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

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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 information 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 if 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

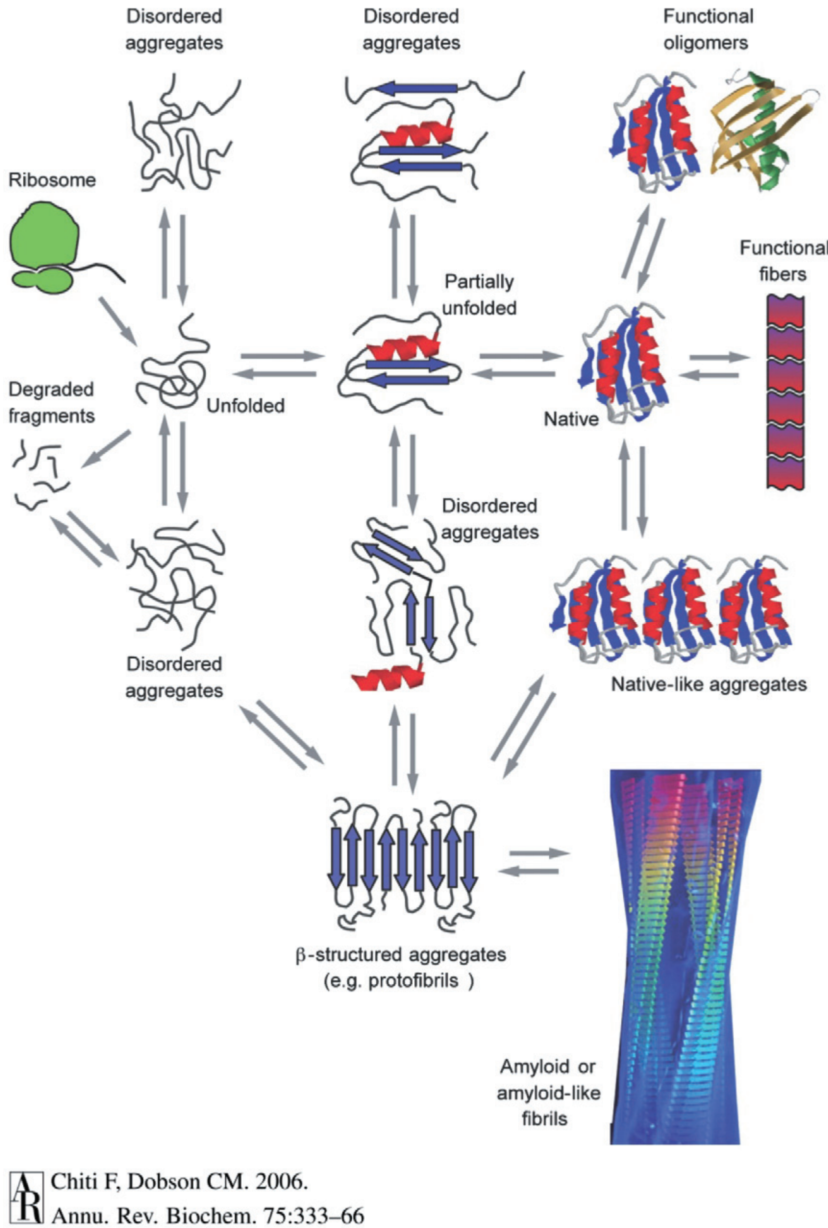

Fig. 2.1 Model for protein folding, unfolding and aggregation proposed by Chiti and Dobson (2006). Reproduced with permission from Annual Review of biochemistry by Richardson, Charles C. Reproduced with permission of Annual Reviews in the format Republish in a book via Copyright Clearance Center.

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).

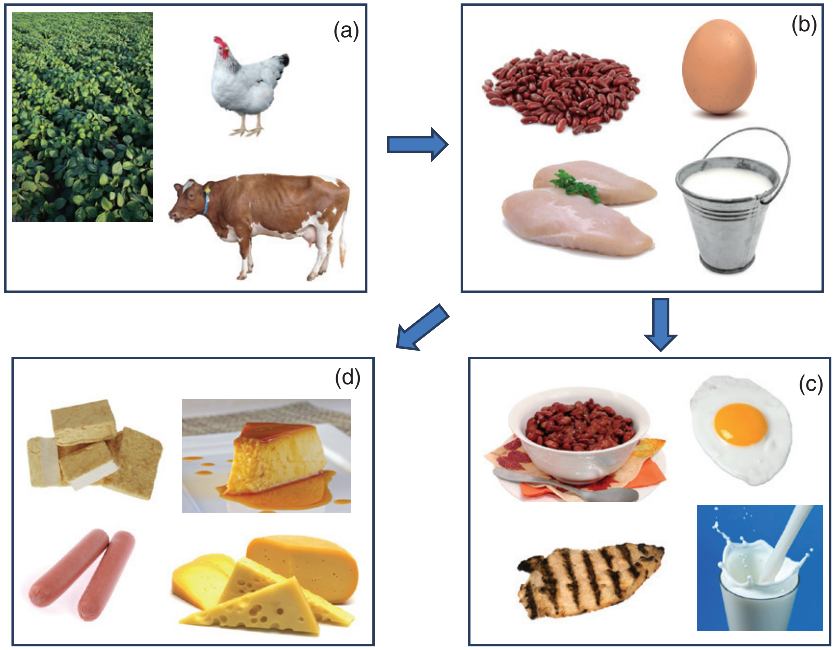

Fig. 2.2 Transformation of protein-rich crops and livestock into food products. Plants and animals produced through agriculture (a) are initially converted to raw food materials (b). The raw food materials can be converted to food by thermal processing to kill undesirable micro-organisms and at the same time produce desirable sensory characteristics (c). Alternatively, the raw food materials can be combined with other ingredients and processed into food products (d).

