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Food Protein Functionality—A New Model

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C: Food Chemistry

Abstract: Proteins in foods serve dual roles as nutrients and structural building blocks. The concept of protein functionality has historically been restricted to nonnutritive functions—such as creating emulsions, foams, and gels—but this places sole emphasis on food quality considerations and potentially overlooks modifications that may also alter nutritional quality or allergenicity. A new model is proposed that addresses the function of proteins in foods based on the length scale(s) responsible for the function. Properties such as flavor binding, color, allergenicity, and digestibility are explained based on the structure of individual molecules; placing this functionality at the nano/molecular scale. At the next higher scale, applications in foods involving gelation, emulsification, and foam formation are based on how proteins form secondary structures that are seen at the nano and microlength scales, collectively called the mesoscale. The macroscale structure represents the arrangements of molecules and mesoscale structures in a food. Macroscale properties determine overall product appearance, stability, and texture. The historical approach of comparing among proteins based on forming and stabilizing specific mesoscale structures remains valid but emphasis should be on a common means for structure formation to allow for comparisons across investigations. For applications in food products, protein functionality should start with identification of functional needs across scales. Those needs are then evaluated relative to how processing and other ingredients could alter desired molecular scale properties, or proper formation of mesoscale structures. This allows for a comprehensive approach to achieving the desired function of proteins in foods.

Keywords: foam, gel, protein, protein functionality, sol

Introduction

It could be argued that proteins are biology's most versatile polymers. The other main biological polymers—polysaccharides and nucleic acid polymers (DNA and RNA)—are structurally restricted because being composed of just a few monomers limits the chemical properties that can be imparted in the final structure. In contrast, proteins with 20 amino acids have the ability to take on a range of structures that provide catalytic activity (enzymes), building material for tissues (collagen), and mechanisms for movement (myosin and actin), to name a few of the numerous biological functions. The history of proteins has been eloquently covered in a book by Charles Tanford and Jacqueline Reynolds titled "Nature's Robots (Tanford and Reynolds 2001)." As the name implies, proteins are molecules that play an active role in biology and the concept of activity is usually expressed as *biological function*. The central dogma of protein chemistry is that the sequence of amino acids determines the 3 dimensional structure, and the 3-dimensional structure determines the biological function (Creighton 1993). This is fitting for biological functions where protein structure is the result of evolutionary pressure to survive and be passed on to the next generation. A fascinating example of this is the umami receptor in hummingbirds evolving to essentially become a sweetness receptor, allowing hummingbirds to adapt to a different food source (Baldwin and others 2014). Proteins take on a broader *functionality* role in foods. They can function in foods by an extension of their biological activity, by

being building blocks for food structures that biology had not intended, or they can bind a variety of small molecules and alter their availability during ingestion.

One example of proteins functioning in foods as an extension of their *biological structure and function* is enzymes. The ability of enzymes to catalyze reactions can produce desirable (meat tenderness altered with proteolytic enzymes) or undesirable (fruit browning caused by polyphenol oxidase) changes in food. In other cases, the *polymer and colloidal properties* of proteins are manipulated to produce desirable food structures, as is the situation with dairy foods. The biological function of casein micelles is not to make cheese or yogurt; however, the colloidal and polymer properties of casein micelles allow for the production of a wide range of dairy products. Finally, protein surfaces have different degrees of polarity and *potential binding sites* for small molecules such as flavor compounds and polyphenols (Franzen and Kinsella 1974; O'Connell and Fox 2001; Bandyopadhyay and others 2012). This binding could alter the ability to perceive a flavor (Reiners and others 2000) or could be used to decrease loss of a bioactive polyphenol (Livney 2010).

The function of proteins in foods, and therefore what is implied by the term *protein functionality*, becomes a matter of the task proteins perform in a specific food application. Moreover, proteins may be added with the intent to form specific colloidal structures but have an unintended undesired secondary effect. For example, in beverages containing whey proteins the pH is adjusted to pH 3.2 to 3.5 to increase the colloidal stability of the protein sol but also imparts undesirable astringency (Beecher and others 2008). An ideal goal would be to have a "functionality label" on a food protein ingredient that provides measures of how effective the ingredient would be in a range of food applications. The following discussion provides a perspective on how to view protein functionality in foods with the goal of a complete functionality

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assessment. It builds and expands on a previous evaluation of protein functionality (Foegeding and Davis 2011).

