JFS R: Concise Reviews/Hypotheses in Food Science

Protein Stabilization of Emulsions and Foams

SRINIVASAN DAMODARAN

ABSTRACT: Proteins play an important role as macromolecular surfactants in foam and emulsion-type food products. The functioning of proteins in these applications is determined by their structure and properties in the adsorbed layers at air-water and oil-water interfaces. In addition, because typical food proteins are mixtures of several protein components, interaction between these components in the adsorbed layer also impacts their ability as surfactants to stabilize dispersed systems. In this paper, recent progress in our understanding of the molecular mechanisms involved in the formation and stability of protein-stabilized foams and emulsions has been reviewed.

Keywords: foams, emulsions, interfacial properties, proteins, surface activity

Introduction

Processed foods are very complex multi-component systems consisting of polymeric proteins and polysaccharides, fats, water, sugars, flavors, and other small molecular-weight compounds. In most cases, some of these components, for example fat and aqueous phases, do not mix with each other and exist as different phases within the food matrix. Although proteins and polysaccharides are soluble in the aqueous phase, they often exhibit incompatibility of mixing and undergo coacervation, a phenomenon often termed as water-in-water emulsion (Tolstoguzov 1998; de Kruif and Tuinier 2001; Stokes and others 2001). Other small molecular-weight organic substances, such as flavors, partition between the aqueous and the oil phases depending on their relative solubility in these 2 phases (Meynier and others 2003). Such phase separation, aggregation, and partitioning phenomena profoundly affect the organoleptic properties, especially the textural and mouth-feel properties, of food products (Tolstoguzov 2000). The phase separation phenomenon is particularly of concern in liquid-type food emulsions and foams as compared with solid foods because of greater molecular mobility inherent in liquid-type foods. In products such as ice cream, salad dressings, spreads, infant milk formulas, geriatric foods, and so on, the stability of the dispersed fat phase, which is present in the form of micron-sized dispersed particles, during storage is essential for product acceptance at the time of consumption.

Food dispersions generally are of 3 types, namely, oil-in-water and water-in-oil emulsions, in which 1 liquid phase is dispersed in another liquid phase; foam, in which air (gas) bubbles are dispersed in an aqueous medium; and sol, which is small solid particles dispersed in a liquid medium. In oil-in-water emulsions, an aqueous medium is the continuous phase and oil is the dispersed phase. Most food emulsions, including mayonnaise, fall under this category. In the case of water-in-oil emulsions, the oil is the continuous phase and water is the dispersed phase. A good example of this type of emulsion is margarine. In both cases, the dispersed phase is distributed in the form of micron and submicron-size particles or droplets and hence such systems are often called colloidal dispersions.

Food emulsions and foams are essentially lyophobic colloidal dispersions (Walstra 1996); that is, the continuous phase of these systems does not have the thermodynamic desire to wet the dispersed phase. This is characterized by the interfacial tension between the 2 phases. As a result, when oil is dispersed in water, the increase in interfacial area between the continuous and dispersed phases increases the overall free energy of the system compared with its free energy before dispersion. In accordance with the thermodynamic dictum that all systems should be at their global energy minimum state, such colloidal dispersions rapidly separate into 2 phases to minimize the interfacial contact area and the free energy. The rate of phase separation or de-emulsification can be controlled or manipulated by minimizing the interfacial tension between the continuous phase and the dispersed droplets. This is accomplished by adding an amphiphilic molecule that acts as surfactant by adsorbing to and orienting itself at the interface in such a way that its nonpolar (hydrophobic) segment is partitioned into the oil (or gas) phase and its hydrophilic segment exposed to the aqueous phase. Even in the presence of a surfactant adsorbed at the interface, lyophobic colloids are never truly in a state of thermodynamic equilibrium. The thermodynamic state of these systems continuously changes with time as a result of various physical processes and interactions. The only possible equilibrium state for these systems is complete separation into 2 phases with the minimum possible interfacial area. Therefore, even in the presence of a surfactant, these systems are only kinetically stable. This innate quality of 2-phase dispersed systems poses a challenge to the keeping qualities of food emulsions and foams during storage.

MS 20040646 Submitted 9/22/04, Revised 10/26/04, Accepted 12/15/04. *Author is with Dept. of Food Science, Univ. of Wisconsin-Madison, 1605 Linden Drive, Madison, WI 53706. Direct inquiries to author Damodaran (E-mail:sdamodar@wisc.edu)*

Key Issues in Emulsion Stability

Various physical forces affect the kinetic stability of emulsions. These are related to density difference between the dispersed and the continuous phases, interparticle interactions between droplets, and the structure and viscoelastic properties of the surfactant film. These physical forces influence the rates of various processes, such as creaming, flocculation/aggregation, and coalescence of emulsion droplets during storage.

Creaming

Creaming refers to the tendency of oil droplets in an emulsion to rise to the top against gravity. This is essentially related to the difference in the density between the dispersed phase and the continuous phase. Using the Stokes law, it can be shown that the rate of creaming follows the equation:

$$v = 2r^2(\Delta\rho)g/9\eta_o \quad (1)$$

where r is the radius of the oil droplets, $\Delta\rho$ is the difference in the

densities of the dispersed and the continuous phases, g is acceleration due to gravity, and η_o is the viscosity of the medium. Equation 1 can predict the creaming rate of a dilute emulsion with monodispersed particles. For dilute emulsions with polydispersity, such as infant milk formula (Howe and others 1985), the following equation holds true:

$$v = \frac{(d^2 + \sigma^2)\Delta\rho g}{18\eta_o} \quad (2)$$

where d and σ are the weight average particle diameter and standard deviation, respectively. It should be emphasized that both Eq. 1 and 2 are applicable only to dilute emulsions; they cannot predict creaming rate in concentrated emulsions, such as mayonnaise or salad dressings. Nevertheless, Eq. 1 and 2 state that the rate of creaming can be greatly reduced if the density difference between the dispersed oil phase and the aqueous continuous phase is minimized, if the radius of oil droplets is reduced, and the viscosity of the continuous phase is increased. However, while it is possible to control the radius of the oil droplets and the viscosity of the medium, it is not possible to manipulate the density difference between the oil and the aqueous phases, which is about 0.05 g cm^{-3} for most of the food grade oils and fats. Brominated vegetable oil is used as a weighting agent to compensate for the density difference between the oil and the aqueous phases in soft drink emulsions. When brominated vegetable oil is mixed with regular vegetable oil at 25 wt% level, the density of the oil phase is almost the same as the aqueous phase (Chanamai and McClements 2000). However, the use of brominated oil in typical food emulsions is not common because it is suspected to adversely alter lipid metabolism in rats (Lombardo and others 1985). Therefore, in most food emulsions, the size of the oil droplets and the viscosity of the medium are the only parameters that can be manipulated to decrease the rate of creaming. If the density difference between oil and water is about 0.05 g cm^{-3} and the viscosity of the continuous phase is 0.001 Pa s, then Eq. 1 predicts that the rate of creaming is about 0.32 mm h^{-1} for a droplet having a radius of 5 μm, about 0.012 mm h^{-1} for a droplet having a radius of 1 μm, and about 0.12 μ h^{-1} for a 0.1-μm radius particle. Thus, the size of oil droplets has an enormous influence on the rate of creaming. It is easy to visualize that if the radius of the oil droplet is further reduced to a very small size at which the creaming rate of the particle is roughly equal to its Brownian motion, then creaming will not occur in such an emulsion. However, in practical situations as encountered in food systems, it is difficult to reduce the size of the oil droplets to such a small size without incurring enormous amount of energy input.

Dispersion of oil in water in the form of tiny droplets is an energy intensive process. The average size of droplets produced during homogenization of a mixture of oil and water is a complex function of the energy input per unit volume per unit time (E), the interfacial tension between the dispersed and continuous phases (γ_i), and the density of the continuous phase (ρ), and it follows an empirical relation (Walstra 1988):

$$d_{av} \propto \frac{\gamma_i^{3/5}}{E^{2/5}\rho^{1/5}} \quad (3)$$

Equation 3 states that smaller droplets can be obtained by increasing the energy input and the density of the continuous phase, and by decreasing the interfacial tension. Decrease of interfacial tension is achieved by adding low molecular-weight surfactants, such as lecithin, monoacylglycerol, and sorbitan esters, or by adding proteins. The ability of these surfactants to rapidly adsorb and lower the interfacial tension during the emulsification process (that is, as new interfacial area is created) is critical to both the formation and stability of emulsions. In this respect, small molecular-weight surfactants generally perform better than macromolecular surfactants, such as proteins, owing to their high diffusivity. However, although proteins adsorb and reorient themselves at a slower rate at the newly created interfaces during the emulsification process compared with small molecular surfactants because of size and structural complexities, other properties, such as their ability to form a strong viscoelastic film around oil droplets and the arrangement of the protein chain configurations in the form of "loops and trains" in the film, introduce additional forces that help formation of a stable emulsion (Dalgleish 1997; Wilde 2000; Dickinson 2001).

The rate of creaming can be decreased further by increasing the viscosity of the medium, for example by adding thickening agents such as hydrocolloids and polysaccharides to the continuous phase. However, while these macromolecules can retard creaming at high concentrations, they tend to destabilize emulsions at low concentrations. Two molecular phenomena, namely polymer bridging between adjacent droplets and depletion flocculation, are involved in this destabilization process (Cao and others 1990). The mechanisms of these processes will be discussed later.

Creaming is essentially a reversible process. Until the time flocculation or coalescence of droplets commences in the cream, a thin liquid film between the droplets keeps them separated. The droplets can be redispersed into the continuous phase by gentle shaking.

Flocculation

Flocculation refers to loose association of oil droplets facilitated by a net force of interparticle attraction. The structure and properties of the flocks depend on the magnitude of the net attractive force between the droplets and the oil volume fraction. At low oil volume fraction (dilute emulsions) and with weak attractive forces, a weakly flocculated emulsion with small number of droplets in flocks will result; however, if the attractive forces are very strong, then large aggregates can occur even in dilute emulsions.

The major noncovalent interactions that contribute to flocculation in emulsions are the van der Waals forces (which are attractive), electrostatic forces (which are mostly repulsive), and steric forces (which are also repulsive). Depending on the type of surfactant employed, additional interactions, such as hydrophobic interactions and hydration-repulsion interactions, between the adsorbed surfactant films may also become important in the promotion or retardation of flocculation of oil droplets.

The van der Waals attraction between 2 oil droplets arises due to dispersion interactions between the oil phases as they approach each other. According to the DLVO (Derjaguin, Landau, Verwey and Overbeek) theory, when 2 spherical colloidal particles with radius a approach each other, the net force of attraction is (Israelachvili 1992)

$$U_{vdw} = \frac{A_H a}{12d} \quad (4)$$

where A_H is the Hamaker constant and d is the surface-to-surface distance of the 2 particles. In an emulsion, the van der Waals forces act as long-range forces and their influence can extend from 0.2 nm to greater than 10 nm. For a typical 10- to18-carbon chain triacylglycerol oil phase, the Hamaker constant is about 5×10^{-20}J (Israelachvili 1992).

The repulsive interactions between oil droplets stabilized by a low molecular-weight anionic or cationic surfactant essentially arise

from electrostatic forces between the adsorbed surfactant films. If ψ_o is the surface charge potential around oil droplets coated with a saturated monolayer of the surfactant, then when the 2 droplets approach each other, the compression of the counter ions layers around each droplet induces a repulsive force. This repulsive force is roughly equal to (Walstra 1993)

$$U_R = 4.3 \times 10^{-9} a \ln(1+e^{-\kappa d}) \quad (5)$$

where κ is the Debye-Huckel parameter in m^{-1}. In simple emulsions stabilized by small molecule surfactant, the net force of attraction is given by the sum of the van der Waals forces of attraction and the repulsive electrostatic forces. The typical profiles of repulsive, attractive, and net-interaction energy between a pair of oil droplets as a function of surface-to-surface distance are shown in Figure 1A. The net-interaction energy typically shows a secondary energy minimum in the range of 4 to 10 nm and a maximum around 1 to 3 nm, and a primary minimum below 1 nm, depending on the values of the various parameters used. It is obvious from the interaction energy profiles that emulsion droplets can approach each other up to a distance of separation corresponding to the secondary minimum. However, the primary maximum acts as an energy barrier and prevents further approach of the particles. In most cases, the primary maximum is typically about 25 to 50 kT at low ionic strength (I < 0.01 M^{-1}), and this energy barrier is large enough to prevent aggregation. Depending on the position of the secondary minimum as well as its depth, however, a weak flock of droplets can occur in certain type of emulsions, subject to structural properties of the surfactant film. In the example shown in Figure 1a, the primary maximum in the potential energy profile is due to strong electrostatic repulsive interactions. In a typical food emulsion, the ionic strength of the continuous phase is greater than 0.01 M^{-1}. This effectively neutralizes the electrostatic repulsive potential between the droplets (shown in Figure 1a). Consequently, for all practical purposes, the electrostatic energy barrier for flocculation of emulsion droplets does not exist in emulsions solely stabilized by small molecular-weight emulsifiers and, therefore, the oil droplets readily approach the primary minimum, resulting in strong flocculation.