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:

$$v_p = \frac{2(\rho_f - \rho_p)gr^2}{9\eta_0} \tag{2.1}$$

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 (η_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; Vardhanabhuti *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

Table 2.1 Role of proteins in colloidal structures used in designing food products.

Colloidal Structure	Protein location in Structure	Stability Goals	Food
Sol, solid dispersed in a liquid (s/l)	Proteins or protein aggregates serve as solid particles	Prevent phase separation to retain dispersed, homogeneous appearance and consistency; maintain desirable rheological properties	Beverages containing just protein particles
Gel, a continuous network surrounded by a liquid (s & l)	Continuous protein network surrounded by fluid	Maintain appearance, water holding and sensory textural properties	Protein gel-based desserts (e.g. gelatin gels), cooked egg white, no-fat yogurt
Emulsion, liquid dispersed in a liquid, (l/l)	Protein coated lipid droplet dispersed in fluid	Prevent phase separation to retain dispersed, homogeneous appearance and consistency; maintain desirable rheological properties	Usually a component of a complex food such as a beverage or ice cream mix
Foam, gas dispersed in a liquid, (g/l)	Protein coated air bubbles surrounded by fluid or solid matrix	Prevent phase separation to retain dispersed, homogeneous appearance and consistency; maintain desirable rheological properties	Fluid structure that is converted to a solid in meringue, bread, cake, confectionary products and some meal bars
Sol & Emulsion, Solid and liquid dispersed in a liquid, (s + l/l)	Solid protein particles and protein coated lipid droplets	Prevent phase separation to retain dispersed, homogeneous appearance and consistency; maintain desirable rheological properties	Beverages designed for general nutrition, muscle recovery, weight loss, prevent sarcopenia
Filled gel, particles dispersed in a gel matrix (s or l/s)	Gel network filled with particles (lipid, protein or polysaccharide)	Maintain appearance, water holding and sensory textural properties	Cheese, processed meats
Jammed particles	Close packed protein particles and an adhesive phase	Prevent hardening reactions	Meal replacement bars

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 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 several perspectives. Condensed-matter physics considers the liquid–solid transition of amorphous materials (glasses, foams and emulsions) as a jamming transition (Xu, 2011). This concept describes materials that are amorphous, viscoelastic and out of thermal equilibrium. An example would be a system increasing in volume fraction like a high phase volume emulsion. However, in foods with high protein content, low-moisture systems often contain some other compounds that assist in sticking the particles together (i.e. we seldom eat a protein bar that is only protein particles!). These are generally combinations of fats and various forms of carbohydrates (sugars, sugar alcohols, corn syrups and polysaccharides). Air and macroscopic inclusions (e.g. nuts) are used to disrupt the connectivity of the structure and provide weak spots that soften the texture.

Location and structural state of proteins: These products have a wide range of structures so proteins can be found: (1) in a small aqueous phase, (2) at air–water or lipid–water interfaces, (3) coating inclusion particles or (4) as close-packed particles. In all locations, there is the possibility of native and denaturated/aggregated proteins.

2.4 PROTEINS IN NUTRITION AND HEALTH

The English word protein originates from the Greek word, proteios, meaning first or primary. This is a fitting term, given protein's central role in nutrition. Proteins are amino-acid polymers composed of 20 separate amino acids. Of these, nine are considered essential nutrients for humans, meaning they cannot be synthesised from other dietary components. They are: phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, histidine, leucine and lysine. The amino acids arginine, cysteine, glutamine, glycine, proline, serine, tyrosine and asparagine are considered conditionally essential, meaning that under certain conditions (illness, intense bouts of exercise, pregnancy) the body may not be able to make enough of them (Insel *et al.*, 2012). Foods that contain all of the essential amino acids are considered "complete" proteins. Proteins that, when combined, make up for the lack of essential amino acids in the other food are referred to as "complimentary". A common example of this is the consumption of beans with rice. Bean protein lacks methionine, while rice protein lacks lysine. When eaten together, they form a complete protein (Centers for Disease Control and Prevention, 2012a). The cost of producing and utilising complimentary, plant-based proteins, relative to animal proteins, presents new applications in food structure design, providing that functionalities, such as foaming and gelling, can be maintained.

2.4.1 Protein quality

Nutritional protein quality relates to the presence, concentration, ratios and digestibility of essential amino acids. The standard method for determination of protein quality in the United States, and for the World Health Organization, is PDCAAS (Protein Digestibility-Corrected Amino Acid Scoring). PDCAAS combines the use of analytical instruments (to determine amino-acid content) with rodent models (to determine the percentage of protein absorbed during digestion). This involves the chemical determination of the amino-acid content in the food and the comparison of these values to those required for humans from birth up to three years of age (the life stages where protein needs are highest). Amino-acid values are adjusted for digestibility using a rodent model, as follows: (1) Young rodents are fed a test diet, (2) The amount of nitrogen excreted in the rodent faeces, and therefore not absorbed, is measured, (3) The amount that is absorbed is then determined by difference (Schaafsma, 2000).