Understanding How Proteins Function in a Food from the Basis of Food Structure

Proteins are generally part of a complex structure in foods that determines hedonic properties and may contribute to the bioavailability of nutrients and other bioactive compounds. Some examples of foods and properties associated with proteins are seen in Table 1. Each food property can be explained by structural elements according to scale (Figure 1 and Table 1). From this perspective, *Food structure* is the combined representation of a food starting with the molecules, the primary structures formed from the molecules, and the overall structure observed by the consumer (Figure 1). The *molecular scale* (<1 nm to ~150 nm) is the structure of the individual molecule. In proteins, this encompasses primary, secondary, tertiary and, when applicable, quaternary structure (Creighton 1993). The *macroscopic level* is the highest level and represents the overall organization of molecules and substructures in a food. This structural level is observed when viewing food and evaluated when eating. Intermediate between molecular and macroscopic scales is the *mesoscale*. This has also been referred to as microscale due to features with sizes in the range of micrometers, but *meso* meaning “middle or intermediate” (from ancient Greek for “middle”) is more accurate as nano size structures can also be involved. Structures viewed at the microscale have historically been the focus of “food structure analysis,” for example see review by Heertje (2014), but a more recent and comprehensive approach to food structure is to take on the perspective of soft matter physics and view structures across length scales (Ubbink and others 2008; van der Sman 2012). Foods can be defined on a soft matter physics length-scale perspective as a collection of molecules (molecular scale) assembled into various mesostructures (mesoscale) that are assembled into the overall food structure (macroscale). Hedonic sensations of a food—those associated with appearance, taste, aroma, and texture—are therefore based on molecules and structures occurring at different length scales. For example, the basic taste of sweet occurs at a molecular scale while hardness in gels is due to mesoscale gel networks (Kaneko and Kitabatake 2001; Çakır and others 2012).

The role of mesostructures can be further illustrated by examples of milk and meat products. The function of proteins in meats can be subdivided into 2 general categories: those based on *biological structure/function* or those that are *polymer/colloidal property* based. An example of the former is the role of muscle proteins in producing the texture of a grilled beef steak. An individual muscle is composed of muscle fibers containing contractile proteins (molecular structure), formed into myofilaments (mesostructures) that are aligned into sarcomeric structures (a collection of mesostructures) (Aberle and others 2001). The sarcomeres are the smallest functional unit undergoing muscle contraction achieved by the myofilaments interacting and shortening the sarcomere. Muscle fibers are grouped into bundles by connective tissue, and bundles are collected into a muscle (macrostructures) (Aberle and others 2001). The proteins in the myofilaments, sarcomeres, and connective tissue all contribute to the texture of cooked meats (Quali 1992). This form of protein functionality is directly related to the biological mesostructures (for example, myofilaments and sarcomeres) and how physiological events occurring during conversion of muscle to meat alter those mesostructures. This often requires *in situ* investigations to understand structural changes associated with meat tenderness. Forma-

tion of processed meats such as a hot dog/frankfurter is a different matter. Myofibrillar proteins are extracted (dispersed) from their biological structures and form interfacial films around dispersed fat droplets (colloidal/polymer mesostructure) and, upon heating, a gel network (colloidal/polymer mesostructure) is formed that traps the dispersed fat phase (Foegeding and Ramsey 1987). In processed meats, protein functionality is related to the ability of muscle proteins to form and stabilize colloidal structures. However, post-mortem events may alter the proteins and/or the ability to extract the proteins from the biological structures for use in forming colloidal structures.

A contrast to meats would be milk and milk products. The biological structure of milk is actually a colloidal dispersion of proteins (whey and casein micelles) and fat globules in a true solution (serum phase) containing salts, lactose, and other compounds. Casein micelles are naturally dispersed in milk and stabilized against aggregation by steric and electrostatic mechanisms that are also used to stabilize synthetic colloidal systems (Tuinier and de Kruif 2002; de Kruif and others 2012). Cheese making involves controlled destabilization of the casein micelles so that they aggregate into a continuous gel network that, like in hot dogs, traps the dispersed fat phase. Subsequent steps decrease the amount of solution/dispersion (water, minerals, lactose, protein, and some lipid) surrounding the casein gel network, confining the fat inside the network, and by dehydration and other means, lower water activity to preserve the system. The biological structural changes are at the molecular and supramolecular (casein micelles) level to create a mesostructure of a protein gel network entrapping fat particles—a so-called emulsion gel (Dickinson 2012).

Historic Approaches to Understanding Protein Functionality

The role of proteins in forming food structure and imparting texture and other properties has been investigated based on 2 general approaches. Scientists interested in specific food products, such as processed meats, cheese, and bread, often take a *top down* approach where the contribution of individual components of a system are determined by changing 1 or a few variables in the overall system and determining the effects. Butchers and bakers have used this approach for centuries to optimize their processes by close observation and adjustments—the difference being that practitioners typically have a goal of understanding *how* to make a better product as opposed to scientists asking *why* it is a better product. A second approach is *bottom up*, where simple systems are fully investigated and progressively assembled into more complex structures. This is the hallmark of reductive experimental science focused primarily on the molecular and mesoscales. An early food example of this approach is Sammis and Hart (1909) determining the effect of various acids (lactic, oxalic, acetic, and phosphoric) on the precipitation of casein relative to the formation of cheese curds. This is a bottom up approach to understand the complex process of cheese making. The bottom up approach is applicable to understanding how food protein ingredients function in a variety of application.

