have an average size of 150 to 200 nm (Walstra et al., 1999; Dalgleish and Corredig, 2012).

Stability of dilute dispersions under the influence of gravity (g) is based on the Stokes’ equation:

\[ v_p = \frac{2(\rho_f - \rho_p)gr^2}{9\eta_0} \]  

(2.1)

where the velocity of the particle \(v_p\) is determined by the particle radius \(r\), the density difference between the fluid and particle \(\rho_f - \rho_p\) and the Newtonian viscosity of the fluid \(\eta_0\).

Most strategies used to increase protein sol stability are based on minimising particle size or increasing continuous-phase viscosity. Some of the approaches developed to decrease aggregation (i.e. minimum particle radius) are: covalent and non-covalent complexing with polysaccharides (Mitchell and Hill, 1995; Oliver et al., 2006; Vardhana-bhuti et al., 2009); forming soluble aggregates by controlled denaturation/aggregation (Ryan et al., 2012) or using water-in-oil emulsions to create nano-particles (Zhang and Zhong, 2010); covalent crosslinking (Buchert et al., 2010) and addition of aggregation-inhibiting solutes (LaClair and Etzel, 2010).
2.3.1.2 Emulsions

Emulsions are a liquid dispersed in a liquid, and for foods, the most common form is an oil-in-water emulsion. However, it should be noted that the many food lipids have melting points within the temperature range for common food use so an oil-in-water emulsion may contain semi-solid or solid fat at refrigeration temperatures and then be liquid at room temperature. Since proteins contain polar and non-polar amino acids, they are amphipathic molecules that can adsorb at the oil–water interface, lowering surface tension and thereby aiding in reduction of dispersed phase particle size during emulsification (Walstra, 2003). Based on Stokes’ considerations, the protein’s first contribution to stability is in facilitating decreased particle size. Once formed, the nature of the protein interfacial film will determine, in part, the resistance to destabilisation processes not described by the Stokes’ equation, such as by flocculation, coalescence and Oswald ripening (Dickinson, 1992; Murray, 2011).

2.3.1.3 Foams

Foams are a gas dispersed in a liquid. As with emulsions, proteins adsorb at the interface and aid in formation and stabilisation (Foegeding et al., 2006). The movement of dispersed phase gas from small bubbles to large bubbles, called disproportionation, is a problem with protein foams and can be regulated by the permeability and rigidity of the interfacial film (Murray, 2011). Ideally, proteins form an interfacial film that resists the passage of gas and bubble shrinkage. Another approach is to immobilise gas bubbles in a gel network (Zúñiga and Aguilera, 2008).

2.3.1.4 Gels

Sols are always liquids, whereas emulsions and foams can exist in liquid or solid states (more on this when discussing food structures). Protein gels can be considered the solid form of a sol as they are generated by a sol-to-gel transition that links proteins into a three-dimensional network that immobilises the surrounding fluid. While definitions may vary, food protein gels are generally defined as semi-solid or solid material consisting of mainly water and a continuous protein network. Key factors are an aqueous phase much greater than the protein phase and an elastic structure. The elastic structure is commonly defined rheologically as having a storage modulus much greater than loss modulus, \( G' \gg G'' \), that has a plateau in frequency dependence (Almdal et al., 1993). Textural and water-holding properties are determined by
the gel network structure. Factors determining protein gel properties have been extensively reviewed (for example, Clark and Ross-Murphy, 1987; Clark et al., 2001; Bromley et al., 2006; van der Linden and Foegeding, 2009).

### 2.3.2 Food structures

Protein functionality in foods has traditionally been defined based on formation and stabilisation of colloidal structures (Cherry, 1981; Hall, 1996; Foegeding and Davis, 2011). Indeed, the literature is full of examples where a protein (or protein ingredient) is evaluated based on simple tests predicting foaming, emulsifying and gelling ability (Morr and Foegeding, 1990). However, protein ingredients also contribute to the flavour of foods (Wright et al., 2009). The term “flavour” has different meanings to consumers and scientists; however, most consumers would think of it as “the blend of taste and smell sensations evoked by a substance in the mouth” (defined by Merriam-Webster, http://www.merriam-webster.com/dictionary/flavor). Scientists define flavour compounds as those that primarily stimulate the olfactory system: this requires volatility. Based on that definition, proteins are unlikely to have any direct flavour due to their low volatility (things may change when converting proteins to peptides). Protein ingredients contribute to flavour by: (1) containing flavour compounds that were not removed during processing or generated during processing (Wright et al., 2009), (2) binding flavour compounds (Kinsella, 1982; Guichard, 2006; Kühn et al., 2008) and (3) forming structures that regulate texture, flavour release and flavour perception (Gwartney et al., 2000; Visschers et al., 2006; Gierczynski et al., 2011). Here again, there is extensive literature on how proteins contribute to flavor, but the important point to convey is that successful applications of proteins in foods depends on a combination of factors, one being not diminishing the overall flavour quality. To summarise, proteins are biopolymers that can be used to form and stabilise colloidal structures used in foods. A successful application of a protein ingredient: (1) forms and/or stabilises desirable structures, (2) does not have a negative flavour contribution, (3) maintains bioactivity and (4) produces an overall desirable sensory sensation (e.g. appearance, flavour and texture). Key elements associated with specific food categories will be discussed in the following section, and a summary of the role of proteins in colloidal structure used in designing foods is seen in Table 2.1.