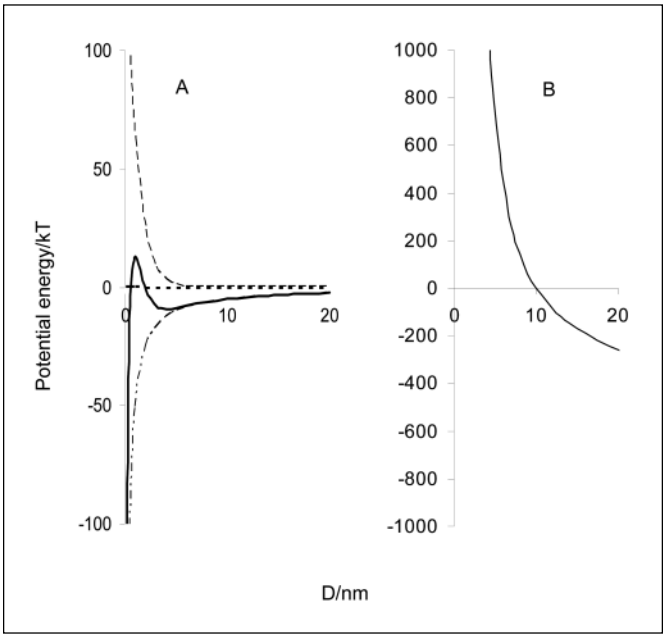

Figure 1—(a) Potential energy profiles of electrostatic (dashed [at I = 0.001 M] and dotted [at I = 0.1 M] lines) van der Waals forces (dash-dot line) between 2 oil droplets coated with an adsorbed protein film. The solid line represents the sum of repulsive and attractive forces at I = 0.001 M. (b) Steric repulsive force between adsorbed protein layers. The following assumptions were used: Diameter of droplets = 1 μm; Hamaker constant = 5 × 10^{-21} J; interfacial concentration of adsorbed protein = 2 mg/m^2; surface potential = 20 mV; thickness of the protruding layers = 5 nm; area of contact between the droplets = 3.14 × 10^{-15} m^2 (this corresponds to overlapping of about 157 molecules from each droplet surface).

The major force that prevents or significantly retards flocculation of oil droplets in food emulsions is the steric repulsive force. This is particularly very dominant in emulsions stabilized by proteins and other polymeric surfactants. The origin of this force is attributed to configurations of polymer chains in the adsorbed layer around oil droplets (Israelachvili 1992). Typically, flexible polymers often assume "train," "loop," and "tail" configurations at the oil-water interface (Israelachvili 1992). The segments in tail and loop configurations protrude into the aqueous phase and the segments in the train configuration lie in the interface (Damodaran 1997). The various configuration states of polymers adsorbed to an interface are shown in Figure 2. If the volume or the size of polymers does not change upon adsorption (for example, at low coverage), then overlapping between the polymer films is less likely when 2 particles approach each other. If the polymer becomes expanded up on adsorption, which usually is the case at high surface coverage, then this expansion creates a density profile of polymer segments perpendicularly from the interface to the bulk phase. When 2 droplets with such adsorbed polymer layers approach each other, the overlapping of the outer segments of the adsorbed layers produces an osmotic pressure gradient, which effectively prevents further approach of the droplets. This osmotic repulsive force arises due to constraints on the freedom of mobility of the overlapping segments, causing a decrease in entropy of the chains. The magnitude of this steric repulsive energy per unit area of the interacting surface is approximately (de Gennes 1987)

$$U_{SR} = \frac{kT\Gamma}{s}\left[(2L/d)^{2.25} - (d/2L)^{0.75}\right] \quad (6)$$

where k is the Boltzman constant, T is the temperature, Γ is the number of adsorbed polymer molecules per unit area of the interface, L is the thickness of the protruding chains, s is the mean distance between polymers in the adsorbed layer ($s = \sqrt{1}/_{\Gamma}$), and d is the surface-to-surface distance of the interacting surfaces. The 1st term in Eq. 6 is the osmotic repulsion force, which opposes the approach of the 2 particles, and the 2nd term is the elastic energy of the chain, which favors the approach of the 2 particles. An example of the influence of steric forces on the interaction energy profile of 2 oil droplets of 1 μm each in diameter with a 2-mg m^{-2} adsorbed β-casein layer having 5-nm thick protruding chains is shown in Figure 1b. It should be noted that steep energy barrier created by steric repulsion below 10-nm distance effectively eliminates the secondary as well as the primary minima in the interaction energy profile. Thus, steric forces play a predominant role against flocculation in polymer-stabilized emulsions.

Hydrocolloids are often used as thickening agent in food emulsions to retard the creaming rate (see review by Dickinson 2003; Leroux and others 2003; Parker and others 1995). However, while hydrocolloids, such as guar gum, gum Arabic, xanthan gum, and so on, retard creaming at high concentration, they tend to create instability in emulsions at low concentrations. This seemingly contradictory phenomenon occurs in protein-stabilized, as well as in low molecule

surfactant-stabilized emulsions (Cao and others 1990, 1991; Gunning and others 1986; Reiffers-Magnani and others 2000; Singh and others 2003a, 2003b). Two different suggestions have been proposed to explain this phenomenon. When 2 particles coated with a surfactant are in close proximity, a hydrocolloid polymer chain may make contact with the surfaces of the particles, creating a bridge between the 2 particles (de Gennes 1987; Dickinson and others 1989). Formation of multiple contacts of this type will promote flocculation and enhance the rate of creaming. This is commonly known as "bridging flocculation," and it is more likely to occur when the hydrocolloid used is a weak emulsifier, for example, gum Arabic, which can adsorb to the oil-water interface (Syrbe and others 1998). The 2nd explanation, known as "depletion flocculation," originally proposed by Asakura and Oosawa (1954, 1958) and later adopted by others (Dickinson 1995; Warren 1997; McClements 1999) is related to the excluded volume effect of polymeric materials. When a nonadsorbing hydrocolloid is added to a moderately concentrated emulsion, exclusion of the polymer from the space between droplets, owing to its hydrodynamic size, sets up a local osmotic pressure gradient. This osmotic force drives the oil droplets to aggregate. This excluded volume effect and the consequent development of osmotic depletion force is fundamentally related to the conformational entropy of the polymer. When the space between adjacent droplets is smaller than the entropically most favorable hydrodynamic size of the flexible polymer, the polymer excludes itself from that space to maintain its thermodynamically favorable low free energy (high entropy) state. This sets up a concentration gradient, and, therefore, an osmotic pressure gradient, in the system, which forces the emulsion droplets to flock. Depletion flocculation is more prominent with hydrocolloids having a stiff backbone (Syrbe and others 1998). A theoretical phase diagram relating creaming instability in emulsions to molecular structure and concentration of polysaccharides that induce creaming instability has been reported (McClements 2000).

It should be noted that both bridging and depletion flocculation can also occur in protein-stabilized emulsions containing no added hydrocolloids. This has been observed especially in caseinate-stabilized emulsions, depending on the concentration of caseinate (Singh and others 2003a). The creaming stability of emulsion prepared using 0.5% caseinate solution is better than the one prepared using 3% caseinate solution (Singh and others 2003a). The low stability of the latter emulsion is due to the presence of excess amount of unadsorbed casein molecules, which promote flocculation of the emulsion particles via the bridging and depletion mechanisms.

Although nonadsorbing polysaccharides induce depletion flocculation in emulsions at all concentrations, the creaming stability of the flocculated emulsion is dependent on the concentration of the polysaccharide: They destabilize at low concentrations, but stabilize at high concentrations (Dickinson,Ma, and Povey 1994; Singh and others 2003a, 2003b; Parker and others 1995; Quintana and others 2002a, 2002b). The stabilizing effect against creaming at high concentrations is not simply due to the viscosity enhancement in the aqueous phase of the emulsion, but is believed to be due to formation of a weak gel-like network of the emulsion particles (Parker and others 1995). The viscoelastic properties of such depletion-induced gels in emulsion have been studied (Tadros 1994; Meller and others 1999). It is the high yield stress of the weak particle network, and not the viscosity of the continuous phase, that retards the creaming rate. A weak particle network can occur in the cases of both uncharged and charged polysaccharides under appropriate pH and ionic strength conditions (Parker and others 1995; Dickinson and Pawlowsky 1997; Singh and others 2003a, 2003b).

In most cases, flocculation is an irreversible process. Because interdroplet interactions are involved in the flocculation process, it is affected by solution conditions, such as pH, ionic strength, and temperature.

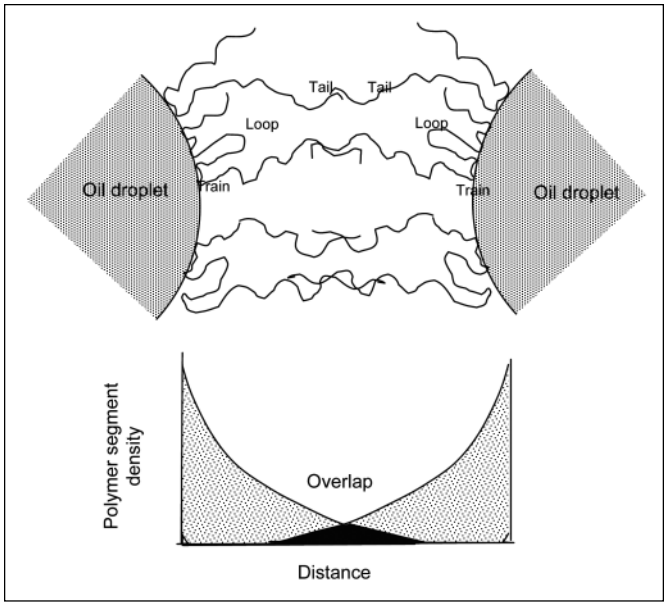

Figure 2—Schematics of steric repulsion between adsorbed protein layers at the oil-water interface of emulsion droplets.

Coalescence

Coalescence refers to merging of 2 or more oil droplets to form a single large droplet. For coalescence to occur, the droplets need to be in close proximity for a long period of time. This situation occurs in creamed or flocculated emulsions.

Coalescence is an irreversible process. It involves rupture of the thin liquid film of the continuous phase that separates dispersed oil droplets in a concentrated emulsion or in a cream. The stability of this film (also known as lamella) as 2 droplets approach each other depends upon the magnitude of 2 opposing forces. The 1st is the capillary pressure. In foams and emulsions, the pressure in the dispersed phase is always higher than the pressure in the continuous phase, and this pressure difference is given by the Laplace equation:

$$\Delta P = 2\gamma/r \tag{7}$$

where γ is the interfacial tension and r is the radius of the oil droplets or gas bubbles. This capillary pressure causes drainage and thinning of the liquid film and eventual collapse of the film. The other force is the disjoining pressure. This arises from forces between the 2 interfaces of the thin liquid film (Bergeron 1999; Stubenrauch and Klitzing 2003). When the 2 interfaces are empty of any adsorbed surfactant molecules (that is, when the dispersed and continuous phases are pure fluids) (Figure 3a), the disjoining pressure between the interfaces is negligible, and the film collapses readily owing to the Laplace capillary pressure. However, when the 2 interfaces of the thin liquid film contain adsorbed surfactant molecules (Figure 3b), interaction forces generated between these surfactant layers create disjoining pressure (Π), which tends to increase the thickness (h) of the liquid film and prevents its thinning. Several intermolecular forces contribute to this disjoining pressure and the most important ones are (Bergeron 1999; Stubenrauch and Klitzing 2003)

$$\Pi(h) = \Pi_{ele} + \Pi_{vdW} + \Pi_{steric} + \ldots\ldots \tag{8}$$

The contribution of van der Waals interactions (Π_{vdW}) to disjoining pressure is always negative, and the contributions of Π_{ele} and Π_{steric} are always positive. The typical Π versus film thickness (h) profiles of these forces are shown in Figure 4. As the interfaces of the thin liquid film approach each other, the local concentration of counter ions in the lamella film increases compared with that in the plateau region. In addition, overlapping between the protruding chains of adsorbed polymeric surfactants also increases local concentration gradient. Both these factors set up an osmotic pressure gradient that retards fluid drainage and further thinning of the film.