Peter and Bell addressed a food functionality challenge in 1930 that is still relevant in 2015—substituting proteins and maintaining food properties such that, hopefully, the reformulated product is indistinguishable from the original. They stated that “The foaming and heat-coagulative properties of egg albumin (egg white) add greatly to the commercial importance of the product;” establishing the functional properties of foaming and heat-induced aggregation (Peter and Bell 1930). They went on to introduce the specific

Table 1–Properties of foods associated with proteins and their related structural element.

Food	Food property	Structural elements by scale		
		Molecules	Mesostructures	Macrostructure
Skeletal meat	Texture	Myofibrillar proteins and collagen	Sarcomere, connective tissue	Amount and location of connective tissue and muscle fibers
	Color	Myoglobin		
	Water holding	Myofibrillar proteins and collagen	Sarcomere, connective tissue	Amount and location of connective tissue and muscle fibers
Cheese	Texture	Caseins and whey proteins	Emulsion, Gel	Phase volumes of emulsion and gel; gel structure; emulsion droplet size
Tofu	Melting and flow	Caseins and whey proteins	Gel	Phase volume and structure of gel
	Texture	Soy proteins	Gel	Gel structure
	Water holding	Soy proteins	Gel	Gel structure
Beverages	Texture (mouthfeel)	Various proteins	Sol	Size and shape of solid particles
	Clarity	Various proteins	Sol	Size and shape of solid particles
Bread and Cakes	Texture	Various proteins	Foam, Emulsion	Phase volume of foam and emulsion; bubble (foam) and droplet (emulsion) size

topic by stating that “The possession of these characteristics by a much lower-priced product made from whey, either naturally or as the result of permissible physical or chemical treatment, would result, therefore, in its extended use” (Peter and Bell 1930). This type of investigation continues today with additional motivations (beyond lower cost) of dietary restrictions (gluten free or vegetarian) and a more sustainable ingredient source, to name a few goals. Based on the food structure model (Figure 1), successful substitution of one protein for another (changing the molecular

scale) is based on the ability of various proteins to form the same mesostructures. In other words, Peter and Bell worked on the hypothesis that if they could show how to produce whey protein foams with properties similar to egg white foams, then whey proteins could be substituted for egg white. This is a logical yet often incomplete approach that will be discussed in subsequent sections. Scientists have used both top down and bottom up approaches to understand protein functionality, and this has produced confusion on the precise meaning of *protein functionality*. Difficulties