### 2.3.2.1 Low-solids phase; fluids

Beverage is a collective term for foods we drink. Many are clear, thin fluids, while others take on a thicker consistency and are approaching
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The characteristics of semi-solids. This is an especially relevant food category for protein application, as there are an increasing number of protein-containing beverages designed to meet specific nutrition and health needs. Besides classical products such as infant formula, beverages are being designed to: (1) aid in muscle recovery after strenuous exercise, (2) aid in weight reduction and control and (3) prevent muscle loss with aging (see Section 2.4). Products are designed based on nutritional and bioactive compounds delivered per serving and overall product quality. They can be clear or opaque, thick or thin, and come

<table>
<thead>
<tr>
<th>Colloidal Structure</th>
<th>Protein location in Structure</th>
<th>Stability Goals</th>
<th>Food</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sol</strong>, solid dispersed in a liquid (s/l)</td>
<td>Proteins or protein aggregates serve as solid particles</td>
<td>Prevent phase separation to retain dispersed, homogeneous appearance and consistency; maintain desirable rheological properties</td>
<td>Beverages containing just protein particles</td>
</tr>
<tr>
<td><strong>Gel</strong>, a continuous network surrounded by a liquid (s &amp; l)</td>
<td>Continuous protein network surrounded by fluid</td>
<td>Maintain appearance, water holding and sensory textural properties</td>
<td>Protein gel-based desserts (e.g. gelatin gels), cooked egg white, no-fat yogurt</td>
</tr>
<tr>
<td><strong>Emulsion</strong>, liquid dispersed in a liquid, (l/l)</td>
<td>Protein coated lipid droplet dispersed in fluid</td>
<td>Prevent phase separation to retain dispersed, homogeneous appearance and consistency; maintain desirable rheological properties</td>
<td>Usually a component of a complex food such as a beverage or ice cream mix</td>
</tr>
<tr>
<td><strong>Foam</strong>, gas dispersed in a liquid, (g/l)</td>
<td>Protein coated air bubbles surrounded by fluid or solid matrix</td>
<td>Prevent phase separation to retain dispersed, homogeneous appearance and consistency; maintain desirable rheological properties</td>
<td>Fluid structure that is converted to a solid in meringue, bread, cake, confectionary products and some meal bars</td>
</tr>
<tr>
<td><strong>Sol &amp; Emulsion</strong>, Solid and liquid dispersed in a liquid, (s + l/l)</td>
<td>Solid protein particles and protein coated lipid droplets</td>
<td>Prevent phase separation to retain dispersed, homogeneous appearance and consistency; maintain desirable rheological properties</td>
<td>Beverages designed for general nutrition, muscle recovery, weight loss, prevent sarcopenia</td>
</tr>
<tr>
<td><strong>Filled gel</strong>, particles dispersed in a gel matrix (s or l/s)</td>
<td>Gel network filled with particles (lipid, protein or polysaccharide)</td>
<td>Maintain appearance, water holding and sensory textural properties</td>
<td>Cheese, processed meats</td>
</tr>
<tr>
<td>Jammed particles</td>
<td>Close packed protein particles and an adhesive phase</td>
<td>Prevent hardening reactions</td>
<td>Meal replacement bars</td>
</tr>
</tbody>
</table>
in a variety of flavours. They can be a sol, emulsion, foam or combination of two or all three. For example, a milkshake is a combination of all three. No matter what the goal, they have the common problem of maintaining stability during processing and storage. Instability can be due to a variety of factors, including solvent quality (pH and ionic solutes), thermal processing and addition of bioactive compounds that favour aggregation (e.g. polyphenols; O’Connell and Fox, 2001; Jöbstl et al., 2006).

Location and structural state of proteins: aqueous phase – native, denatured/aggregated or phase separated and suspended; possibly bound with polysaccharides, polyphenols or other molecules; air/water and lipid/water interfaces – varying degrees of unfolding and aggregation into a film; possibly bound with polysaccharides, polyphenols or other molecules.

2.3.2.2 Low solids phase; semi-solid and soft-solid foods

Cooked egg white (albumen), processed meats, some cheeses and gelatin-based desserts have the common structure of a gel network. Unlike beverages, this structural designation does not encompass one main food category. Also, there is not a clear demarcation between moving from a high-moisture system, such as cooked egg white with 10% protein and 89% moisture, to a low-moisture gummy bear. Moreover, many of these foods contain a dispersed lipid phase. For example, cheddar cheese contains approximately 25% protein, 32% fat and 37% moisture. In this case, the system can be viewed as different phase volumes of gel (protein + water) and fat. The remaining 6% of ash (salts), carbohydrates and other materials would be partitioned between the two phases, depending on their relative solubility. Cakes and breads are solid foams that also fit into this category.

Location and structural state of proteins: aqueous phase – native, denatured/aggregated or phase separated and suspended; possibly bound with polysaccharides, polyphenols or other molecules; air/water and lipid/water interfaces – varying degrees of unfolding and aggregation into a film; possibly bound with polysaccharides, polyphenols or other molecules; gel network – aggregated into strands of proteins alone or possibly co-aggregated with other molecules.

2.3.2.3 Low-aqueous phase; aggregated particles, semi-solid and hard-solid foods

Foods such as gummy bears and high-protein bars fit into this category. These products are chewy (semi-solid) or crunchy (hard-solid) depending on composition, especially water content. They can be viewed from