Another important factor affecting coalescence is the viscoelastic properties of the surfactant layer. When the lamella film thins below a critical thickness, hydrodynamic forces can cause ripples in the film (Sharma and Ruckenstein 1987). The larger the droplet size and the lower the interfacial tension, the more pronounced will be the ripples. The ripples, which affect local film thickness, can cause holes in the film, leading to film rupture and coalescence. A highly viscoelastic surfactant film can greatly diminish the propagation of ripples and the probability of formation of holes. In this respect, proteins, which form a thicker interfacial layer with higher viscoelasticity than small molecular-weight surfactants, are better suited as emulsifiers.

The forgoing discussions clearly indicate that although the stability of oil-in-water emulsion is seemingly affected by several factors, such as the droplet size, the magnitude of attractive and repulsive forces between particles and interfaces, interfacial tension, and so on, 2 factors, namely, the steric repulsion between adsorbed surfactant layers and the viscoelastic properties of the surfactant film play a much larger role than the others. The steric repulsion retards flocculation, the initial step that leads to coalescence. The viscoelastic properties of the surfactant film retard the rupture of the lamella film and thereby affect the rate of coalescence of emulsion droplets. These 2 factors are fundamentally related to the physicochemical properties of the surfactant molecules and, therefore, attention to these properties of the surfactant is critical for producing a more kinetically stable emulsion.

Key Issues in Foam Stability

Foam is a colloidal system containing tiny air bubbles dispersed in an aqueous continuous phase. Typical foam-type food products are whipped cream, ice cream, cakes, mousses, and marshmallows. Depending on the volume fraction of gas bubbles in a foam system, the bubbles may exist in spherical or polyhedral shapes.

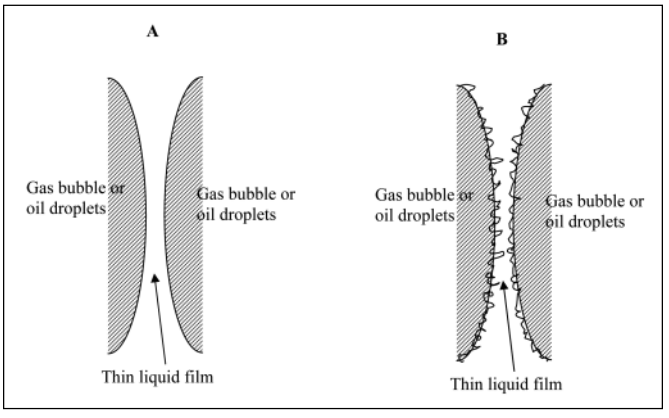

Figure 3—Schematic representation of thin liquid film (lamella) between 2 dispersed particles with (a) and without (b) surfactant layers at the 2 interfaces.

Liquid drainage

The factors affecting the stability of foams are very similar to those that affect emulsion stability. These are the disjoining pressure, viscoelasticity of the surfactant film, and the interfacial tension. Liquid drainage and gas disproportionation are 2 macroscopic processes that contribute to instability of foams (Monsalve and Schechter 1984; Yu and Damodaran 1991). Coalescence of bubbles occurs because of drainage of liquid from the lamella film as 2 gas bubbles approach each other, leading to film thinning and rupture. The rate of film drainage is given by

$$V = 2h^3(\Delta P)/3\mu R^2 \quad (9)$$

where h is lamella film thickness, μ is dynamic viscosity, R is the radius of the bubble, and ΔP is the difference between the capillary hydrostatic pressure (P_c) and the disjoining pressure (Π_d) pressure between the interfaces of the lamella film. In all food foams, P_c is always greater than Π_d and, therefore, film drainage is inevitable. However, the rate can be significantly decreased by increasing the viscosity of the medium and by increasing the disjoining pressure. As in the case of emulsions, the disjoining pressure is related to the development of an osmotic pressure difference between the lamella fluid and the bulk phase as the local solute concentration increases when 2 bubbles approach each other. The major contributors for this osmotic pressure development are the protruding chains of the surfactant film, counter ion cloud around the surfactant layer, and hydration repulsion forces. These forces are inherently dependent upon the physicochemical properties of the adsorbed surfactant film.

Ostwald ripening

The physicochemical properties of the surfactant film play a vital role in countering several of the physical processes that impact foam stability. One of the processes is the Ostwald ripening, also known as disproportionation. This process refers to diffusion of gas from small bubbles to large bubbles. The gas bubbles in foams are polydispersed. The Laplace pressure of gas in small bubbles is greater than

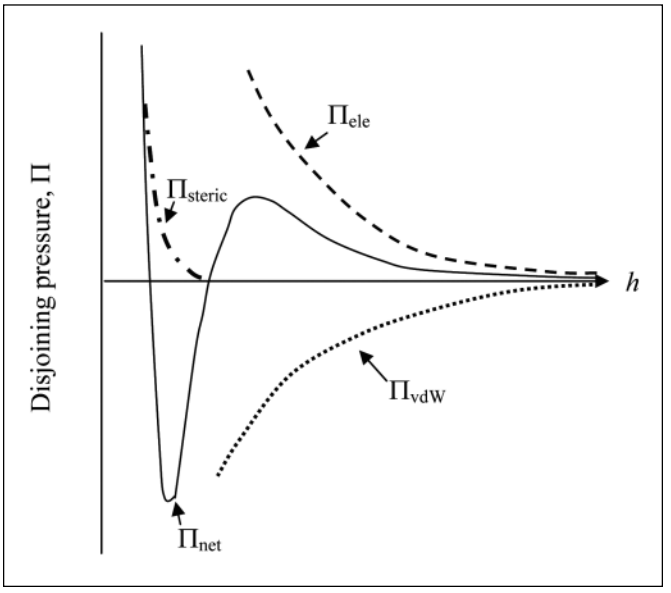

Figure 4—Contribution of electrical double layer, van der Waals, and steric forces to disjoining pressure (Π) between the interfaces of a thin liquid film as a function of film thickness (h). (Adopted from Stubenrauch and von Klitzing 2003).

that in large bubbles (Eq. 7). Because the solubility of gas in the continuous phase is affected by pressure, the gas in small bubbles is more soluble than that in large bubbles. This causes shrinkage of small bubbles, which eventually disappear, and expansion of large bubbles. The viscoelastic properties of the surfactant film can exert control over the rate of shrinkage and eventual collapse of small bubbles. For instance, if the surfactant molecule in the film can be readily displaced or dissolved into the bulk phase as the bubble shrinks, then disproportion can proceed without any change in the interfacial tension. This occurs normally in foams made with small molecule surfactants. However, when the rate of desorption of the surfactant is extremely slow, as is the case with proteins, the increase in the concentration of surfactant in the adsorbed layer as the bubble shrinks increases its surface rheological properties, notably the surface dilatational modulus of the adsorbed layer. This significantly retards the rate of disproportionation.

Another factor that significantly influences drainage is the Marangoni effect. The Marangoni effect relates to the ability of a surfactant layer to respond rapidly to local fluctuations in interfacial tension. When liquid drainage occurs in a lamella film, it exerts a shearing stress on the surfactant layer and as a result an interfacial tension gradient is created at the surfaces of the lamella film. If excess surfactant is present in the liquid core of the lamella and if the surfactant can adsorb rapidly to the regions of the lamella surface where the interfacial tension is high, then stretching and thinning of the lamella film can continue. However, when the rate of adsorption of the surfactant is slow, the adsorbed surfactant layer from the low interfacial tension region moves toward the high-tension region of the lamella. As the surface layer moves upward, it drags the lamella fluid along with it. This process retards the rate of drainage of liquid from the lamella. The ability of the surfactant layer to stretch toward the high-tension region is related to its surface dilatational elasticity. Small molecular-weight surfactants possess poor surface dilatational elasticity compared with proteins and, therefore, thinning and breakage of the lamella film occurs more commonly and at a faster rate in foams stabilized by small surfactants.

Proteins and Emulsion stabilization

Formation and stabilization of an oil-in-water emulsion requires the presence of a surfactant that can effectively reduce the interfacial tension between the oil and aqueous phases. This can be achieved by using either small surfactants, such as lecithins, monoacylglycerol, and so on, or macromolecules, such as proteins. Proteins are generally less surface active than small surfactants, and at equivalent interfacial concentration small surfactants are more effective than proteins in reducing the interfacial tension between oil and water. Typically, most proteins decrease the tension at air-water and oil-water interfaces by about 15 to 20 mN m^{-1} at saturated monolayer coverage (Razumovsky and Damodaran 1999a) compared with 30 to 40 mN m^{-1} for small surfactants. In any case, recently, based on theoretical considerations, it has been pointed out that pure proteins cannot decrease the surface tension of water below 50 mN/m (Damodaran 2004). The inability of proteins to greatly reduce the interfacial tension is related to their complex structural properties. Although proteins contain hydrophilic and hydrophobic groups in their primary structure, there are no clearly defined hydrophilic head and hydrophobic tail as found in lecithin or monoacylglycerol. These groups are randomly spread all over the primary structure of proteins and in the tertiary folded conformation some of them exist as segregated patches on the surface of the protein molecule. Thus, when a protein adsorbs to the oil-water interface, only a fraction of the hydrophobic residues are positioned in the interface facing the oil phase and most the protein molecule is suspended into the bulk aqueous phase. Because of conformational constraints to properly orient the hydrophilic and hydrophobic groups at the interface and improper packing at the interface proteins are unable to greatly reduce the interfacial tension. That is, even in a saturated monolayer of protein at the interface, a large fraction of water in the interfacial region remains in a high free energy state.

Although proteins are not highly effective in reducing the interfacial tension, a requirement that is absolutely essential for dispersing oil into the aqueous phase, protein-stabilized emulsions are generally more stable than those stabilized by small surfactants. Obviously, other properties of the protein film seem to more than compensate for their low surface activity. Specifically, at saturated monolayer and multilayer coverage at an interface, proteins form a gel-like film around oil droplets via noncovalent interactions (Dickinson 2001). When a protein contains sulfhydryl and disulfide groups, conformational changes in the protein at the interface promote polymerization via the sulfhydryl-disulfide interchange reaction (Dickinson and Matsumura 1991; Monahan and others 1993; Damodaran and Anand 1997). These interactions, apart from making the protein irreversibly adsorbed to the interface, provide a highly viscoelastic film that resists coalescence (Dickinson 1998, 1999, 2001). The segments of the protein that remain suspended into the aqueous phase in the form of loops provide steric stability against flocculation and coalescence of oil droplets. The excellent stability of casein-stabilized emulsions is attributed to this phenomenon. It has been shown that the thickness of the protruding layer of caseinate adsorbed at the oil-water interface is about 10 nm at saturated monolayer coverage (Fang and Dalgleish 1996). This steric force, which is a major factor against coalescence, does not exist in small molecular surfactant-stabilized emulsions.