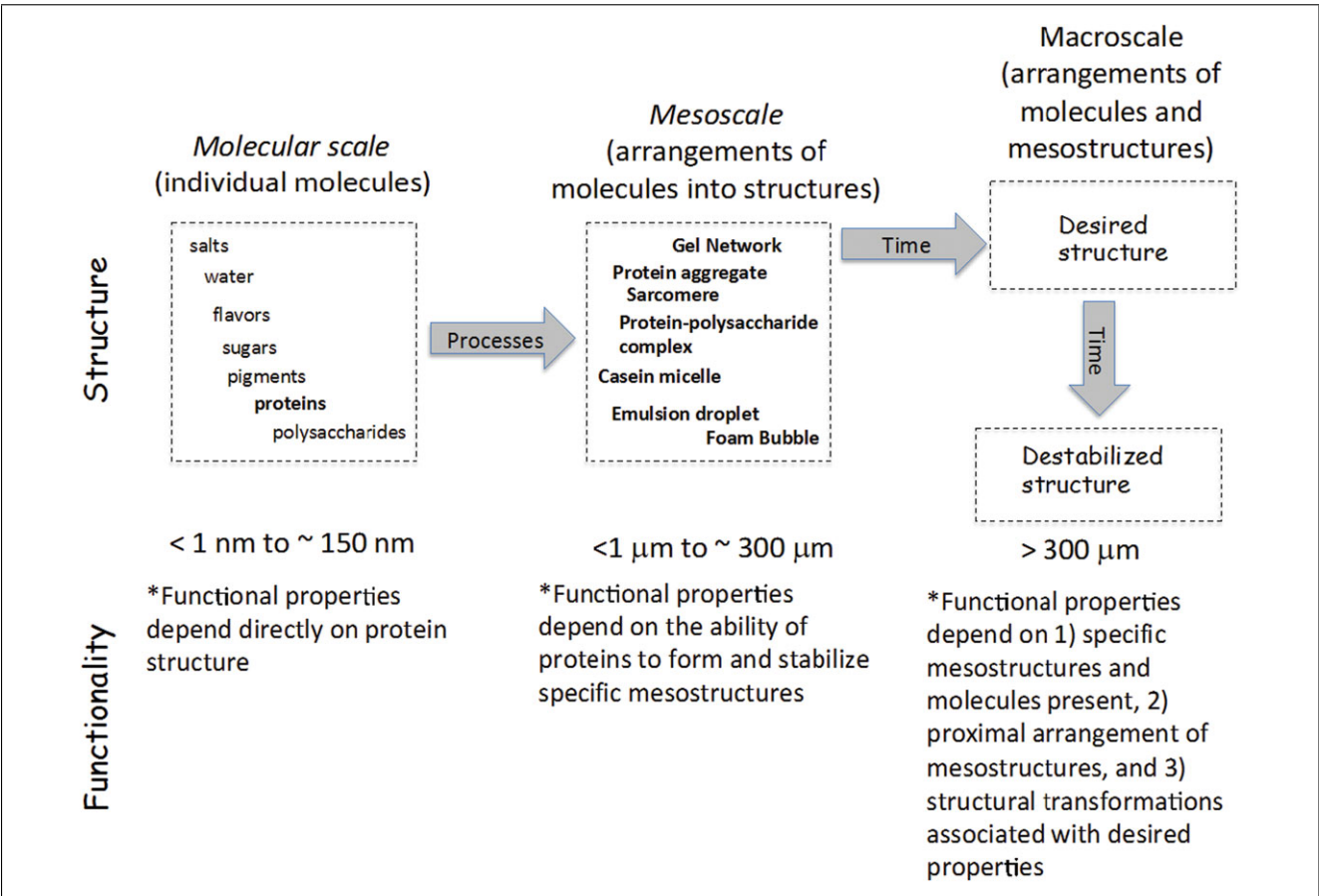


Figure 1–Protein functionality based on length scale of structural elements determining functionality.

Table 2—Molecular scale functional properties of proteins.

Molecular property	Protein example	Food use or concern
Color	Myoglobin	Fresh meat color
Sweetness	Thaumatin	High intensity sweetener
Enzymatic activity	Chymosin	Used in making cheese
Ligand binding—flavor	Many proteins	Lower volatility of desired or undesired aroma compounds
Ligand binding—polyphenol	Many proteins	May protect bioavailability of a bioactive polyphenol
Allergenicity	Many proteins	Cause food allergy
Bioactive peptides	Angiotensin-converting-enzyme inhibitor (ACE inhibitor)	Lower heart rate

in applying the term “functionality” to food proteins and food applications were addressed by Pour-El (1981) in stating that “No subject in the general area of food chemistry and technology has suffered more from inconsistency, confusion, and ambiguity than the field of functionality.” Pour-El (1981) proposed a list of food types and associated protein functionalities. Some of the so-called functionalities mentioned by Pour-El (1981) are directly related to molecular or mesoscale structures that can be characterized by a range of chemical and physical properties associated with molecular structure (color and complex formation) or formation and stabilization of colloidal mesostructures. The colloidal systems covered are sol (solid dispersed in liquid), emulsion (liquid dispersed in a liquid), foam (gas dispersed in a liquid), and a gel (continuous solid network surrounded by a fluid). Other functionalities were a bit more ambiguous and dependent on specific empirical tests (solubility, fat holding capacity, liquid holding capacity, and water absorption). In addition, some were associated with sensory perception of macroscale structures (hardness, chewiness, cohesion, and adhesion). If a protein has a function, and the function is due to chemical/physical aspects of the protein structure or mesostructure formed, then there should be a way to assay that function. Hall (1996) proposed that protein functionality can be analyzed by methods that cover solubility, viscosity, gelation, foam formation and stabilization, emulsion formation and stabilization, water and fat holding properties, and surface hydrophobicity. These can be grouped into molecular (hydrophobicity and viscosity), molecular and mesostructure (solubility of a powdered protein), and mesostructure (gel, foam, emulsion, and fat and water holding) scale properties. This is generally valid as a point of comparison that is independent of a specific food application. However, these tests must cover a range of solution conditions (protein concentration, pH, ions, and ionic strength) to be robust enough to cover a broad range of food applications. The advantage of this approach is that there are numerous physical and chemical tests that can be used to characterize molecular and mesostructure properties. Still, it is a bottom up approach and omits how mesostructures interact with other components in forming the macrostructure. In addition, the ultimate evaluation of a food structure is a sensory response. A more comprehensive approach is needed that allows for understanding how proteins form specific structures and how humans perceive those structures.

Model for Protein Functionality

A model to explain how proteins function in food must be comprehensive enough to cover enzymatic activity producing tenderness in meats; the formation of gel, emulsion, and foam structures; and the human perception of texture. In addition, it should be adjustable to account for how nonprotein molecules modify protein functionality. This is accomplished by defining functionality based on the critical structural scale determining functionality (Figure 1).