PDCAAS values range from 0.0 to 1.0, with 1.0 being considered the highest-quality protein. Animal proteins, including casein, whey and egg white all rank as 1.0. Despite the fact that vegetable protein is generally of lower quality than animal protein, soy protein also possesses a 1.0 score, while whole soybean and beef scores are nearly identical, at 0.91 and 0.92, respectively. Fruit, vegetable and grain products generally have low PDCAAS scores (0.40–0.80). Low PDCAAS scores of plant foods, such as cassava and sorghum, can be increased via processing to remove anti-nutritional factors or by combining lower-quality proteins with higher-quality proteins (Muoki *et al.*, 2012). Another, more controversial way of improving protein quality is through genetic engineering to produce more of a limiting amino acid or less of a digestive inhibitor (Henley *et al.*, 2010).

Several questions remain with regard to the use of PDCAAS as a standard method for determining protein quality. There is debate as to whether the PDCAAS method overestimates protein quality if foods have been heated, alkaline treated or if they contain anti-nutritional factors (Sawar, 1997). Heat or alkaline processing products, including Maillard browning products and D-amino acids, have been reported to inhibit protein digestibility by one-quarter in animal models. The anti-nutritional factors widely distributed in plant foods, such as glucosinolates in cruciferous vegetables, oxalates and goitrogens in vegetables and tree nuts, and trypsin inhibitors in legumes, may decrease nutrient digestibility even more than processing. Trypsin inhibitors, found in soy, may inhibit protein digestibility by half (Gilani *et al.*, 2005). This has led to questions about the high PDCAAS scores for soy protein, which contains trypsin inhibitors. Trypsin inhibitors can be inactivated by heating and other types of food processing, but inactivation may not be complete. Young animals, which absorb protein efficiently even in the presence of anti-nutritional factors, are typically used to determine digestibility for the PDCAAS assay. Older animals do not absorb proteins as easily (nearly 20% less efficiently) in the presence of anti-nutritional factors, as compared with young animals. This finding may have implications for the digestibility of protein in elderly humans and has led to proposals to use older test animals to determine a more broadly applicable estimate of protein digestibility in the presence of anti-nutritional factors (Gillian and Sepehr, 2003). The use of an *in vitro* system designed to more closely simulate the human ileum (the part of the intestine where amino-acid absorption occurs) has been suggested as an alternative to the current rodent faecal digestibility model (Schaafsma, 2000). Anti-nutritional factors can have important implications for overall health, because decreasing protein digestibility may not only result in fewer calories consumed, but also in less protein consumed as a percentage of total calories. In this sense, anti-nutritional factors have the potential to convert protein into a non-carbohydrate-based form of fibre.

2.4.2 Recommended versus actual protein intake

There is considerable debate about the optimal levels of protein intake for humans, although it is generally accepted that protein needs vary based on age, weight, physical condition and athletic performance. The USDA provides recommendations in terms of grams of protein consumed per kilogram of bodyweight. The Reference Dietary Intakes (RDI), based on age and activity level are: ~1.0 g/kg for infants and 0.8 g/kg for adults (USDA, 2010, 2011a). In contrast, a food-industry-sponsored summit on protein intake concluded that the 0.8 g/kg level of protein intake for adults was minimal, and that this level could be safely doubled without increased disease risk in all but those already afflicted with impaired kidney function (Wolfe, 2008). Adults in the United States are reported to consume about 90 grams of protein per day (Grosvenor and Smolin, 2010). Given an average weight of 165 and 195 pounds (75 and 89 kilograms) for women and men, respectively (Centers for Disease Control and Prevention, 2012b), this means that US adults are consuming between 1.0 and 1.2 grams of protein per kilogram of bodyweight. A study of strength-trained male athletes found that their intake was even higher, at approximately two grams per kilogram per day (Fox *et al.*, 2011).

Overall, it appears that adults in the United States consume sufficient levels of protein for basic needs; however, their intake does not greatly exceed recommended levels on a gram per kilogram basis. From one perspective, consuming 90 grams of protein per day may only be "adequate", because US adults are, generally, overweight. This is not necessarily an indication that 90 grams represent a moderate protein intake. From a sustainability perspective, would it be possible to provide 90 grams of high-quality protein to the entire adult world population, either from animal, complimentary plant, or combined animal and plant sources? From another perspective, the "adequate" consumption levels indicate an opportunity for the food industry to produce and provide highly palatable, healthy, high-protein foods as a means to deliver the satiety and other health benefits that proteins can provide (see Section 2.5 on Protein Intake and Satiety for more information).

2.4.3 Protein deficiency effects

When considering the dietary effects of protein as a nutrient, it is important to consider the full spectrum of effects that it may have, from deficiency to sufficiency to toxicity effects, as Fig. 2.3 illustrates. Marasmus, a deficiency of all calorie-containing nutrients including protein, is characterised by a generalised wasting, leaving those that suffer from it painfully thin and highly susceptible to infections. In contrast, kwashiorkor is severe protein malnutrition, independent of the number of calories consumed. Kwashiorkor is distinguished from marasmus by the characteristic abdominal edema (swelling) it produces (Rolfes *et al.*, 2009). Like marasmus, those suffering from kwashiorkor are at increased risk of infection, because antibodies and other immune-system components are formed from protein. Given that the enzymes that catalyse the formation of structures like muscle and bone, as well as immune cell components are themselves proteins, a long-term lack of protein inevitably leads to death because essential protein-based systems cannot be repaired or maintained. Although these conditions