While it is possible to predict surface activity of small molecular-weight emulsifiers, for example from their hydrophilic-hydrophobic balance (HLB) values, this is not possible in the case of proteins. This is due to the complexity of protein structure. Each protein possesses unique conformational characteristics imparted by its primary amino acid sequence. Even proteins that contain similar overall content of hydrophobic and hydrophilic residues exhibit entirely different structural characteristics. The differences in structural and other physicochemical properties of proteins greatly affect their surface activity. Results of several studies have shown that for a protein to be a good surfactant, it should have the following attributes: (1) it should be able to readily adsorb to interfaces, (2) should be able to readily unfold at the interface, and (3) should be able to form a cohesive film at the interface via intermolecular interactions.

The rate of adsorption of proteins to the oil-water interface is greatly affected by the physicochemical properties of the proteins. For instance, Figure 5a shows the rates of adsorption of 3 structurally very different proteins, namely β-casein, bovine serum albumin, and lysozyme at the triolein-water interface. This difference in their adsorption behavior arises from differences in the pattern of distribution of hydrophobic and hydrophilic residues on the proteins' surfaces and consequently their affinity to the oil-water interface. It has been shown that apart from electrostatic and hydrophobic interactions, dispersion interactions between proteins and the oil phase, which include London dispersion interactions and Debye-Keesom interactions, significantly contribute to adsorption of proteins at the oil-water interface (Sengupta and Damodaran 1998; Sengupta and others 1999). While several proteins exhibit an energy barrier to adsorption at the air-water interface, no energy barrier has been found for adsorption at the oil-water interface; this is due to stronger dispersion interactions between protein and the oil phase than between protein and the gas phase (Sengupta and Damodaran 1998). Dispersion interactions between proteins and

the oil-water interface are always attractive whereas that between proteins and the air-water interface are generally repulsive. Because of this difference, proteins are adsorbed much more readily at the oil-water interface than at the air-water interface.

About 1% to 3% protein concentration is typically used in the making of protein-stabilized emulsions. At this concentration range and under turbulent homogenizing conditions, the rate of adsorption of the protein at the oil-water interface is not a factor in the formation of an emulsion. However, the rate at which the adsorbed protein decreases the interfacial tension is very crucial for stabilizing the interface. Figure 5b shows differences in the rate of increase of surface pressure of 3 proteins at the triolein-water interface. It should be noted that while the rates of change of surface concentration and surface pressure of β-casein follow a parallel behavior, the rate of increase of surface pressure lags behind the rate of increase of surface concentration in the cases of BSA and lysozyme (Figures 5a and 5b). These observations, which are similar to those found at the air-water interface (Xu and Damodaran 1993; Anand and Damodaran 1995), suggest that while the disordered β-casein could rapidly expand and reorient at the oil-water interface, the highly ordered BSA and lysozyme unfold and reorient slowly at the interface. Recent investigations have shown that the delay in the surface pressure evolution of lysozyme solution is related to a surface phase transition in the adsorbed film (Erickson and others 2003).

The rheological properties of protein films are very critical for stabilizing the dispersed oil phase under dynamic conditions of emulsion formation. For instance, at 1% protein concentration most food proteins reach equilibrium surface pressure very rapidly and the final surface pressure is almost the same, that is, about 15 to 20 mN m^{-1}. Yet, the size of oil droplets and the total interfacial area of the emulsion created under identical emulsification conditions are not the same for proteins. The oil droplet size increases in this order: soy proteins > whey proteins > sodium caseinate > soluble wheat proteins > blood plasma proteins (Walstra and de Roos 1993). This difference is due principally to differences in the viscoelastic properties of the protein films formed at the oil-water interface (Dickinson 1999) and to the amount of protein adsorbed to the interface (Γ, mg m^{-2}) (Tcholakova and others 2003). Under dynamic conditions of homogenization, when interfacial gradients are continuously generated as new interfacial areas are created, the ability of the nascent protein film to flow from low-tension regions to high-tension regions (the Marangoni effect) is critical for the initial stabilization of emulsion droplets.

The single most important property that impacts surface activity of proteins is its molecular flexibility, that is, a protein's innate ability to undergo rapid conformation change when it is transferred from one environment to another. Investigations on several unrelated proteins have shown that the dynamic surface activity of proteins, that is, the instantaneous change in surface pressure per milligram of protein, during adsorption from the bulk phase to the air-water interface, is positively and linearly correlated to the adiabatic compressibility (that is, molecular flexibility) of proteins (Razumovsky and Damodaran 1999a). Molecular flexibility is a manifestation of the sum of all positive and negative interactions as well as steric forces within a protein molecule, and it represents the susceptibility of a protein's conformation to altered environment. Rapid conformation change at an interface is essential for the protein to reorient its hydrophobic and hydrophilic residues toward oil and aqueous phases and also to maximize the exposure and partitioning of these residues toward the 2 phases. A highly rigid protein cannot decrease the interfacial tension even at a saturated monolayer condition because such a protein will continue to remain in a gaseous state; a phase transition from a surface gaseous phase to a liquid expanded phase through protein-protein interactions, which requires protein unfolding, is essential for interfacial tension reduction (Erickson and others 2003; Rao and Damodaran 2000).

Apart from molecular flexibility, several other physicochemical properties of proteins affect the formation and stability of emulsions. Solubility in the aqueous phase is a prerequisite for a protein to be a good emulsifier. Less soluble or highly hydrophobic proteins may precipitate at the oil-water interface and thus cause instability in emulsions. Surface hydrophobicity plays an important role in the initial anchoring of a protein to the oil-water interface (Kato and Nakai 1980). The greater the number of hydrophobic patches on a protein's surface, the higher is the probability of its adsorption and retention at the interface. For instance, recently Wierenga and others (2003) studied the rates of adsorption of native and caprylated ovalbumin at the air-water interface. It was found that whereas the rate of adsorption of native ovalbumin was much lower than its diffusivity in the bulk phase, the rate of adsorption of caprylated ovalbumin (with 4 capryl groups attached) was comparable to its bulk diffusivity. The slower-than-diffusional transport of native ovalbumin to the air-water interface was interpreted as due to the existence of an energy barrier and the magnitude of this energy barrier was removed almost completely by increasing the exposed hydrophobicity of the protein via attaching 4 capryl groups on its surface. Wierenga and others (2003) also reported that even though the rate of adsorption of caprylated ovalbumin was faster than native ovalbumin, the surface pressure (Π) at any given surface concentration (Γ) was the same in both cases. Thus, surface hydrophobicity might increase the probability of anchoring of the protein to the interface, but the ability of the protein to reduce interfacial tension largely depends on its other physicochemical properties discussed earlier.

The initial structural state of a protein impacts its surface activity. Compact and highly ordered proteins possess poorer surface activity and emulsifying capacity than the ones that have disordered structure. In this respect, partial heat denaturation of proteins generally improves their surface activity (Dickinson and Hong 1994; Zhu and Damodaran 1994a). Excessive heat denaturation may impair the emulsifying properties by rendering the protein insoluble (Voutsinas and others 1983).

Small molecular-weight emulsifiers, such as phospholipids, which are generally found in foods, can compete with proteins for adsorption at the oil-water interface during the emulsification process (Kiosseoglou and Perdikis 1994; Tomas and others 1994; Dickinson and Hong 1994; Fang and Dalgleish 1996; Dalgleish and others 1995; Euston and others 1995, 1996; Gunning and others 2004; Mackie and others 2003, 2000). Because small surfactants can dif-

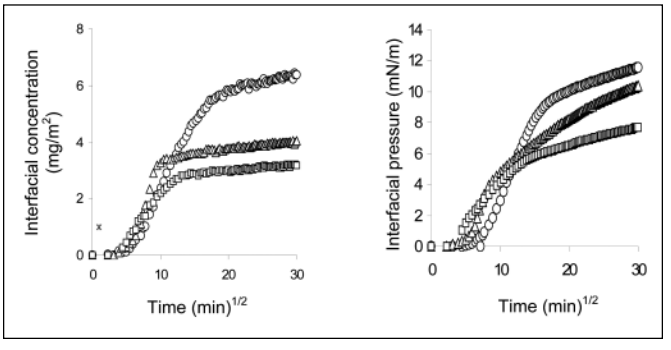

Figure 5—Kinetics of adsorption of β-casein (○), bovine serum albumin (ϵ), and lysozyme (□) at triolein-water interface at pH 7.0 and 25 °C. The initial concentration of the protein in the bulk phase was 1.5 μg/mL.

fuse rapidly to the interface and lack conformational constraints for reorientation at the interface, at sufficiently high concentrations they can effectively inhibit protein adsorption to oil droplets. This behavior is seen during coadsorption of protein and small molecular surfactant at the planar oil-water interface (Gunning and others 2004; Mackie and others 1999a 2000, 2003). A review of these studies has been published recently (Bos and van Vliet 2001; Pugnaloni and others 2004)

In real food emulsions, when small molecular-weight surfactant and proteins are mixed together prior to homogenization with the oil phase, the amount of protein incorporated at the oil droplet surface decreases as the surfactant to protein concentration ratio is increased. However, only partial displacement of protein occurs even at high surfactant concentrations (Euston and others 1995, 1996). This suggests that in emulsion systems, protein in adsorbed layers around oil droplets might be present in the form of a protein-surfactant complex, formed as a result of the homogenization process. On the other hand, when a small molecular surfactant is added to a preformed protein-stabilized emulsion, the surfactant displaces the protein from the oil droplet surface. Complete displacement of the protein occurs at high surfactant to protein ratios (Courthaudon and others 1991). This phenomenon is explained using an orogenic displacement mechanism (Mackie and others 1999a). According to this orogenic mechanism, because of inherent steric factors, packing of protein molecules in the film formed at an interface is not homogeneous and thus it contains void spaces. When a low molecular surfactant is added to the continuous phase, the surfactant initially adsorbs to these void spaces owing to its small size and these nucleated surfactant domains grow with time (Mackie and others 2003). It is believed that as the surfactant domains expand, they compress the protein film. At sufficiently high surface pressures, created by the adsorbing surfactant molecules, the protein film loses its integrity and the protein desorbs into the bulk phase. An alternative mechanism involving changes in interfacial water activity has been proposed to explain this phenomenon (Damodaran 2004).

Another factor that affects protein-stabilized emulsions is the protein composition. Food proteins in general are mixtures of several protein components. For instance, egg protein is a mixture of 5 major proteins and several minor protein components. Likewise, whey protein is a mixture of α-lactalbumin and β-lactoglobulin and several other minor proteins. Seed storage proteins, such as soy protein isolate, contain at least 2 major protein fractions, viz., legumins and vicillins. During emulsification, the protein components of the mixture compete with each other for adsorption to the interface. The composition of the protein film formed at the interface is dependent on relative surface activities of various protein components of the mixture. For instance, when a 1:1 mixture of α_s- and β-caseins are allowed to adsorb to the oil-water interface, the amount of α_s-casein in the protein film at equilibrium is almost twice that of β-casein (Damodaran and Sengupta 2003) (Figure 6). At the air-water interface, however, an exactly opposite behavior is observed (Anand and Damodaran 1996). Preferential adsorption of protein components of various food proteins at the oil-water interface of emulsions has been reported. Anton and Gandemer (1999) studied the effect of pH on emulsifying properties of egg yolk. The interfacial protein concentration was higher at pH 6 than at 3 and 9. At pH 6, all the proteins of yolk, except phosvitin, were adsorbed at the emulsion interface. At pH 3.0, phosvitin was the main protein, and at pH 9 only the apoproteins of low-density and high-density lipoproteins were found. Thus, the pH-dependent net charge of proteins seems to affect the interfacial protein composition of yolk-stabilized emulsions. Dickinson and others (1988) showed that oil-in-water emulsions prepared with a 1:1 mixture of β-casein and α_{s1}-casein contained more β-casein than α_{s1}-casein in the adsorbed protein layer. The ratio of α_{s1}-casein to β-casein in the adsorbed layer increased with increasing ratio of α_{s1}-casein to β-casein in the bulk phase; however, under no circumstances was the ratio in the adsorbed layer the same as the ratio in the bulk phase, indicating that there was preferential adsorption of β-casein at the oil-water interface. In another study involving sodium caseinate, the composition of various caseins in the adsorbed layers of freshly prepared emulsions was very similar to the composition in the bulk phase, but on aging, β-casein from the bulk phase displaced some of the α_{s1}-casein from the interfacial layer (Robson and Dalgleish 1987). Competitive adsorption of caseins and whey proteins at the oil-water interface emulsions has been reported (Dalgleish and others 2002a, 2002b). At temperatures above 40 °C, addition of whey protein isolate to casein-stabilized emulsions caused displacement of caseins from the interface. Both α_{s1}-casein and β-casein were displaced as α-lactalbumin and β-lactoglobulin from the bulk phase adsorbed to the interface, but κ-casein was not displaced. The exchange was temperature dependent as there was no detectable exchange at room temperature (Dalgleish and others 2002a). However, when purified individual whey proteins and caseins were used, only β-lactoglobulin was able to displace caseins but α-lactalbumin could not (Dalgleish and others 2002b). These results indicated that some minor protein components of whole caseinate or whey protein isolate or some contaminant phospholipids might account for exchange between bulk phase whey proteins and adsorbed caseins. Nevertheless, the competitive adsorption studies on protein mixtures in model as well as real emulsion systems clearly indicate that preferential adsorption of protein components occurs at the oil-water interface, and variations in protein composition of the bulk phase would affect protein composition of the adsorbed film, which in turn may affect the stability of the emulsion.