The model consists of 3 structural scales and functional properties associated with each structural scale.

Molecular scale properties are those that can be explained primarily on protein structure considerations. Some examples of molecular scale properties are seen in Table 2. Enzymatic activity, binding of proteins to biological receptors, color, and binding of ligand compounds (for example, flavor compounds or polyphenols) are the result of specific properties of protein structure. These functions are analogous to biological structure–function relationships and amenable to a broad range of physical, chemical, and molecular biology tools that are used to determine how proteins function in biology. For example, a molecular docking computational tool can be used to evaluate how ligands (flavor compounds or polyphenols) bind to proteins (Erickson and others 2004). There are molecular properties that have a direct impact on health and nutrition. Proteins are the source of amino acids and bioactive peptides; therefore, it is essential that modifications that reduce these contributions be avoided. Likewise, proteins can have epitopes that illicit an allergic response. This restricts sensitive populations from consuming certain foods and the modulation of allergenicity should be considered with any modification of food protein structure. Any functional property that can be fully explained based solely on the structure of a protein molecule, and is not reliant on forming a mesostructure, is termed a *molecular scale functionality*.

The next level of scale is the arrangement of protein molecules into definable *mesoscale* structures. Mesoscale structures must be formed, so a process or self-assembly is needed to connect the molecules into structures. Biological properties such as muscle contraction—likewise texture of gels, emulsions, and foams—are properties originating from mesostructures. The formation and stabilization of individual mesostructures have classically been the main focuses of measuring protein functionality. As discussed previously, Hall (1996) has proposed methods for analyzing gelation, foam formation and stability, emulsion formation and stability, and water and fat holding (Hall, 1996). Protein functionality as the ability to form and stabilize colloidal mesostructures is a good way to compare *relative functionality* among proteins, or the result of protein modification, but may miss a critical element needed to perform in a specific food product. If there were standardized conditions for forming colloidal structures, then the properties of those structures could be considered an *inherent functionality* of a protein.

The final level is the *macroscale*, which accounts for all elements comprising a food. The science of soft matter physics is based on the premise that the behavior of soft matter materials cannot be predicted from molecular constituents and originates from mesostructures (Ubbink and others 2008; van der Sman 2012). The macrostructure can be viewed as the amount and spatial distribution of definable mesostructures and molecules. It can be very simple, as in a gelatin gel, or the complex structures found in ice cream, cakes, or cheese, to name a few. Furthermore, there are

Table 3—Locations and properties of proteins in colloidal systems relative to formation and stabilization.

Colloidal system	Continuous phase	Dispersed phase	Interface
Sol	Viscosity relative to a second dispersed phase	Protein aggregate size, shape, and density	NA
Foam	Viscosity, formation of a network, interaction with interface	NA	Protein film thickness, gas permeability; surface properties (electrostatic or steric stabilization); and rheological properties
Emulsion	Viscosity, formation of a network, interaction with interface	NA	Protein film thickness; surface properties (electrostatic or steric stabilization); dispersed phase permeability; and rheological properties
Gel	Solid continuous phase Continuous gel network of characteristic strand thickness, connectivity, and phase volume	Liquid continuous phase Viscosity and interactions with the gel network	NA

NA, indicates not applicable.

some foods where processing produces an initial “green” structure, but the target structure is the result of slow changes during aging, such as in cheese. Products like cheese require an aging process to allow for slow transitions at the molecular and mesoscales to produce the desired macroscale structure.

Macroscale Structure Based on Forming and Stabilizing the Appropriate Mesostructures

As seen in Table 1, processed foods that rely on proteins for food structure can be modeled as one or a combination of the basic colloidal structures of sol, emulsion, foam, or gel. This has led to producing and stabilizing said colloidal structures as being a primary focus in measuring protein functionality. Moreover, forming and stabilizing colloidal structures have a solid foundation in theoretical and experimental science (for example, Dickinson 1992; Birdi 2009) that provides useful models to determine mechanisms responsible for formation and stability in foods (Dickinson 1992; Walstra 2003). Each colloidal structure has various elements. Sols, foams, and emulsions have a *dispersed phase* surrounded by a *continuous phase* and the *interface* where the 2 phases meet. Proteins can function as being the dispersed phase (sol), forming an interfacial film (emulsion or foam) or as part of the continuous phase (sol, emulsion, or foam) (Table 3). This means that several parameters need to be determined when evaluating protein functionality, even in a simple colloidal system.