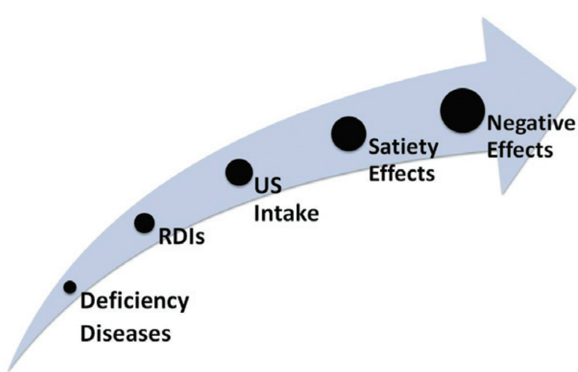

Fig. 2.3 Effects of increasing protein intake on health.

can occur at any age, marasmus and kwaskiorkor are most likely to affect children under five years of age, given their greater relative nutritional needs. It should be noted that a variety of conditions and diseases, including age-related sarcopenia (muscle loss), cancer-related cachexia, HIV-AIDS and diabetes-associated malnutrition may be viewed as forms of either marasmus or kwashiorkor, given their effects on protein absorption and storage.

Low-protein diets during the pre-natal period are not only a matter of short-term nutrition, but may also set the stage for diabetes and heart disease later in life. Based on animal data, low-protein pre-natal diets impair glucose and cholesterol metabolism. Female mice born to low-protein-fed mothers had increased abdominal fat deposition and impaired glucose tolerance (Han *et al*., 2012). Underfeeding protein to pregnant sows results in underweight offspring with disregulated cholesterol metabolism. The basis of this disregulation was the epigenetic increase in HMG-CoA reductase, CYP7α1 and SREBP1 protein expression. These are the rate-limiting enzymes for the formation of cholesterol, of bile acids from cholesterol and fatty-acid synthesis, respectively (Cong *et al*., 2012). Pre-natal low-protein diets have been reported to limit the number of beta-cells formed in the pancreas in rats, potentially setting the stage for type 2 diabetes later in life. These beta-cells, which produce insulin, are key to glucose metabolism (Rodriguez-Trejo *et al*., 2012). In summary, low-protein diets during prenatal development and early childhood have devastating health effects and may prime the metabolism for diabetes and heart disease in those that survive to adulthood.

2.4.4 Excess protein effects

Protein deficiencies are rarely seen in Western societies. Instead, protein intake is generally adequate or slightly high, as noted earlier. The question then becomes, how much protein is too much? Epidemiological data indicates that high protein intake is associated with diabetes, with renal and prostate cancers, and with fractures, but it is not clear how much protein is too much, whether protein is the sole culprit or how much other diet and lifestyle factors of high protein consumers contribute to these conditions. Overconsumption of protein has been reported to have negative effects on kidney and bone health, based on short-term feeding studies where protein intakes ranged from 1.6–2.9 g/kg (Metges and Barth, 2000). Note that the low end of this range conflicts with the reported safe levels in Section 2.4.2 (Recommended versus actual protein intake). This points to the debate over the safe upper limits of protein intake in the scientific literature. There is evidence for an effect on kidney physiology with chronic high protein consumption, but it is unclear whether the changes observed are indicative of damage or simply evidence of adaptation to a high protein diet (Martin *et al*., 2005). Very high-protein diets may be counterproductive in infants. A study of 41 infants found that those fed high-protein formula gained weight, particularly fat mass, faster than infants fed either a low protein formula or those that were breast fed (Escribano *et al*., 2012). An animal study examined the effects of switching rats from either a high-protein or a high-fibre diet to a high-fat, high-sugar diet. Rats fed the high-protein diet before switching over to the high-fat, high-sugar diet gained more fat than those that started on the high-fibre diet (Maurer *et al*., 2008). The available data indicates that excess protein intake can have negative effects, just as any other nutrient in excess. On the other hand, the "ceiling" for protein consumption has not been well established and merits further study, especially given the popularity of high-protein diets. This is due to the fact that studies of essential nutrients primarily focus on avoiding deficiency, rather than on toxicity.

2.4.5 Health implications of protein source

Not only does the quantity and quality of the protein in question appear to be important to health outcomes, but the source of the protein (dairy, meat, plant-based, etc.) may be important as well. The consumption of meat, especially red and processed red meat, has been associated with an increased risk of diabetes, cancer, heart disease and other health concerns in several studies, while in other studies no association was observed. A prospective study that followed over 4000 Dutch individuals reported a correlation between the consumption of processed meats, but not poultry or beef, with a near doubling of the risk for type 2 diabetes (van Woudenbergh *et al*., 2012). Combined data from the Health Professionals and Nurses Health Studies, representing over 120 000 individuals surveyed every four years for 25 years reported increases in cardiovascular disease and cancer risks for individuals consuming either processed red meat or fresh red meats. The authors concluded that 9.3% of the deaths in men and 7.6% of the total observed deaths over that 25-year period could have been prevented if red meat consumption had been limited to one half serving, or approximately 43 g of red meat per day (Pan *et al*., 2012). Increased dietary protein was associated with increased heart disease risk in a group of 853 women and 878 men whose protein consumption was an average of 5% higher than their peers (Hatoum *et al*., 2010). A French study examining the effects of animal protein consumption in over 67 000 women reported increased risks of inflammatory bowel disease with increased meat and fish consumption. Dairy products and eggs were not associated with increased risks, however (Jantchou *et al*., 2010).