Polymer mixtures generally exhibit incompatibility of mixing in solution. This is also true with protein mixtures at high concentrations (Polyakov and others 1979, 1985, 1997). In adsorbed layers of protein mixtures at the oil-water interface, where the local protein

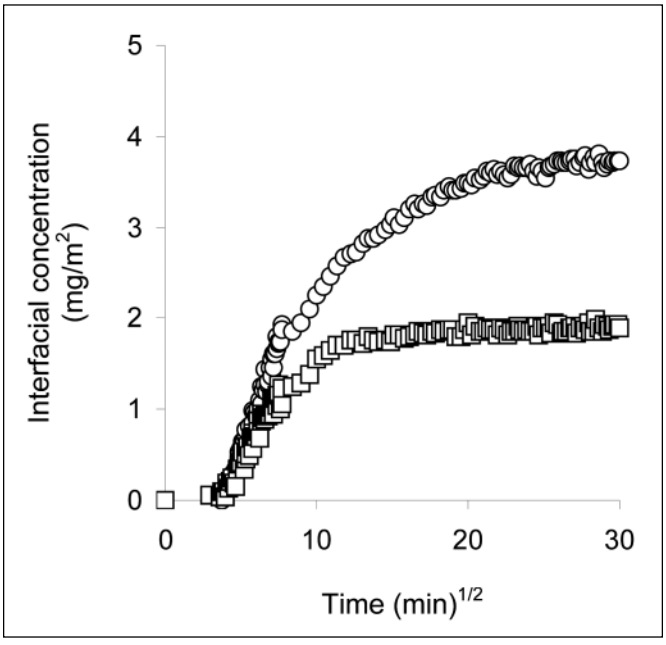

Figure 6—Kinetics of competitive coadsorption of α_s-casein (○) and β-casein (□) at the triolein-water interface from a binary bulk solution containing 1.5 μg/mL each protein.

concentration is typically in the range of 15% to 30%, conditions do exist for nonideal mixing between protein constituents. If the system is sufficiently mobile, which is the case at the air-water and oil-water interface, and if the proteins in the adsorbed layer are not kinetically trapped by covalent cross-linking, then incompatibility of mixing may lead to two-dimensional phase separation of the proteins in the adsorbed layer. Evidence for this at the air-water (Razumovsky and Damodaran 1999b, 2001; Sengupta and Damodaran 2000 2001; Sengupta and others 2000) and oil-water (Damodaran and Sengupta 2003) interfaces has been reported. Rheological studies on adsorbed β-casein + β-lactoglobulin binary films at the air-water interface also have provided evidence for the existence of nonideality of mixing between these 2 proteins (Rideout and others 2004). Epifluorescence microscopy of several mixed protein films at the air-water interface also has shown distinct phase separated regions within the film (Sengupta and Damodaran 2000, 2001; Sengupta and others 2000). An example of this phenomenon in acidic subunits of soy 11S and β-casein mixed film at the air-water interface is shown in Figure 7. If two-dimensional phase separation occurs in mixed protein films around oil droplets, it is conceivable that the interface of such phase-separated regions might act as source of instability in emulsions. However, a direct correlation between thermodynamic incompatibility of mixing of proteins in mixed protein films at the oil-water interface and the kinetic stability of emulsions made of protein mixtures is yet to be determined.

Proteins and foam stabilization

The molecular properties requirements of proteins to stabilize foams and emulsion are very similar. However, because the interfacial tension at the air-water interface is much greater than at the oil-water interface and the magnitude of dispersion interactions between protein and the nonaqueous phase in the interfacial region is different (Sengupta and Damodaran 1998), some variations are observed in molecular requirements for foaming properties of proteins. For instance, the emulsifying capacity of proteins shows a good positive correlation with their surface hydrophobicity (Kato and Nakai 1980), but no such correlation is found with the foaming capacity of proteins (Townsend and Nakai 1983). Instead, foaming capacity exhibits a positive correlation with the average hydrophobicity of proteins (Kato and others 1983). While surface hydrophobicity refers to the free energy change for binding of nonpolar fluorescent probes such as *cis*-parinaric acid or 1-anilino-8-naphthalene sulfonate to hydrophobic patches or cavities on the surface of proteins, the average hydrophobicity refers to the average free energy change for transfer of amino acid side chains from a nonpolar solvent to water. It appears that because the interfacial free energy at the oil-water interface is lower than that at the air-water interface, proteins may be denatured only to a limited extent at the oil-water interface and, therefore, the surface hydrophobicity becomes crucial for anchoring proteins at the oil-water interface. At the high free energy air-water interface however, proteins are denatured to a greater extent, which exposes buried hydrophobic residues to the interface. Thus, although surface hydrophobic patches facilitate initial anchoring of the protein, the properties of the unfolded protein rather than the native protein dictates the behavior of the protein at the air-water interface. This seems to be the reason for the positive correlation between average hydrophobicity and foaming capacity.

Partial denaturation of proteins generally improves their foaming properties. A 5% w/v whey protein isolate (WPI) solution heated for 1 min at 70 °C exhibited significantly better foamability and foam stability than the native and extensively denatured WPI (Zhu and Damodaran 1994a). Extensive heat-induced polymerization of proteins impairs their foaming properties. However, in certain proteins, an optimum ratio of monomeric to polymeric protein improves their foaming properties. For example, foams prepared using a 40:60 mixture of monomeric to polymeric WPI shows better foam stability than those prepared using the native or polymerized WPI (Zhu and Damodaran 1994a). However, the foaming capacity was better at a monomer to polymer ratio of 60:40. Recent investigations on the rheological properties of foams formed with mixtures of monomeric and polymeric WPI have essentially confirmed these findings (Davis and Foegeding 2004).

Divalent cations, that is, Ca^{++} and Mg^{++}, significantly influenced the foaming properties of WPI (Zhu and Damodaran 1994b). This has been attributed to time-dependent aggregation of the protein in the presence of low (0.02 to 0.4 M) concentrations of these ions. The effect was more dramatic when the protein solution was foamed immediately after the addition of the cation, and it decreased progressively with longer incubation time with the cation prior to foaming. It appears that cation-induced slow aggregation of the protein in situ in the film produces a better viscoelastic film that retards liquid drainage by preventing thinning of the lamella film, whereas solution-phase aggregation impairs foamability by decreasing the adsorption of protein to the air-water interface during whipping/bubbling (Zhu and Damodaran 1994b). It is not clear whether the effects of divalent cations on foaming properties are specific only to WPI or to other proteins as well.

Molecular flexibility affects foaming properties of proteins. Cleavage of intramolecular disulfide bonds in proteins can increase molecular flexibility. It has been shown that cleavage of the 17 disulfide bonds in bovine serum albumin dramatically improved its foamability and foam stability (Yu and Damodaran 1991). Apart from improving the molecular flexibility, re-oxidation of the sulfhydryl groups in the protein film at the air-water interface might also be a reason for improvement in the foaming properties as it might enable formation of a cohesive film (Yu and Damodaran 1991). Recently, it has been reported that the rate of adsorption soy glycinin and surface tension reduction at the air-water interface was greater at pH 3 than at pH 6.7 (Martin and others 2002). This has been attributed to dissociation of the 11S form glycinin oligomer to 3S form subunits. Surface shear viscosity of the glycinin film after aging for 2 h at the air-water interface was higher at pH 3 than at pH 6.7; however, the shear viscosity was the same for both the films after 24 h of aging (Martin and others 2002), suggesting that the 11S form of glycinin may slowly dissociate into subunits at the inter-

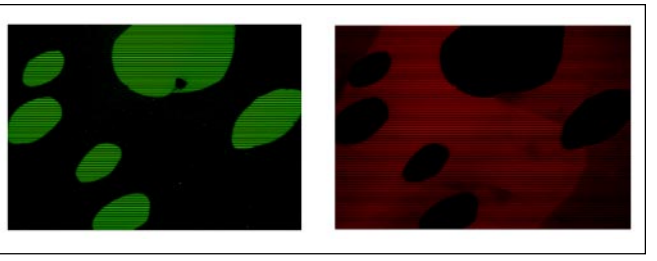

Figure 7—Epifluorescence microscopic images of adsorbed mixed protein film of acidic subunits of soy 11S (A11S) and β-casein at the air-water interface. The film was transferred from the air-water interface on to a glass slide using the Langmuir-Schafer technique. The proteins were labeled differently with red (β-casein) and green (A11S) fluorescent dyes before coadsorption from a bulk phase. The concentration ratio of A11S to β-casein in the bulk phase was 2.5:1.5. The red regions are β-casein and the green regions are A11S in the binary film. Note that β-casein forms the continuous phase and A11S forms the dispersed phase in this laterally phase-separated film.

face. The foaming properties of glycinin were better at pH 3 than at pH 6.7, confirming a direct correlation between conformational state of the protein, rheological properties of the protein film, and the foaming properties.

Several external factors affect foaming properties of proteins. These include nonprotein additives such as polysaccharides, low molecular-weight peptides, phospholipids, sugars, pH, and ionic strength (Zhu and Damodaran 1994b, 1994c; Murray and Liang 1999; Carp and others 1999; Sarker and others 1996). Phospholipids and low molecular-weight peptides compete with proteins for adsorption at the air-water interface. Because these small emulsifiers produce interfacial films with poor viscoelastic properties and steric stability, foams formed in the presence of excess amounts of these additives adversely affect the stability of the foam (Garofalakis and Murray 2001). This is also one of the reasons why protein hydrolyzates are poor foaming agents than their parent proteins (Mutilangi and others 1996; Perea and others 1993). Increase of ionic strength decreases both foam stability and foaming capacity of whey proteins (Zhu and Damodaran 1994c). This is related to charge neutralization, which may cause protein aggregation and formation of holes in the protein film and acceleration of Ostwald ripening. However, in the case of skim milk powder, an increase in foaming properties with increase of NaCl concentration up to 0.8 M has been reported (Zhang and others 2004). This might be related to the antagonistic effect of NaCl with calcium ions on the dissociation of casein micelles in skim milk powder; the dissociated caseins may be more surface active than the intact micelle.

Sugars generally improve foam stability, however they do not improve foamability (Zhu and Damodaran 1994c). The improvement in foam stability is due to an increase in the specific viscosity of the solution. A direct correlation has been observed between specific viscosity of lactose containing WPI solution and its foam stability (Zhu and Damodaran 1994c).

Improving protein functionality for stabilizing emulsions and foams

Traditional proteins, such as caseins, egg, and meat proteins, possess excellent functional properties suitable for several applications in the food industry. It should be recognized that each of these traditional proteins are, in fact, a mixture of proteins and, therefore, their desirable functional properties may arise from specific contribution of each component in the mixture to the formation and stability of foams and emulsions. In this context, the poor emulsifying and foaming properties of nontraditional plant proteins, such as cereal and legume proteins, and whey proteins might be due to lack of protein components that can contribute to various functions at interfaces.