Emulsions

One of the most common approaches for evaluating protein functionality in an oil-in-water emulsion is to compare several proteins at concentrations where there is sufficient protein to coat oil droplets and minimal remaining in the dispersed phase. If one protein produces smaller droplets, does it have a higher emulsifying ability? Yes, if your goal is to just produce small droplets. However, most emulsions have to be stable for an extended time, so the stability of the system is often more important than the initial physical state (that is, droplet size distribution). Do small droplets make for a more stable emulsion? Yes, if the destabilization mechanism is just based on the creaming rate of the oil droplets as described by the Stokes’ Law (Dickinson 1992). However, flocculation (surface association of droplets) and coalescence (forming 2 droplets into 1 droplet) can increase particle size and cause destabilization, and these phenomena are regulated by surface properties (Dickinson 1992). In the least, a measure of a protein’s ability to form and stabilize an emulsion—often lumped together as its emulsifying

ability—will not be a simple number but a measure of how it aids reducing particle size during emulsification and the properties of the protein film to resist flocculation and coalescence.

Foams

Formation and stabilization of foams are also complex and not described by 1 simple test or physical property. Take for example the ability to form a stable egg white meringue. Foam formation depends on egg white proteins creating interfacial films that trap gas in bubbles that are dispersed in a continuous aqueous phase. Destabilization processes involve properties of the dispersed phase (gas composition, bubble size, and bubble phase volume), continuous phase (water and soluble molecules), and the interfacial protein film (gas permeability, strength, viscosity, and elasticity) (Dickinson 1992; Koehler and others 2000; Yang and Foegeding 2010, 2011). The ability to form an interfacial film with specific properties may be somewhat predictable from knowledge of protein structure. At the least, the understanding of how protein structure is changed when adsorbed at a gas/water interface and how these changes allow for film properties would be useful (Barbana and Pérez 2011; Audebrand and others 2013). Even if understood at the single molecule level, this process can be altered by cofactors such as other surfactants or sugars. For example, increasing sucrose concentration in egg white foams will increase the interfacial dilatational elasticity of egg white protein films and foam stability (Yang and Foegeding 2010, 2011). Therefore a key functional property, forming a protein film with high dilatational elasticity, depends on inherent properties of the protein and how other ingredients modify them. This would suggest that a *primary functionality* to compare among proteins is properties of an air–water interfacial film from a standard aqueous solution—with application to a specific food formulation requiring accounting for additional constituents. This is evident when comparing the ability of egg white proteins or whey proteins to be used in making angel food cakes. A complete replacement of egg white with equal amounts of whey protein produces a cake that collapses upon baking (Pernell and others 2002). Both proteins have relatively similar abilities to form wet foams (Yang and Foegeding 2010, 2011) and have similar structural transitions when heated (Berry and others 2009). They differ when flour and sugar are folded into the foam to make the cake batter. This initiates destabilization in the whey protein foam and has minimal effect on the egg white foam (Berry and others 2009). In this case, the critical difference in protein functionality is not observed until a mesostructure is

mixed with other ingredients to form the batter (collection of mesostructures and molecules to form the macrostructure).

Sols

Protein-containing beverages can be considered sols, with the dispersed solid phase being protein aggregates. Beverages designed for meal replacement or workout recovery contain other ingredients that may affect protein interactions. The effect of nonprotein ingredients need to be accounted for when determining how a protein will function in a given beverage. A functional concept that is used regarding beverages is “thermal stability.” This evokes the notion of comparing protein denaturation temperatures (molecular scale), but in reality it is the stability of a colloidal sol of protein aggregates (mesoscale) that determines producing a satisfactory product and shelf life. Denaturation and aggregation are somewhat linked, but there are exceptions as with α -lactalbumin that denatures at $\sim 64^\circ\text{C}$ but with minimal aggregation (McGuffey and others 2005). An acceptable quality beverage is one that after thermal processing has protein aggregates that create the desired clarity, viscosity, and mouthfeel. Structures produced from protein aggregation (molecular scale interactions producing mesoscale structures) will depend on pH, ionic strength, type of ion, and protein concentration, in addition to other molecules such as sugars (Nicolai and Durand 2013).

Protein functionality in a beverage can be represented based on a state diagram that shows relationships between 2 or more variables. With the goal of producing a fluid, a general approach is to use a fixed thermal process and vary the solution conditions to determine critical concentrations to produce a stable sol. This has been done for β -lactoglobulin (Ako and others 2009) and whey protein isolate (Wagoner and others 2015). As seen in Figure 2, this allows for a comparison of zones of stability—based on forming a sol—and the sol region can be further subdivided to show properties such as solubility. The state diagram approach is applicable to

comparison of relative stability among various protein ingredients or could be used to determine the stabilizing/destabilizing effect of other beverage components (for example, salts and polyphenols). However, as with the example of emulsions, this only shows conditions relevant to formation of a sol after thermal processing, and further analysis is need to determine shelf stability. Shelf life is based in part on the protein particle phase remaining dispersed and not forming a precipitate, gel, or 2-phase system over time. At the molecular scale, most proteins are 1 to a few nanometers in diameter and sedimentation would be so slow as to not be a factor with a shelf life of up to a year or 2. Therefore, forming a visible precipitate or gel network will depend on the size and amount of aggregates formed during thermal processing and their propensity to undergo secondary aggregation at storage temperatures (Ryan and others 2013). In theory, these elements should be predictable based on how molecular structure (ability to form intermolecular interactions during thermal processing) determines the type of mesostructure (particle) formed. The desired mesostructure (particle) is small in size, had a density approaching that of the continuous phase, and has a higher amount of repulsive than attractive surface forces to inhibit or prevent secondary aggregation.

