

Abstract

Davis, Jack Parker. Investigations into the mechanisms responsible for the yield stress of protein based foams. (Under the direction of Dr. E. Allen Foegeding)

Proteins function as natural surfactants in many industrial processes involving foam formation. Egg white proteins have traditionally served this role in the food industry, although substitution with other proteins, including those derived from bovine milk is becoming more prevalent. Above a critical gas phase (ϕ), foams transition from viscous liquids to semi-solid materials that display a yield stress (τ_0). Measurements of τ_0 relate well to the empirical concept of foam robustness and more robust foams are generally desirable from a food science perspective, in order to withstand the rigors of processing, including pumping, heating, coating, etc. Accordingly, the general goal of this research was to investigate mechanisms responsible for foam τ_0 on a fundamental level, in order to more efficiently utilize whey proteins (derived from bovine milk) as a foaming ingredient, with an emphasis on their capacity to regulate foam τ_0 .

In the first study, the yield stress of whey protein isolate (WPI) foams as affected by electrostatic forces was investigated by whipping 10% w/v protein solutions prepared over a range of pH levels and salt concentrations. Measurements of foam overrun, protein adsorption kinetics at the air/water interface, and dilatational rheological characterization, aided data interpretation. Interfacial measurements were also made with the primary whey proteins, β -lactoglobulin (β -lg) and α -lactalbumin (α -la). Yield stress of WPI foams was dependent on pH, salt type and salt concentration. In the absence of

salt, τ_0 was highest at pH 5.0 and lowest at pH 3.0. The addition of NaCl and CaCl₂ significantly increased τ_0 at pH 7.0, with equivalent molar concentrations of CaCl₂ as compared to NaCl increasing τ_0 to greater extents. Salts minimally affected τ_0 at pH 3.0 or 5.0. Comparisons with interfacial rheological data suggested the protein's capacity to contribute towards τ_0 was related to its potential at forming strong, elastic interfaces throughout the structure. Dynamic surface tension data for β -lg and α -la were similar to WPI, while the interfacial rheological data displayed several noticeable differences.

In the second study, polymerized WPI (pWPI) was investigated for its potential as a functional foaming ingredient. Note that pWPI is a soluble complex of covalently bound whey protein formed via controlled heating. Foam τ_0 displayed a parabolic response to increasing concentrations of pWPI to native WPI, peaking at 50%. Foam air phase volume steadily decreased with increasing pWPI content, whereas equilibrium surface tension steadily increased. Dynamic surface tension measurements revealed that native WPI adsorbed much more rapidly than pWPI, presumably due to the former's smaller size. Dilatational elasticity (E') also displayed a parabolic trend with increasing pWPI content, peaking at 50%. This suggested that pWPI coadsorbs with native WPI, bolstering E' of native WPI interfaces. However, too much pWPI caused a weakening of the network. A positive, curvilinear relationship between E' and τ_0 was observed, consistent with earlier data, further suggesting a general link between these parameters.

In the third study, β -lactoglobulin (β -lg), which is the primary whey protein, was hydrolyzed with three different proteases and subsequently evaluated for its foaming

potential. Two heat treatments designed to inactivate the enzymes, 75°C/30 min and 90°C/15 min, were also investigated for their effects on foam functionality. All unheated hydrolysates improved τ_0 as compared to unhydrolyzed β -lg, with those of pepsin and Alcalase 2.4L® being superior to trypsin. Heat inactivation negatively impacted foam τ_0 , although heating at 75°C/30 min better preserved this parameter than heating at 90°C/15 min. The previously observed relationship between E' and τ_0 was generally confirmed for these hydrolysates. Additionally, the three hydrolysates imparting the highest τ_0 not only had high values of E' , they also had very low phase angles (essentially zero). This highly elastic interfacial state is presumed to improve foam τ_0 indirectly by improving foam stability and directly by imparting resistance to interfacial deformation.

In the final study, the foaming and interfacial properties of WPI and egg white protein (EWP) were directly compared. The highest τ_0 and resistance to drainage were observed for standard EWP, followed by EWP with added 0.1% w/w sodium lauryl sulfate, and then WPI. Previously observed relationships between τ_0 and interfacial rheological measurements did not hold across the protein types; however these interfacial measurements did effectively differentiate foaming behaviors within EWP-based ingredients and within WPI. Addition of 25% w/w sucrose to the solutions increased τ_0 and drainage resistance of the EWP-based ingredients, but it decreased τ_0 of WPI foams and minimally affected their drainage rates. These differing sugar effects were reflected in the interfacial measurements, as sucrose addition increased the dilatational elasticity and decreased the interfacial phase angle for both EWP-based ingredients, while sucrose addition imparted the exact opposite effects on WPI.