The factors affecting the foaming and emulsifying properties of proteins are fairly well understood. However, imparting these functionalities in plant proteins is easier said than done. The difficulty arises not from lack of knowledge, but because of the complexity of the protein structure, which, in certain cases, is not amenable to precise alteration/modification. Nevertheless, the foaming and emulsifying properties of proteins can be improved through physical, chemical, enzymatic, and genetic modifications.

Physical modifications involve partial denaturation or unfolding of the protein under controlled heating and shear conditions (for example, see Bals and Kulozik 2003; Mitidieri and Wagner 2002). This is essentially a trial and error approach because "partial denaturation" cannot be defined in absolute terms. Furthermore, the "partially denatured state" may be affected by several parameters, such as heating rate, shear rate, protein concentration, pH, ionic strength, the presence of other food constituents such as lipids and polysaccharides and their concentrations, and so on. One also has to take into account the impact of partial denaturation on polymerization and/or aggregation of the protein. The protein in the partially denatured state should be soluble as insoluble proteins cannot adsorb and form a viscoelastic film at air-water and oil-water interfaces. Heating of β-lactoglobulin and α-lactalbumin at 80 °C in the dry state (7.5% moisture content) for an extended period of time (up to 5 d) significantly improved the foaming and emulsifying properties of the proteins (Ibrahim and others 1993). During this heating period, the surface hydrophobicity of β-lactoglobulin decreased and that of α-lactalbumin increased, suggesting structural changes in these proteins. Partial denaturation of proteins also can be achieved by subjecting proteins to high hydrostatic pressure. Studies on soy proteins have shown that the emulsion stability index of high pressure–treated soy proteins decreased with increasing pressure in the range of 200 to 600 Mpa (Molina and others 2001). This was attributed to aggregation of the proteins as a result of pressure-induced denaturation. A similar effect also was observed in the case of whey protein isolate (Ibanoglu and Karatas 2001).

Protein-protein interactions play a crucial role in the formation of protein films at interfaces and in the stabilization of foams and emulsions. The excellent foaming properties of egg albumen are attributed to interactions between 5 major protein components of egg albumen at the air-water interface (Poole and others 1984). The fact that the foaming properties of individual protein components of egg albumin are poor compared with the composite mixture suggests that specific protein-protein interactions play an important role in this system. It has been proposed that electrostatic interaction of the positively charged lysozyme with other negatively charged proteins of egg white at the interface stabilizes the foam (Poole and others 1984; Clark and others 1988). However, competitive adsorption of the 5 major protein components of egg white at the air-water interface showed that while at 0.002 ionic strength all 5 components could adsorb to the interface, at 0.1 M ionic strength only ovalbumin and ovoglobulins were able to adsorb and the other 3 components, that is, lysozyme, ovotransferrin, and ovomucoid, were excluded from the interface (Damodaran and others 1998). Thus, the detrimental effect of sodium chloride on the stability of egg white foam (Poole and others 1984) could be attributed to inhibition of lysozyme adsorption and other compositional changes in the protein film at the interface.

The interfacial properties of proteins can be improved by mixing proteins that complement each other in terms of various molecular properties required to create a stable foam or emulsion. In this respect, addition of basic proteins, such as lysozyme and clupeine, to acidic proteins at appropriate ratio might be a promising strategy (Poole and others 1984). However, while this strategy may work with some proteins, it may not be effective with others (Poole 1989). Mixing of caseins with other globular proteins, such as whey proteins and soy proteins, may also improve the stability. In this case, while the loops of caseins protruding into the aqueous phase can be expected to provide steric stability, the globular protein may impart viscoelasticity to the protein. Likewise, mixers of native and limitedly polymerized proteins (as shown in the case of whey protein isolate (Zhu and Damodaran 1994a) may also be a strategy worth pursuing. However, because competitive adsorption occurs between proteins in a mixture, the concentration ratio of the proteins in the bulk phase would impact the composition in the interfacial film. This needs to be optimized for each mixed proteins system.

The emulsifying and foaming properties of proteins can be improved using appropriate chemical modification strategies. Improvements in functionality occur potentially via 2 mechanisms: First, chemical modification induces structural changes in proteins

at the secondary, tertiary, and quaternary structural levels, and second, it alters the hydrophobicity-hydrophilicity balance. Several chemical modification strategies, including acylation using acetic and succinic anhydrides, phosphorylation using phosphorous oxychloride ($POCl_3$) or sodium trimetaphosphate (STMP), sulfitolysis using sodium sulfite, alkylation using N-hydroxy succinimidyl esters of fatty acids, and amino-carbonyl reaction (Maillard reaction) with reducing sugars, have been reported (Kato 2002; Schwenke 1997; Damodaran 1996). Of these strategies, phosphorylation and amino-carbonyl reactions (Kato 2002) seem to be more preferable than the others on the basis of nutritional safety of the modified proteins. Many food proteins, for example, caseins and ovalbumin, are phosphylated proteins, and chemical phosphorylation does not adversely affect the nutritional value and safety of proteins (Damodaran 1996). The initial product of the carbonyl-amine reaction between a reducing sugar and the amino groups of proteins is the Schiff base, which is acid labile. Thus, under conditions that do not accelerate nonenzymatic browning, the modified lysine residues might be bio-available as the Schiff base is hydrolyzed in the acidic stomach. A stable glycosylated protein can be obtained when the Schiff base is reduced using a reductant. Polyphenol-mediated protein-protein cross-linking in situ in adsorbed protein films in foams has been shown to significantly improve the stability of the foam (Sarker and others 1995). Conjugation of β-lactoglobulin with carboxymethyl dextran and other cationic saccharides has been shown to improve its emulsifying properties (Hattori and others 1994, 2000). Sulfhydryl-disulfide interchange reaction in adsorbed films at interfaces leads to polymerization and formation of a highly viscoelastic film. This enhances emulsion and foam stability (Dickinson and Matsumura 1991; Monahan and others 1993; Damodaran and Anand 1997). Introduction of thiol groups in α_{s1}-casein, which is devoid of cysteine residues, has been shown to significantly increase the foam stability (Okumura and others 1990). Thus, thiolation of cysteine-deficient proteins, such as soy proteins might improve their foaming and emulsifying properties.

The major limitations of chemical modification to improve functionality of proteins are the economics as well as the nutritional safety of the derivatized proteins.

Enzymatic modification is a promising approach to improve the foaming and emulsifying properties of proteins. Although a number of enzymatic modifications can be envisaged, the most practical ones are the hydrolysis and polymerization reactions. Limited enzymatic hydrolysis of proteins using proteases, such as pepsin, trypsin, chymotrypsin, and other nonspecific proteases, often increases the emulsifying activity index and produces smaller oil droplets than the intact protein (Huang and others 1996). However, highly hydrolyzed proteins produce very unstable emulsions and foams (Nielsen 1997). Another promising enzymatic approach is controlled polymerization of proteins using transglutaminase. Several studies have shown that transglutaminase catalyzes homopolymerization of several proteins and the functional properties of the polymerized proteins are distinctly different from those of the native proteins (Ikura and others 1980; Motoki and others 1984, 1987a; Nio and others 1986; Liu and Damodaran 1999; Babiker and others 1996). It has been shown that although the emulsifying activity index of β-casein decreased with the extent of polymerization, emulsion stability increased with increasing degree of polymerization (Liu and Damodaran 1999). Mixing of polymerized β-casein with monomer β-casein increased the emulsion stability with increasing percentage of polymerized β-casein in the mixture, confirming that the polymerized β-casein imparts better steric stabilization than the native β-casein. Heterologous cross-linking between 2 different proteins by transglutaminase also has been reported; however, there is no convincing evidence that this actually occurs in the protein systems studied (Motoki and others 1987b; Kurth and Rogers 1984). In fact, a comprehensive study on several pairs of globular proteins has shown that while certain pairs of proteins can form heterologous polymers, most of the proteins pairs cannot (Han and Damodaran 1996). It has been hypothesized that this inability to form heterologous cross-linking might be related to incompatibility of mixing of the proteins in the active site of the enzyme. Further research is needed to improve the efficiency of heterologous polymerization of proteins by transglutaminase.

A long-term objective of research in this area should be genetic modification of plant proteins to improve their functional properties, especially the foaming, emulsifying, and gelling properties. Protein engineering is often used by biochemists to elucidate structure-function relationship of enzymes. This approach has been successfully employed to improve thermostability, to alter temperature and pH optima, and to change substrate specificities of enzymes. Application of this technique to improve the functional properties of food proteins is in its infancy. In one of the earliest studies, it has been shown that the foaming and emulsifying properties of tryptophan synthase α-subunits improved significantly when a glutamate residue at position 49 was substituted by isoleucine and phenylalanine (Kato and Yutani 1988). The surface activities of the 6 mutants studied showed a good correlation with the free energy change for unfolding in water. Deamidation of hen egg-white lysozyme at positions 103 and 106 by site-directed mutagenesis also significantly improved surface activities of mutant lysozymes compared with the wild-type (Kato and others 1992). Significantly, although the surface activities of the mutants and the wild-type were different, the free energy changes of unfolding of the mutants and the wild-type were almost the same, suggesting that properties other than structural stability may influence surface activities of proteins. Significant advances in genetic modification of soybean proteins and other proteins are being made to improve their functional properties in food systems (Kim and others 1990; Utsumi and others 1993, 2002; Adachi and others 2004; Momma and others 2000).

Molecular flexibility is a quintessential factor for the surface activity of proteins. This is borne out of the fact that of all the molecular properties of 19 different proteins investigated, only molecular flexibility showed a linear correlation with their surface activities at the air-water interface (Razumovsky and Damodaran 1999a). Thus, any genetic engineering approach to improve the functional properties of proteins, especially plant proteins, should involve judicious selection of regions of the native protein which, when mutated, would potentially increase the flexibility of the protein. The interior core of plant proteins typically is made of β-barrel-type structure (Lawrence and others 1994; Adachi and others 2003). The high thermal stability of the legume proteins might be attributable to this β-barrel core. Thus, single residue mutations or cassette mutations at appropriate locations in the β-barrel core might produce mutants with high molecular flexibility. It should be pointed out that β-lactoglobulin variants A and B, which differ by only 2 amino acid residues at positions 64 and 118 in their amino acid sequences, show significant difference in their surface activity (Mackie and others 1999b). Thus, it is quite possible to significantly alter the physicochemical properties of proteins by targeting mutations at specific locations in proteins.

References

Adachi M, Kanamori J, Masuda T, Yagasaki K, Kitamura K, Mikami B, Utsumi S. 2003. Crystal structure of soybean 11S globulin: Glycinin A3B4 homohexamer. Proc Nat Acad Sci USA 100:7395–400.