Gels

Forming a protein gel network can be considered under the same context as sol formation. In both cases, the primary concern is at the molecular scale in factors that favor interprotein aggregation. They differ in that the goal of forming a stable sol is to minimize aggregation while gelation requires directed aggregation of proteins into a 3-dimensional gel network. The mesostructure of the gel network will determine macroscopic physical properties of the gel (van der Linden and Foegeding 2009). Phase diagrams can be used to evaluate relationships among protein concentration, pH, salt concentration, and other ingredients regarding combinations that produce macroscale structures of sols, precipitates, or gels (Ako and others 2009; Wagoner and others 2015). One of the unique aspects of gel formation is that it requires a critical minimum of protein to form a continuous gel network (Clark and Lee-Tuffnell 1986). This may be an inherent molecular property but that would have to be established under conditions that are maximized for favoring aggregation that produces a gel network. For example, exhaustive heating (overnight at 80°C) of β -lactoglobulin solutions (4 to 6% w/v at pH 7) produced sols, homogeneous gels, micro-phase separated gels, or precipitates as the concentration of NaCl is increased from 0.01 to 1 M (Ako and others 2009). The state diagram presenting the relationship between NaCl concentration and protein concentration at pH 7.0 shows a minimal concentration for gelation of around 2% w/v; however, this may differ with a change in pH. A comprehensive approach for an individual protein would be to first generate state diagrams that cover a broad range of pH values and protein concentrations to determine critical points, and then evaluate how other factors, such as added ingredients, change the critical points in the state diagram.

Macroscale properties associated with gels include appearance (clear or opaque), firmness (force/deformation), strength (force to cause fracture), and water holding. These are all relevant to appearance and stability of the food product and possibly associated with sensory perception (Szczeniak 2002; Pascua and others 2013). They are related to elements at molecular and mesoscales (van Vliet 2002; van den Berg and others 2007a,b). A recent example is the finding that the micrometer length scale (mesostructure) is the most important factor determining water

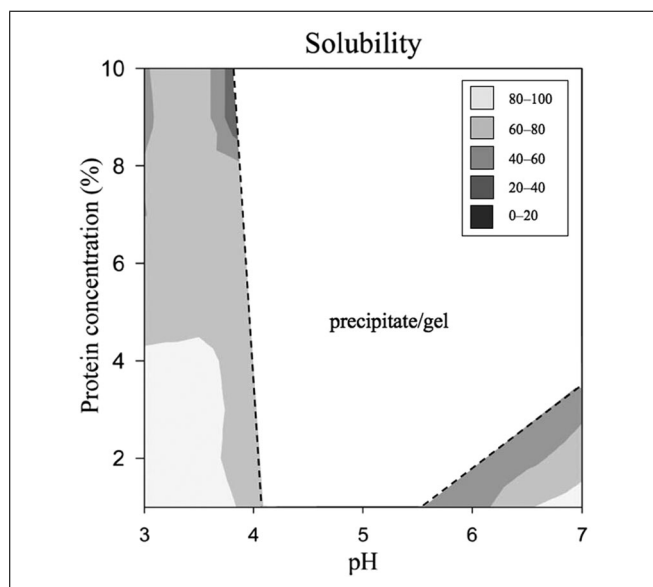


Figure 2—State diagram for whey protein isolate in a beverage after thermal processing. Image based on Wagoner and others (2015). Shaded areas are protein concentration and pH regions where a sol is formed after thermal processing. Within the sol regions, the percent solubility is shown according to the gray scale legend within the figure.

loss from soy protein gels under deformation (Urbonaite and others 2014). A unique challenge with physical properties of food gels is that there are numerous methods available to determine water holding, rheological or fracture properties; however, the relevance to perception during consumption may be more complex. That requires an understanding of how food molecules and structures are perceived by humans.