As with the association of high-protein diets with various diseases, it is not clear whether processed or red meat causes these conditions directly or whether it is a "symptom" of other lifestyle factors. As an aside, the Caerphilly study observed a positive association between black tea consumption and heart disease in Welsh men (Hertog *et al.*, 1997). It was also observed that black tea consumption was associated with smoking and heavy alcohol consumption, which probably had greater effects on heart disease outcome than black tea. Similarly, the association of red and processed red meat with disease may be due to the co-consumption of fat, cholesterol, Maillard compounds, heat-related carcinogens and excess sodium, rather than protein itself.

In contrast to reports of negative effects of meat in general, and red meat in particular, some studies have reported positive effects of meat consumption. A controversial study, aptly titled BOLD (Beef as part of an Optimal Lean Diet) reported positive effects of beef on cholesterol. In the study, 36 subjects with high cholesterol consumed four different diets: HAD (Healthy American Diet), DASH (Dietary Approaches to Stop Hypertension), BOLD (Beef as part of an Optimal Lean Diet), and BOLD+ (a higher protein version of the BOLD diet). The HAD, DASH and BOLD diets were all comprised of 17–19% protein, while the BOLD+ diet contained 27% protein. Beef consumption was limited to 20 and 28 grams per day in the HAD and DASH diets, while in the BOLD and BOLD+ diets, beef consumption was increased to 113 and 153 grams per day. The HAD diet had the highest levels of saturated fat, at 12%, while all other diets contained 6% saturated fat. Each subject consumed all four diets in order to compare their effects. The DASH, BOLD and BOLD+ diets lowered total and LDL cholesterol levels relative to the HAD diet. The greatest effects were observed in the BOLD+ diet, in which subjects consumed the most beef (Rousell *et al.*, 2012).

Meat consumption as a major portion of the diet was not found to be associated with cardiovascular disease in observational studies of 229 hunter-gatherer societies, where an average of two-thirds of calories came from meat sources and one third of calories came from plants. Depending on the society in question, these meat-based diets were reported to contain as much as 35% protein and 58% fat (Cordain *et al.*, 2002). Studies like these have led to the proliferation of meat-based, high-protein "paleo" diets in the popular media. Published reports of both negative and positive health effects for meat-based diets are difficult to reconcile. Perhaps the reports of both positive and negative effects of meat consumption reflect the importance of dietary moderation (the "BOLD+" diet described above contained only 113 to 153 g of beef) and physical activity in relatively sedentary Western societies. It may also reflect the difficulty of obtaining and storing food, the high levels of physical activity, the low corresponding rates of obesity and the relatively short life spans of individuals living in hunter-gatherer societies.

2.5 PROTEIN INTAKE AND SATIETY

Recent reports indicate that proteins may play a role in improving overall health in “overfed” populations via positive influences on satiety. For the purpose of this chapter, satiety is defined as the state of being content with a given level of food intake. Both physical and psychological factors play a role in satiety. Physical factors include sensory cues, such as food appearance, texture and viscosity, as well as hormonal responses and the feeling of fullness associated with an adequate food intake. Protein intake tends to increase levels of the satiety hormones peptide tyrosine-tyrosine, or PYY, as well as glucagon-like protein-1, or GLP-1 (Veldhorst *et al*., 2008). Psychological factors include the desire to consume foods because they are both appealing and readily available. A dish invitingly full of candy and freely accessible to everyone in an office would be a good example. This psychological “craving”-based eating is often quite separate from feelings of hunger. Dr. Brian Wansink has conducted extensive research on the psychology of eating patterns and overeating. Much of his research points to the strong influence of environmental and social cues on food intake, especially in relation to portion size and eating frequency. His research is summarised in the book entitled *Mindless Eating: Why We Eat More Than We Think* (Wansink, 2007).

2.5.1 Sensory cues important to satiety

Food forms, flavours and textures play important roles in satiety. Liquids are generally less satiating than solid foods, while thick liquids tend to be more satisfying than thin liquids. A study involving 36 normal-weight volunteers examined the effects of calorie content and beverage thickness on satiety (as measured by subsequent food consumption). The study found that thickened high-calorie beverages enhanced satiety, but un-thickened high-calorie beverages did not (Yeomans and Chambers, 2011). Foods which are very chewy or tough, thus requiring extensive mechanical energy to consume tend to be more satisfying than those which are easily swallowed with minimal chewing. Savoury foods (as opposed to sweet foods) may also increase satiety, independent of protein content, when given prior to meals (Griffioen-Roose *et al*., 2011). When the effects of low- and high-calorie beverages (78 versus 279 kilocalories), each at low, medium and high levels of thickness/creaminess were compared in 36 normal-weight volunteers, only the high-calorie beverage with a thickened texture produced satiety when given 30 minutes prior to a meal (Yeomans and Chambers, 2011). Both viscosity and protein content were found to be important to satiety when a low-viscosity whey-protein beverage was compared with a high-viscosity, low-protein alginate-based beverage (Solah *et al.*, 2010). Overall, viscous, creamy, chewy and savoury foods may play a role in augmenting the satiating role of protein.

2.5.2 Effects of timing and pattern of protein intake on satiety

Not only do the protein content and sensory characteristics of foods affect satiety, the timing of protein intake is critical as well. The importance of eating breakfast and of pre-meal high-protein snacks are two major themes emerging from the protein-related satiety literature. Contrary to popular belief, increased meal frequency (beyond three meals per day) does not appear to play a role in satiety. A study of overweight men indicated that a high-protein intake (comprising 25% of the calories for the day), but not frequent meals (six times per day), was effective at inducing satiety when subjects were consuming a weight loss (750 kilocalories less than subjects typically consumed) diet (Leidy *et al.*, 2011).