Adachi M, Ho CY, Utsumi S. 2004. Effects of designed sulfhydryl groups and dis-

ulfide bonds into soybean proglycinin on its structural stability and heat-induced gelation. J Agric Food Chem 52:5717–23.
Anand K, Damodaran S. 1995. Kinetics of adsorption of lysozyme and bovine serum albumin at the air/water interface from a binary mixture. J Colloid Interface Sci 176:63–73.
Anand K, Damodaran S. 1996. Dynamics of exchange between α_{s1}- casein and β-casein during adsorption at air-water interface. J Agric Food Chem 44:1022–8.
Anton M, Gandemer G. 1999. Effect of pH on interface composition and on quality of oil-in-water emulsions made with hen egg yolk. Colloids Surf B Biointerfaces 12:351–8.
Asakura S, Oosawa F. 1954. On interaction between two bodies immersed in a solution of macromolecules. J Chem Phys 22:1255–6.
Asakura S, Oosawa F. 1958. Interaction between particles suspended in solutions of macromolecules. J Polym Sci 33:183–92.
Babiker EFE, Khan MAS, Matsudomi N, Kato A. 1996. Polymerization of soy protein digests by microbial transglutaminase for improvement of the functional properties. Food Res Int 29:627–34.
Bals A, Kulozik U. 2003. Effect of preheating on the foaming properties of whey protein isolate using a membrane foaming apparatus. Int Dairy J 13:903–8.
Bergeron V. 1999. Forces and structure in thin liquid soap films. J Phys Condens Matter 11:R215–38.
Bos MA, van Vliet T. 2001. Interfacial rheological properties of adsorbed protein layers and surfactants: a review. Adv Colloid Interface Sci 91:437–71.
Carp DJ, Bartholamai GB, Pilosof AMR. 1999. Electrophoretic studies for determining soy proteins xanthan gum interactions in foams. Colloid Surf B Biointerfaces 12:309–16.
Cao Y, Dickinson E, Wedlock DJ. 1990. Creaming and flocculation in emulsions containing polysaccharide. Food Hydrocolloids 4:185–95.
Cao Y, Dickinson E, Wedlock DJ. 1991. Influence of polysaccharides on the creaming of casein-stabilized emulsions. Food Hydrocolloids 5:443–54.
Chanamai R, McClements DJ. 2000. Impact of weighting agents and sucrose on gravitational separation of beverage emulsions. J Agric Food Chem 48:5561–5.
Clark DC, Makie AR, Smith LJ, Wilson DR. 1988. The interaction of bovine serum albumin and lysozyme and its effect on foam composition. Food Hydrocolloids 2:209–23.
Courthaudon JL, Dickinson E, Matsumura Y, Williams A. 1991. Influence of emulsifier on the competitive adsorption of whey proteins in emulsions. Food Structure 10:109–15.
Dalgleish DG. 1997. Adsorption of protein and the stability of emulsions. Trends Food Sci Technol 8:1–6.
Dalgleish DG, Srinivasan M, Singh H. 1995. Surface properties of oil-in-water emulsion droplets containing casein and Tween 60. J Agric Food Chem 43:2351–5.
Dalgleish DG, Goff HD, Luan B. 2002a. Exchange reactions between whey proteins and caseins in heated soya oil-in-water emulsion systems—behavior of individual proteins. Food Hydrocolloids 16:295–302.
Dalgleish DG, Goff HD, Brun JM, Luan B. 2002b. Exchange reactions between whey proteins and caseins in heated soya oil-in-water emulsion systems—overall aspects of the reaction. Food Hydrocolloids 16:303–11.
Damodaran S. 1996. Amino acids, peptides, and proteins. In: Fennema OR, editor. Food chemistry. New York: Marcel Dekker. p 321–429.
Damodaran S. 1997. Protein-stabilized foams and emulsions. In: Damodaran S, Paraf A, editors. Food proteins and their applications. New York: Marcel Dekker. p 57–110.
Damodaran S. 2004. Adsorbed layers formed from mixtures of proteins. Curr Opinion Colloid Interface Sci 9:328–39.
Damodaran S, Anand K. 1997. Sulfhydryl-disulfide interchange–induced interparticle protein polymerization in whey protein-stabilized emulsions and its relation to emulsion stability. J Agric Food Chem 45:3813–20.
Damodaran S, Sengupta T. 2003. Dynamics of competitive adsorption of α_s-casein and β-casein at the oil-water interface: evidence for incompatibility of mixing at the interface. J Agric Food Chem 51:1658–65.
Damodaran S, Anand K, Razumovsky L. 1998. Competitive adsorption of egg-white proteins at the air-water interface: Direct evidence for electrostatic complex formation between lysozyme and other egg proteins at the interface. J Agric Food Chem 46:872–6.
Davis JP, Foegeding EA. 2004. Foaming and interfacial properties of polymerized whey protein isolate. J Food Sci 69:C404–10.
de Gennes PG. 1987. Polymers at an interface: a simplified view. Adv Colloid Interface Sci 27:189–209.
de Kruif CG, Tuinier R. 2001. Polysaccharide protein interactions. Food Hydrocolloids 15:555–63.
Dickinson E. 1995. Emulsion stabilization by polysaccharides and protein-polysaccharide complexes. In: Stephen AM, editor. Food polysaccharides and their applications. New York: Marcel Dekker. p 501–15.
Dickinson E. 1998. Proteins at interfaces and in emulsions: Stability, rheology and interactions. J Chem Soc Faraday Trans 88:2973–85.
Dickinson E. 1999. Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology. Colloids Surf B Biointerfaces 15:161–76.
Dickinson E. 2001. Milk protein interfacial layers and the relationship to emulsion stability and rheology. Colloids Surf B Biointerfaces 20:197–210.
Dickinson E. 2003. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food Hydrocolloids 17:25-39.
Dickinson E, Rolfe SE, Dalgleish DG. 1988. Competitive adsorption of α_{s1}-casein and β-caseinin oil-in-water emulsions. Food Hydrocolloids 2:397–405.
Dickinson E, Flint FO, Hunt JA. 1989. Bridging flocculation in binary protein stabilized emulsions. Food Hydrocolloids 3:389–97.
Dickinson E, Ma J, Povey MJW. 1994. Creaming of concentrated oil-in-water emulsions containing xanthan. Food Hydrocolloids 8:481–97.
Dickinson E, Matsumura Y. 1991. Time-dependent polymerization of β-lactoglobulin through disulphide bonds at the oil-water interface in emulsions. Int J Biol Macromol 13:26–30.
Dickinson E, Pawlowsky K. 1997. Effect of k-carrageenan on flocculation, creaming, and rheology of a protein-stabilized emulsion. J Agric Food Chem 45:3799–806.
Dickinson E, Hong ST. 1994. Surface coverage of β-lactoglobulin at the oil-water interface: influence of protein heat treatment and various emulsifiers. J Agric Food Chem 42:1602–6.
Erickson JS, Sundaram S, Stebe KJ. 2003. Evidence that the induction time in the surface pressure evolution of lysozyme solutions is caused by a surface phase transition. Langmuir 16:5072–8.
Euston SE, Singh H, Munro PA, Dalgleish DG. 1995. Competitive adsorption between sodium caseinate and oil-soluble and water-soluble surfactants in oil-in-water emulsions. J Food Sci 60:1124–31.
Euston SE, Singh H, Munro PA, Dalgleish DG. 1996. Oil-in-water emulsions stabilized by sodium caseinate or whey protein isolate as influenced by glycerolmonostearate. J Food Sci 61:916–20.
Fang Y, Dalgleish DG. 1996. Competitive adsorption between dioleoylphosphatidylcholine and sodium caseinate on oil-water interfaces. J Agric Food Chem 44:59–64.
Garofalakis G, Murray BS. 2001. Dilatational rheology and foaming properties of sucrose monoesters in the presence of b-lactoglobulin. Colloids Surf B Biointerfaces 21:3–17.
Gunning PA, Hennock MSR, Howe AM, Robbins MM. 1986. Stability of oil-in-water emulsions—the effect of dispersed phase and polysaccharide on creaming. Colloids Surf 20:65–80.
Gunning PA, Mackie AR, Gunning AP, Wilde PJ, Woodward NC, Morris VJ. 2004. The effect of surfactant type on protein displacement from the air-water interface. Food Hydrocolloids 18:509–15.
Han XQ, Damodaran S. 1996. Thermodynamic compatibility of substrate proteins affects their cross-linking by transglutaminase. J Agric Food Chem 44:1211–7.
Hattori M, Nagasawa K, Ametai A, Kaminogawa S, Takahashi K. 1994. Functional changes in b-lactoglobulin by conjugation with carboxymethyl dextran. J Agric Food Chem 42:2120–5.
Hattori M, Numamoto K, Kohayashi K, Takahashi K. 2000. Functional changes in β-lactoglobulin by conjugation with cationic saccharides. J Agric Food Chem 48:2050–6.
Howe AM, Mackie AR, Richmond P, Robins MM. 1985. Creaming of oil-in-water emulsions containing polysaccharides. In: Phillips GO, Wedlock DJ, Williams PA, editors. Gums and stabilizers for the food industry 3. London: Elsevier Applied Science. p 323–36.
Huang XLL, Catignani GL, Swaisgood HE. 1996. Improved emulsifying properties of b-barrel domain peptides obtained by membrane-fractionation of a limited tryptic hydrolysate of b-lactoglobulin. J Agric Food Chem 44:3437–43.
Ibrahim HR, Kobayashi K, Kato A. 1993. Improvement of the surface functional properties of b-lactoglobulin and a-lactalbumin by heating in a dry state. Biosci Biotech Biochem 57:1549–52.
Ibanoglu E, Karatas S. 2001. High pressure effect on foaming behavior of whey protein isolate. J Food Eng 47:31–6.
Ikura K, Kometani T, Yoshikawa M, Sasaki R, Chiba H. 1980. Crosslinking of casein components by transglutaminase. Agric Biol Chem 44:1567–73.
Israelachvili JN. 1992. Intermolecular and surface forces. Academic Press: New York. p 176–8.
Kato A. 2002. Industrial applications of Maillard-type protein-polysaccharide Conjugates. Food Sci Technol Res 8:193–9.
Kato A, Yutani K. 1988. Correlation of surface properties with conformational stabilities of wild-type and six mutant tryptophan synthase alpha-subunits substituted at the same position. Protein Eng 2:153–6.
Kato A, Tanimoto S, Muraki Y, Kobayashi K, Kumagai I. 1992. Structural and functional properties of hen egg-white lysozyme deamidated by protein engineering. Biosci Biotech Biochem 56:1424–8.
Kato A, Nakai S. 1980. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. Biochim Biophys Acta 624:13–20.
Kato A, Osako Y, Matsudomi N, Kobayashi K. 1983. Changes in emulsifying and foaming properties of proteins during heat denaturation. Agric Biol Chem 47:33–8.
Kim CS, Kamiya S, Sato T, Utsumi S, Kito M. 1990. Improvement of nutritional value and functional properties of soybean glycinin by protein engineering. Protein Eng 3:725–31.
Kiosseoglou V, Perdikis A. 1994. Stability of bovine serum albumin-stabilized olive oil-in-water emulsions and the role of the oil minor surface-active lipids. Food Hydrocolloids 8:27–32.
Kurth L, Rogers PJ. 1984. Transglutaminase catalyzed cross-linking of myosin to soya protein, casein, and gluten. J Food Sci 49:573–6.
Lawrence MC, Izard T, Beauchat M, Blagrove RJ, Colman PM. 1994. Structure of phaseolin at 2.2 Å resolution. Implications for a common vicilin/legumin structure and the genetic engineering of seed storage proteins. J Mol Biol 238:748-76.
Leroux J, Langendorff V, Schick G, Vaishnav V, Mazoyer J. 2003. Emulsion stabilizing properties of pectin. Food Hydrocolloids 17:455–62.
Liu M, Damodaran S. 1999. Effect of transglutaminase-catalyzed polymerization of b-casein on its emulsifying properties. J Agric Food Chem 47:1514–9.
Lombardo YB, Chicco A, Basilico MZ, Bernal C, Gutman R. 1985. Effect of brominated vegetable oils on heart lipid metabolism. Lipids 20:425–32.
Mackie AR, Gunning AP, Wilde PJ, Morris VJ. 1999a. Orogenic displacement of protein from the air/water interface by competitive adsorption. J Colloid Interface Sci 210:157–66.
Mackie AR, Husband FA, Holt C, Wilde PJ. 1999b. Adsorption of b-lactoglobulin variants A and B to the air-water interface. Int J Food Sci Technol 34:509–16.
Mackie AR, Gunning AP, Pugnaloni LA, Dickinson E, Wilde PJ, Morris VJ. 2003. Growth of surfactant domains in protein films. Langmuir 19:6032–8.
Mackie AR, Gunning AP, Wilde PJ, Morris VJ. 2000. Competitive displacement of β-lactoglobulin from the air/water interface by sodium dodecyl sulfate. Langmuir 16:8176–81.
Martin AH, Bos MA, van Vliet T. 2002. Interfacial rheological properties and