Interfacing with Human Physiology

There are several *molecular functional properties* that are important to consumers regarding hedonic and health aspects of proteins. Proteins are nonvolatile, so aromas associated with protein ingredients are due to aroma compounds that are either present in the ingredient or formed upon storage. This is more a characteristic of the overall ingredient and should not be considered a property of the protein per se. Likewise, the potential to produce aroma compounds during processing is beyond the scope of protein functionality. However, as mentioned previously, binding flavor compounds is one way that proteins can alter overall aroma of a food. Flavor binding can be modeled as a protein binding to small ligands and the number of binding sites and binding constants can be compared among proteins (van Holde 1985). Sweetness is a molecular property found in specific proteins (Thaumatococcus; Kaneko and Kitabatake 2001) and bitterness is associated with peptides (Matoba and Hata 1972). Peptides are also responsible for a range of bioactivity (Udenigwe and Aluko 2012). Allergenicity is an undesirable molecular property that should be considered, mostly from the standpoint of reducing or eliminating allergenicity (Hefle and others 1996; Poms and others 2004). These molecular properties can be assayed by an array of techniques such as chemical analysis, cell culture assays, animal studies, and human studies.

Colloidal mesostructures are important because they are responsible for textural properties and can modify flavor release (Taylor and Linforth 1996; Visschers and others 2006; van Vliet and Primo-Martín 2011). Terms such as hardness, firmness, and juiciness are directly linked to elements of gel structure (Foegeding 2007; Pascua and others 2013); however, creaminess—associated with emulsions and sols—often contains a flavor element in human assessment (Antmann and others 2011). Moreover, texture perception is determined by the breakdown pattern of foods during oral processing, and a clear relationship of how food structure breakdown patterns translate into sensory perception of texture has not been established (Foegeding and others 2015), but progress is being made. Factors such as if a protein-stabilized emulsion flocculated reversibility or irreversibility in the mouth affect texture perception (Vingerhoeds and others 2009). In emulsion-filled gels, the fat droplets can be bound to the protein network or unbound. The unbound structure produces more fat coalescence in oral processing and an increase in fat-related sensory attributes (Liu and others 2015). These can be viewed as mesoscale properties of an emulsion or gel that can be adjusted to specific transformations during oral preprocessing. In these 2 examples, 1 key factor is coalescence of oil droplets during oral processing. This suggests that besides understanding how to form and stabilize colloidal mesostructures, properties that are essential to consumer acceptance at purchase, transformations in the mesostructure during oral processing need to be understood regarding eliciting texture sensations (Foegeding and others 2015).

Concluding Remarks

The *functional properties* of proteins in foods can be based on the structural length scale required to achieve the function. Properties

such as color, sweetness, enzymatic activity, and ligand binding are observed at the *molecular scale* and should be predictable directly from protein structure. These properties should be invariant from 1 manufacturer to another and could be found in a master list based on common assays. Since food protein ingredients contain a mixture of proteins, the molecular scale properties of the ingredient would be the proportionate sum of the individual proteins.

At the next structural level, forming and stabilizing sols, emulsions, foams, and gels—and their physical and sensory properties—depend on forming specific *mesoscale* structures. The main challenge at this level is creating standardized conditions for forming the structures that do not inherently favor one protein over another. In comparing 2 proteins, the one that forms a stronger gel or more stable foam may depend on pH, therefore comparing across pH and protein concentration is needed. Once standard conditions for formation are established, then a range of tests to assess structure and structural stability can be used. One cautionary note is that processing equipment becomes outdated and conditions should be based on engineering principles that are independent of specific equipment. Also, methods to assess how structures are transformed during oral processing are needed.

The *macroscale* structure, which determines the overall properties of the food, is created by one or several mesoscale structures. As illustrated with angel food cakes, protein functionality differences may not appear until the mesostructures are mixed and processed in the final stage of food production.

An analogy can be drawn with determining the bioactivity of a polyphenol. An isolated molecule can show chemical activity, say antioxidant activity or binding to a protein of interest, which can be conclusively shown at the molecular scale. The activity in a biological system can move from cell culture, to rodent studies, and finally to human studies. Each level of increased complexity provides another potential barrier to it producing the bioactivity predicted from the molecular structure.

Protein functionality should start with defining the length scale and structural basis for the properties of interest. Those defined at the *molecular scale* are inherent to protein structure and can be cataloged for individual food proteins. Formation and stabilization of *mesoscale* structures have been the historic approaches in measuring protein functionality. This is still valid in comparison among proteins and as a first approximation of how they will function in a food. It is most important that common means of forming a structure are established. There are numerous ways of evaluating a structure, so structure formation should be the common point among investigations. “The proof of the pudding is in the eating,” a phrase from the 14th century, is still true when it comes to evaluating the performance at the *macroscale* in the final product (https://en.wiktionary.org/wiki/the_proof_of_the_pudding_is_in_the_eating). This does not negate the importance of understanding across length scales and degree of complexity, as an understanding of how proteins form and stabilize mesostructures is often needed to address failures at the macroscale. Determining the length scale functionality needed for a specific application should allow for a more direct path to choosing which protein and processes are needed to achieve the desired food property.

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Author Contributions

E.A. Foegeding is the sole contributor to the article.

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