Skipping breakfast is becoming increasingly common among adolescents. It has been proposed that skipping breakfast may contribute to obesity by increasing hunger cravings and snacking throughout the rest of the day. The very act of consuming a breakfast, whether one that contained a normal level of protein or one that contained a high level of protein, led to increased subjective reports of "feeling full" as well as increases in the satiety-related hormone PYY in a small study of adolescents who were self-reported breakfast skippers (Leidy and Racki, 2010). A longitudinal study of over 7000 adolescents, 12–19 years of age reported that breakfast consumption was associated with lower risk of obesity and that African American youths, who tend to be at greater risk for obesity, were also less likely to consume breakfast (Merten *et al.*, 2009). Consumption of eggs for breakfast (which would constitute a high-fat as well as a high-protein meal) was associated with lower glucose, insulin and grehlin (a hunger-associated hormone) levels, as well as lower food intake for a full day following the egg breakfast, as compared with a high-carbohydrate, bagel-based breakfast, in a crossover study of a group of adult men aged 21 to 70 years old (Ratliff *et al.*, 2010). Some sources indicate that obesity rates continue to rise in adolescents, while they have plateaued, but remain high for the adult population. For this reason, the consumption of a nutritious, protein-rich breakfast may be especially important for adolescents (Ogden and Carroll, 2010; Ogden *et al*., 2012). In addition, breakfast is the meal where the least protein is typically consumed, so it may be the best meal for a high-protein intervention (USDA, 2008).

2.5.3 Effects of high protein pre-meal snacks on satiety

Although snacking is generally discouraged in the context of weight-loss or weight-maintenance diets, the strategic use of high-protein pre-meal snacks may increase satiety and lower overall calorie intake. There are many examples of this type of study in the satiety literature. The following studies are representative examples. A single-blinded study of 32 male volunteers reported that protein given 30 minutes prior to a meal had a greater effect on satiety than the same amount of protein given as an "appetiser" just before the meal (Abou-Samra *et al*., 2011). Whey-protein beverages containing 10–20 g of protein were effective at inducing reported satiety short-term, but were not effective at decreasing food intake two hours afterwards in a study of 50 overweight women (Poppitt *et al*., 2011). In contrast to appetite-suppressing effects of pre-meal snacks, post-meal snacks (high protein, high fat, or high protein) were reported to delay the onset of hunger, but did not affect the amount of food consumed (Marmonier *et al*., 2000). Overall, it appears that proteins consumed half an hour prior to a meal will decrease calorie intake. Snacks consumed greater than 30 minutes prior to or after a large meal do not appear to affect food intake at the next meal. Long-term studies are needed to determine whether regularly "spoiling one's appetite" using pre-meal snacks might result in fewer calories consumed and weight loss over time.

2.5.4 Permanence of protein-related satiety effects

There are questions in the literature as to whether high-protein diets lose their satiating properties if consumed regularly. In general, protein appears to have its greatest satiating effects when protein intake is initially increased. Some studies have reported that high-protein diets lead to lower calorie intake for several days up to six months, while others have reported only short-term benefits (Veldhorst *et al*., 2008; Larsen *et al*., 2010). In contrast, the idea that increased protein intake only affects satiety in the short term is supported by animal data indicating that the satiating effects of increased protein intake (from 14 to 50%) lasted no longer than one day (Bensaid *et al*., 2003). Since increasing protein intake beyond a certain level may have negative consequences, this may suggest that cycling protein intake between normal and high levels may be an effective way of maximising the satiating effects of protein, while avoiding negative effects (Long *et al*., 2000). Given that most studies examining the effects of proteins on satiety have been short-term in nature, the question of whether protein is satiating over the long term remains largely unanswered and should be further explored.

2.5.5 Protein-related satiety mechanisms

High-protein diets may enhance satiety through effects on glucose and lipid metabolism or via sensory properties. Simpson and Raubenheimer have suggested that protein is the most sought after macronutrient and that overconsumption of fat, carbohydrate and therefore total calories, is partly due to an innate need to regulate protein intake. In other words, a low-protein diet is not "satisfying" and humans tend to overeat in order to compensate for a lack of protein in their diet, if given the opportunity. This is known as the "Protein-Leverage Hypothesis" (Simpson and Raubenheimer, 2005). Human and mouse studies testing this hypothesis appear to bear out the basic idea that low protein intake increases both carbohydrate and fat consumption. A randomised crossover study of 22 subjects comparing a high-protein, high-fat diet to a normal protein diet of equal calorie content found that the rate of intestinal gluconeogenesis (glucose formation) and the levels of the lipid-associated ketone body β-hydroxybutyrate increased, while appetite, as measured by a visual analogue scale, decreased. The satiating effects of the high-protein diet were attributed to elevated levels of the ketone body β-hydroxybutyrate in this study (Veldhorst *et al*., 2012). Other authors have reported that the newly reported phenomena of intestinal gluconeogenesis (which can result from either high-protein diets or from gastric bypass surgery) is most important to satiety (Penhoat *et al*., 2011; Duraffourd *et al*., 2012). The artificial sweetener sucralose has been reported to cause the release of satiety hormones CCK (cholecystokinin) and GLP-1 (glucagon-like protein-1) in human intestinal cells. When administered together with pea protein, a synergistic increase in satiety hormone production was observed (Geraedts *et al*., 2012). This indicates that the satiating effects of protein are still poorly understood and that protein effects are difficult to separate from the intake of other food components.