conformational aspects of soy glycinin at the air/water interface. Food Hydrocolloids 16:63–71.
McClements DJ. 1999. Food emulsions: principles, practice and techniques. Boca Raton, Fla: CRC Press.
McClements DJ. 2000. Comments on viscosity enhancement and depletion flocculation by polysaccharides. Food Hydrocolloids 14:173–7.
Meller A, Gisler T, Weitz DA, Stavans J. 1999. Viscoelasticity of depletion-induced gels in emulsion-polymer systems. Langmuir 15:1918–22.
Meynier A, Garillon A, Lethuaut L, Genot C. 2003. Partition of five aroma compounds between air and skim milk, anhydrous milk fat or full-fat cream. LAIT 83:223–35.
Mitidieri FE, Wagner JR. 2002. Coalescence of o/w emulsions stabilized by whey and isolate soybean proteins. Influence of thermal denaturation, salt addition and competitive interfacial adsorption. Food Res Int 35:547–57.
Molina E, Papadopoulou A, Ledward DA. 2001. Emulsifying properties of high pressure treated soy protein isolate and 7S and 11S globulins. Food Hydrocolloids 15:263–9.
Momma K, Hashimoto W, Yoon HJ, Ozawa S, Fukuda Y, Kawai S, Takaiwa F, Utsumi S, Murata K. 2000. Safety assessment of rice genetically modified with soybean glycinin by feeding studies on rats. Biosci Biotech Biochem 64:1881–8.
Monahan FJ, McClements DJ, Kinsella JE. 1993. Polymerization of whey proteins in whey protein-stabilized emulsions. J Agric Food Chem 41:1826–9.
Monsalve A, Schechter RS. 1984. The stability of foams: Dependence of observation on the bubble size distribution. J Colloid Interface Sci 97:327–35.
Motoki M, Nio N, Takinami K. 1984. Functional properties of food proteins polymerized by transglutaminase. Agric Biol Che. 48:1257–61.
Motoki M, Aso H, Seguro K, Nio N. 1987a. α_{s1}-Casein film prepared using transglutaminase. Agric Biol Chem 51:993–6.
Motoki M, Nio N, Takinami K. 1987b. Functional properties of heterologous polymer prepared by transglutaminase between milk casein and soybean globulin. Agric Biol Chem 51:237–9.
Murray BS, Liang HJ. 1999. Enhancement of the foaming properties of protein dried in the presence of trehalose. J Agric Food Chem 47:4984–91.
Mutilangi WAM, Panyam D, Kilara A. 1996. Functional properties of hydrolysates from proteolysis of heat-denatured whey protein isolate. J Food Sci 61:270-4.
Nielsen PM. 1997. Functionality of protein hydrolyzates. In: Damodaran S, Paraf A, editors. Food proteins and their applications. New York: Marcel Dekker. p 443–72.
Nio N, Motoki M, Takinami K. 1986. Gelation mechanism of protein solution by transglutaminase. Agric Biol Chem 50:851–5.
Okumura K, Miyake Y, Taguchi H, Shimabayashi Y. 1990. Formation of stable protein foam by intermolecular disulfide cross-linkages in thiolated α_{s1}-casein as a model. J Agric Food Chem 38:1303–6.
Parker A, Gunning PA, Ng K, Robins MM. 1995. How does xanthan stabilize salad dressing? Food Hydrocolloids 9:333–42.
Perea A, Ugaide U, Rodriguez I, Serra JS. 1993. Preparation and characterization of whey protein hydrolysates: applications in industrial whey bioconversion processes. Enzyme Microb Technol 15:418–24.
Polyakov VL, Grinberg VY, Antonov YA, Tolstoguzov VB. 1979. Limited thermodynamic compatibility of proteins in aqueous solutions. Polymer Bulletin 1:593–7.
Polyakov VL, Kireyeva OK, Grinberg VY, Tolstoguzov VB. 1985. Thermodynamic compatibility of proteins in aqueous media. Part I. Phase diagrams of some water-protein A-protein B systems. Die Nahrung 29:153–60.
Polyakov VL, Grinberg VY, Tolstoguzov VB. 1997. Thermodynamic compatibility of proteins. Food Hydrocolloids 11:171–80.
Poole S. 1989. Review: The foam-enhancing properties of basic biopolymers. Int J Food Sci Technol 24:121–37.
Poole S, West SI, Walters C. 1984. Protein-protein interactions: their importance in foaming of heterogeneous protein systems. J Sci Food Agric 35:701–11.
Pugnaloni AA, Dickinson E, Ettelaie R, Mackie AR, Wilde PJ. 2004. Competitive adsorption of proteins and low-molecular-weight surfactants: computer simulation and microscopic imaging. Adv Colloid Interface Sci 107:27–49.
Quintana JM, Califano AN, Zaritzky NE, Partal P. 2002a. Effect of salt on the rheological peroperties of low-in-fat O/W emulsions stabilised with polysaccharides. Food Sci Technol Int 8:213–21.
Quintana JM, Califano AN, Zaritzky NE, Partal P, Franco JM. 2002b. Linear and nonlinear viscoelastic behavior of oil-in-water emulsions stabilized with polysaccharides. J Text Studies 33:215–36.
Rao CS, Damodaran S. 2000. Is surface pressure a measure of interfacial water activity? Evidence from protein adsorption behavior at interfaces. Langmuir 16:9468–77.
Razumovsky L, Damodaran S. 1999a. Surface activity—Compressibility relationship of proteins. Langmuir 15:1392–9.
Razumovsky L, Damodaran S. 1999b. Thermodynamic incompatibility of proteins at the air-water interface. Colloids Surf B Biointerfaces 13:251–61.
Razumovsky L, Damodaran S. 2001. Incompatibility of mixing of proteins in adsorbed binary protein films at the air-water interface. J Agric Food Chem 49:3080–6.
Reiffers-Magnani CK, Cuq JL, Watzke HJ. 2000. Depletion flocculation and thermodynamic incompatibility in whey protein stabilised O/W emulsions. Food Hydrocolloids 14:521–30.
Rideout MJ, Mackie AR, Wilde PJ. 2004. Rheology of mixed β-casein/β-lactoglobulin films at the air-water interface. J Agric Food Chem 52:3930–7.
Robson EW, Dalgleish DG. 1987. Interfacial composition of sodium caseinate emulsions. J Food Sci 52:1694–8.
Sarker DK, Wilde PJ, Clark DC. 1996. Enhancement of the stability of protein-based foams using trivalent cations. Colloid Surf A Physicochem Eng Asp 114:227–36.
Sarker DK, Wilde PJ, Clark DC. 1995. Control of surfactant-induced destabilization of foams through polyphenol-mediated protein-protein interactions. J Agric Food Chem 43:295–300.
Schwenke KD. 1997. Enzyme and chemical modification of proteins. In: Damodaran S, Paraf A, editors. Food proteins and their applications. New York: Marcel Dekker. p 393–423.
Sengupta T, Damodaran S. 1998. Role of dispersion interactions in the adsorption of proteins at oil-water and air-water interfaces. Langmuir 14:6457–69.
Sengupta T, Razumovsky L, Damodaran S. 1999. Energetics of protein-interface interactions and its effect on protein adsorption. Langmuir 15:6991–7001.
Sengupta T, Damodaran S. 2000. Incompatibility and phase separation in bovine serum albumin/β-casein/water ternary film at the air-water interface. J Colloid Interface Sci 229:21–8.
Sengupta T, Razumovsky L, Damodaran S. 2000. Phase separation in two-dimensional α_{s1}-casein/β-casein/water ternary film at the air-water interface. Langmuir 16:6583–9.
Sengupta T, Damodaran S. 2001. Lateral phase separation in adsorbed binary protein films at the air-water interface. J Agric Food Chem 49:3087–91.
Sharma A, Ruckenstein E. 1987. Critical thickness and lifetimes of foams and emulsions: role of surface wave-induced thinning. J Colloid Interface Sci 119:14–29.
Singh H, Tamehana M, Hemar Y, Munro PA. 2003a. Interfacial compositions, microstructure and properties of oil-in-water emulsions formed with mixtures of milk proteins and k-carrageenan: 1. Sodium caseinate. Food Hydrocolloids 17:539–48.
Singh H, Tamehana M, Hemar Y, Munro PA. 2003b. Interfacial compositions, microstructure and stability of oil-in-water emulsions formed with mixtures of milk proteins and k-carrageenan: 2. Whey protein isolate (WPI). Food Hydrocolloids 17:549–61.
Stokes JR, Wolf B, Frith WJ. 2001. Phase-separated biopolymer mixture rheology: Prediction using a viscoelastic emulsion model. J Rheol 45:1173–91.
Stubenrauch C, von Klitzing R. 2003. Disjoining pressure in thin liquid foam and emulsion films—new concepts and perspectives. J Phys Condens Matter 15:R1197–232.
Syrbe A, Bauer WJ, Klostermeyer H. 1998. Polymer science concepts in dairy systems—an overview of milk protein and food hydrocolloid interaction. Int Dairy J 8:179–93.
Tadros Th F. 1994. Fundamental principles of emulsion rheology and their applications. Colloid Surf. 91:39–55.
Tcholakova S, Denkov ND, Sidzhakova D, Ivanov IB, Cambell B. 2003. Interrelation between drop size and protein adsorption at various emulsification conditions. Langmuir 19:5640–9.
Tomas A, Courthaudon JL, Paquet D, Lorient D. 1994. Effect of surfactant on some physico-chemical properties of dairy oil-in-water emulsions. Food Hydrocolloids 8:543–53.
Townsend A, Nakai S. 1983. Relationship between hydrophobicity and foaming capacity of proteins. J Food Sci 48:588–94.
Tolstoguzov VB. 1998. Physico-chemical modification of food proteins: Food emulsions. Nahrung-Food 42:205–9.
Tolstoguzov VB. 2000. Foods as dispersed systems. Thermodynamic aspects of composition-property relationships in formulated food. J Thermal Anal Calorimetry 61:397–409.
Yu M-A, Damodaran S. 1991. Kinetics of protein foam destabilization: Evaluation of a method using bovine serum albumin. J Agric Food Chem 39:1555–62.
Utsumi S, Gidamis AB, Kanamori J, Kang IJ, Kito M. 1993. Effects of deletion of disulfide bonds by protein engineering on the conformation and functional properties of soybean proglycinin. J Agric Food Chem 41:687–91.
Utsumi S, Maruyama N, Satoh R, Adachi M. 2002. Structure-function relationships of soybean proteins revealed by using recombinant systems. Enzyme Microb Technol 30:284–8.
Voutsinas LP, Cheung E, Nakai S. 1983. Relationships of hydrophobicity to emulsifying properties of heat denatured proteins. J Food Sc. 48:26–32.
Walstra P. 1988. The role of proteins in stabilization of emulsions. In: Phillips GO, Wedlock DJ, Williams PA, editors. Gums and stabilizers for the food industry. Oxford: IRL Press. p 323–36.
Walstra P. 1993. Introduction to aggregation phenomena in food colloids. In: Dickinson E, Walstra P, editors. Food colloids and polymers: stability and mechanical properties. Cambridge, U.K.: Royal Society of Chemistry. p 3–15.
Walstra P. 1996. Dispersed systems: basic considerations. In: Fennema O, editor. Food chemistry. New York: Marcel Dekker. p 95–155.
Walstra P, de Roos AL. 1993. Proteins at air-water and oil-water interfaces: static and dynamic aspects. Food Rev Int 9:503–25.
Warren PB. 1997. Phase behavior of a colloid + binary polymer mixture: theory. Langmuir 13:4588–94.
Wierenga PA, Meinders MBJ, Egmond MR, Voragen FAGJ, de Jongh HHJ. 2003. Protein exposed hydrophobicity reduces the kinetic barrier for adsorption of ovalbumin to the air-water interface. Langmuir 19:8964–70.
Wilde PJ. 2000. Interfaces: their role in foam and emulsion behavior. Curr Opinion Colloid Interface Sci 5:176–81.
Xu S, Damodaran S. 1993. Comparative adsorption of native and denatured egg-white, human and T_4 phage lysozyme at the air-water interface. J Colloid Interface Sci 159:124–33.
Zhang Z, Dalgleish DG, Goff HD. 2004. Effect of pH and ionic strength on competitive protein adsorption to air/water interfaces in aqueous foams made with mixed milk proteins. Colloid Surf B Biointerfaces 34:113–21.
Zhu H, Damodaran S. 1994a. Heat-induced conformational changes in whey protein isolate and its relation to foaming properties. J Agric Food Chem 42:846–55.
Zhu H, Damodaran S. 1994b. Effects of calcium and magnesium ions on aggregation of whey protein isolate and its effect on foaming properties. J Agric Food Chem 42:856–62.
Zhu H, Damodaran S. 1994c. Proteose peptones and physical factors affect foaming properties of whey protein isolate. J Food Sci 59:554–60.