The satiating effects of protein may vary based on the source and type of protein in question. Some studies have compared the effects of protein-based whole foods (e.g. chicken versus fish versus beef). These studies have reported that fish appears be the most satiating overall, while others have reported little difference in satiety effects across protein sources (Uhe *et al*., 1992; Borzoei *et al*., 2006). Others studies have compared the effects of different proteins within a given food source, such as dairy. There are two basic types of dairy protein, casein and whey. Casein makes up 80% of the protein content of liquid milk and forms they curd that makes up many cheeses. Whey proteins are so named because they remain in solution after casein curd formation and are separated from casein curds in the "whey" that is drained off during cheese making. Glycomacropeptide (a chymosin hydrolysis product of κ-casein) is an exception to this rule, since it also remains in solution during cheese production and thus can become a component of some whey-protein ingredients. Glycomacropeptide-containing whey-protein products may have different satiating effects than either whey or casein proteins alone. A study comparing the effects of a whey-protein-based custard containing glycomacropeptide versus a whey custard without glycomacropeptide reported that only whey with glycomacropeptide was effective at inhibiting food intake at a later meal (Veldhorst *et al*., 2009). A second study comparing the effects of pre-meal whey-protein beverages with or without glycomacropeptide, to a carbohydrate-based beverage, reported no specific effects of whey or glycomacropeptide. Instead, both protein-containing beverages were more satiating overall than the carbohydrate beverage. No effects on total calorie consumption were observed, however (Lam *et al*., 2009).

Dairy proteins may have positive effects on both satiety and weight loss. No weight loss was observed over three months when whey protein was consumed at 15% of total calories by free-living, middle-aged adults (Aldrich *et al*., 2011). This result contrasts with a six-month, double-blind, placebo-controlled study comparing the effects of whey and soy protein to a maltodextrin (carbohydrate) placebo in a similar population. In that study, the whey-protein group lost significantly more body weight and body fat than the maltodextrin group. In addition, waist sizes were smaller and fasting levels of the hunger-associated hormone ghrelin were lower in the whey-protein group (Baer *et al*., 2011). High dairy-protein diets more effectively promoted fat loss (especially visceral fat loss) compared with diets containing adequate protein (with or without dairy protein) in a study of 90 overweight, but otherwise healthy, young women. The authors concluded that beneficial effects of the high-dairy-protein diet were due to the combined effects of protein and calcium (Josse *et al*., 2011). Further studies are needed to determine whether some types of protein-based whole foods, or individual proteins, are substantially more satisfying than others.

In general, proteins could be considered the most "satisfying" of the macronutrients, in the sense that protein staves off hunger for longer periods than either carbohydrates or fats. In addition, increasing the protein content of a food or of an entire diet has the general effect of improving satiety. This improvement in satiety may have two roles.

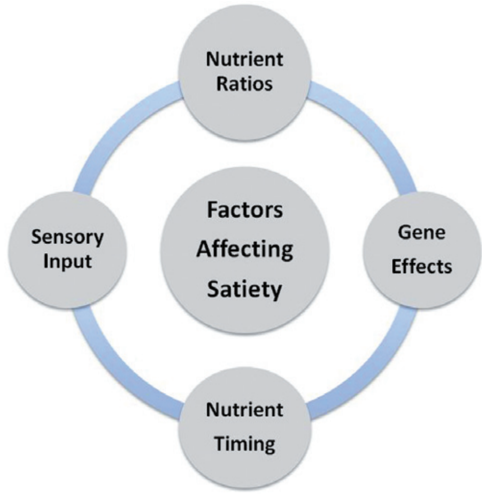

Fig. 2.4 Interactions of multiple factors related to satiety.

Firstly, it may improve mental state, even when calories are limited (as when individuals are dieting). Secondly, it may aid in resisting the temptation of excess calories in affluent societies where food calorie availability is essentially unlimited. The concept of satiety is a simple one, but the sensory, psychological and hormonal elements associated with it make the consistent achievement of satiety a challenging proposition. It is important to keep in mind that protein intake per se is only one of several inter-related components linked to satiety. Nutrient ratios, nutrient timing, sensory effects and gene effects all come into play, as shown in Fig. 2.4. Although it was not the focus of this chapter, scientists are just now beginning to appreciate the profound interaction of human genes with those of our bacterial flora in determining individual responses to food intake.

2.5.6 Thermogenic Effects of food proteins

For individuals seeking to lose or maintain bodyweight, protein provides a second potential benefit in addition to satiety: thermogenesis. Thermogenesis is the temporary increase in calories expended that occurs after eating, reflecting the amount of energy required to digest, store and/or utilise nutrients. This is known as the "thermogenic effect of food". Although all macronutrients have a certain thermogenic effect, the thermogenic effect of proteins is greater than that of either fat or carbohydrate. A review of the scientific literature published by Harvard's School of Public Health reported that proteins enhance both satiety and thermogenesis, but stopped short of saying that these effects caused either weight or fat loss (Halton and Hu, 2004). The thermogenic effect of a high protein intake was more explicitly illustrated in a later study comparing diets at 10% protein versus 30% protein in a group of 12 women. Although both diets contained 30% fat and the same number of total calories, women consuming the higher-protein diet expended more calories both while awake and while asleep (Lejeune *et al*., 2006). The satiety-enhancing effects of protein, along with its thermogenic effects may provide dual benefits to individuals seeking to lose or maintain weight. As with satiety-related effects, further studies are needed to confirm whether the increased thermogenesis observed when protein intakes are increased are permanent or only temporary.

2.6 ALLERGY TESTING OF PROTEINS

Given that some common food proteins, most notably peanut proteins, can also act as allergens, it is important to consider target populations when designing food products. The common foods that make up the "Big Eight" food allergens include: milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat and soybeans (USFDA, 2012). This list presents significant challenges to the food product developer, given the high level of ingredient functionality (foaming, gelling, browning, etc.) these food proteins possess. Although any protein-based food could cause an allergic reaction in a sensitive individual, several other common foods, including buckwheat, celery, sesame seeds and mustard seeds have been identified as foods that may be frequent causes of food allergies. The University of Nebraska-Lincoln maintains an extensive, peer-reviewed database of known allergens and their protein sequences at their FARRP (Food Allergy Research and Resource Program) web page (University of Nebraska-Lincoln, 2012). Potential allergenic proteins are screened in three different ways: resistance to pepsin digestion; screening of amino acid or genes for sequence homology to known allergens; and IgE screening of the serum of individuals with known allergies (van Putten *et al*., 2006; Thomas *et al*., 2009). The primary issue with these screening approaches is that aside from pepsin resistance, it is not possible to accurately predict the allergenicity of novel proteins, whether they come from foods only recently introduced into the human diet or from genetically engineered proteins. In addition, there are no animal models to detect novel allergens, although animal models are available for existing allergens (Ahuia *et al*., 2010). Even with established animal models, responses do not always mirror those of humans suffering from allergies.

2.7 BIOACTIVE PEPTIDES

As mentioned previously, bioactive peptides have a wide range of potential functionalities, including the regulation of blood pressure, cholesterol levels, vascular function, immunomodulation and the correction of inborn errors of protein metabolism (Gilani *et al*., 2008; Ballard *et al*., 2012). These peptides are generated via the hydrolysis of a food protein to varying degrees, followed by the use of bioassays to screen for potential health effects. A number of technologies have recently arisen to carry these peptides through the process of digestion and allow delivery to target organs, including the attachment of glycosylphosphatidylinositol, or GPI to target molecules (Muller, 2010). GPI is a complex lipid and carbohydrate molecule that functions to anchor proteins in cell membranes. It has been theorised that GPI may also facilitate the transport of proteins from the intestine to the bloodstream. That said, one hallmark of allergenicity is a resistance to digestion. Care must therefore be taken to avoid the production of potential allergens when bioactive peptides are produced. PEGylation, or the attachment of a polyethylene glycol molecule to a peptide of interest is one way to decrease the potential for allergenicity (Schellekens, 2002). The attachment of multiple molecules to a peptide might be expected to influence its function, so that further research may be needed to balance the bioavailability, allergenicity and efficacy of bioactive peptides. One question that remains to be answered is whether naturally occurring food proteins produce bioactive peptides during normal digestion and, if so, what the biological effects of those peptides might be.

2.8 RECOMMENDATIONS FOR HIGH-PROTEIN FOOD PRODUCT DEVELOPMENT

As was discussed earlier in the chapter, food scientists use macronutrients and other food components as tools to create products that deliver specific flavours, textures or other desirable properties to the consumer. Based on the current scientific literature and the worldwide need to combat both obesity and hunger, at least four categories of high-protein food products could be envisioned. Firstly, savoury, crunchy and/or chewy "sensory-intense" high-protein snacks could be developed for consumption 30 minutes prior to a meal in order to improved satiety and potentially decrease overall calorie consumption at subsequent meals. Secondly, thickened high-protein beverages could be used for similar purposes as savoury snacks, but could be designed for individuals who find sweet foods more acceptable than savoury ones. Thirdly, a focus on convenient high-protein breakfast foods is warranted, because a high-protein breakfast has been reported to decrease cravings over the rest of the day. In addition, breakfast is the meal most likely to be skipped and where the least amount of protein is consumed. A significant amount of marketing and public service messages may be needed to promote breakfast consumption, since skipping breakfast now appears to be a major cultural trend, at least in the US. Finally, the development of high-quality protein foods using complimentary vegetable proteins addresses several concerns. Foods based on complimentary plant proteins can be more economically and sustainably produced than those based on animal products. The major challenges to the use of plant proteins are the maintenance of food functionality (foaming, gelling, etc.) and the elimination of anti-nutritional factors to maximise protein quality. Development of plant-based high-protein foods must take cultural food norms into consideration in order to produce desirable and sustainable food products.

2.9 CONCLUSION

Food science has provided us with a wealth of information about the functionality of food ingredients and how to form structures that deliver desirable flavour, taste and texture. Likewise, advances in nutrition science (omic technology and advanced analytical techniques) have deepened our understanding of the personal effects of diet on health. Unfortunately, food scientists and nutrition scientists often work in isolation and miss opportunities for producing "nutritious and delicious" foods. Moreover, worldwide problems, such as obesity and diabetes, will require an integrated response. Major challenges include the needs for improved understanding of:

- Individual and long-term health effects of high-protein diets.
- Effects of age, physical condition, and athletic performance on protein needs.
- How to get high nutritional quality and food structure functionality from the same protein-rich plant foods.
- How to produce highly palatable, satiating, protein-rich foods that consumers would choose rather than avoid.

The first two issues could be thought of as primarily "nutritional" questions; however, they have implications as to how the food supply should be moulded to fit societal needs. The second two are good examples of how food science and nutrition can be blended into producing "healthier foods for the future".

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