**Investigations into the mechanisms responsible for the
yield stress of protein based foams.**

by

Jack P. Davis

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Approved by:

E. Allen Foegeding, Ph.D.
(Chair of Advisory Committee)

John Cavanagh, Ph.D.
(minor representative)

Peter Kilpatrick, Ph. D.

Christopher Daubert, Ph.D.

Dedication

This dissertation is dedicated to the memory of Michael Bentley, a hard working man of integrity, intelligence, toughness and compassion. I'm a much better person for having known his company. His spirit undoubtedly lives in Jennifer, his daughter and my wife, and the most spectacular Jacob, his grandson and my son. Thank you God for all my many blessings.

Biography

Jack Davis was born and raised in rural Georgia, a place he still considers home, although North Carolina now runs a close second. After attending the University of Georgia and experiencing an exceptional five years in Athens, Jack moved to Raleigh to begin a Masters degree in Food Science at North Carolina State University under the direction of Allen Foegeding. The experience was so rewarding, Jack continued with his graduate studies with Allen, beginning a PhD in the winter of 2002. Jack looks forward to a career with the USDA, beginning in April of 2005. The most important thing in his life is family, especially his wife, Jennifer and his son, Jacob.

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Literature Review

Whey and Whey Proteins

Whey is the watery fraction of milk remaining after the cheese making process. This material is approximately 93% water and 0.6% protein when derived from bovine milk [1]. Traditionally, whey was considered a waste stream; however, technological advances in areas such as filtration have made it economically beneficial to concentrate and subsequently dry this substance to produce a range of ingredients [2]. Two of the more common products are 1) whey protein concentrates (WPC) which contain between 25% and 80% protein and 2) whey protein isolates (WPI) which contain $\geq 90\%$ protein [1]. There has been a tremendous amount of work characterizing these ingredients for their nutritional, sensorial and especially functional properties, and this information has allowed for a more efficient utilization of these ingredients in a wide range of food products. What immediately follows is a brief summary of some key molecular characteristics of the primary whey proteins. There are several excellent reviews on this subject to which the reader is directed for more in depth discussions [3-6]. The specific aspects of whey protein chemistry thought essential to their foaming and interfacial behaviors will be discussed in detail later.

β -lactoglobulin

β -lactoglobulin (β -lg) is the primary whey protein and one of the best characterized proteins of all time [4, 5]. It's structure has been solved by X-ray crystallography and NMR under a variety of conditions by numerous researchers [7-11]. It shares many

similarities with plasma retinol binding protein, leading some to suggest its involvement in vitamin A transfer, although the exact physiological function of β -lg remains unclear [10]. There are two primary genetic variants of this molecule designated A and B which differ by two amino acids. The monomer of this molecule has a MW of approximately 18,350 kDa and consists of 162 amino acids. It has two disulfide bridges and one free sulfhydryl group that becomes reactive at pH values above ~ 7.5 [12]. The primary tertiary motif is a β -barrel with a single helix lying on its surface. The interior of this barrel is hydrophobic and is the binding site for many small hydrophobic ligands [4]. The protein's isoelectric point (pI) is pH ~ 5.2 , and its quaternary structure is pH sensitive. At physiological pH the protein exists as a dimer; however at pH values less than ~ 3.0 and greater than ~ 8.0 its increased charge density leads to a monomeric form [3]. As the pH approaches its pI, increased associations due to its decreased charge density lead to oligomer formation with a dominant octamer species having been reported [13]

From circular dichroism and infrared spectroscopy, the secondary structure of the molecule is reported to be approximately 15% α -helix, 51% β -sheet, 17% reverse turn, and 17% aperiodic structure [14]. Above 65 °C, β -lg undergoes a time-temperature dependent denaturation, which is accompanied by extensive conformational transitions that expose previously buried hydrophobic regions [5]. Heat denaturation of β -lg is pH dependent, as the protein is most sensitive at pH 4.0 with a maximum stability at pH 6.0 [3]

α -lactalbumin

α -lactalbumin (α -LA) is the second most prevalent protein in whey, accounting for roughly 25% of the whey protein [5]. This compact, globular protein strongly binds calcium and is naturally associated in the biological synthesis of lactose. There are two primary molecular variants with MW's of approximately 14,150 [15]. Its structure shares many similarities with lysozyme [16]. Overall, the molecule has an ellipsoid shape with two distinct lobes divided by a cleft where one lobe is comprised of four helices and the other loop is comprised of two β -strands with a loop-like chain [3, 17]. α -LA has no free thiol groups and four disulfide bonds, which limit its conformational flexibility in certain solvent conditions [15]

Minor Protein Components

The combined concentration of bovine serum albumen and the other minor whey proteins within whey protein ingredients is so low (~5%), they usually aren't considered from a functionality standpoint. Therefore, specifics concerning their molecular characteristics will not be discussed. However, it is worth mentioning, that as separation technologies become more advanced, coupled with advances in genetic engineering for molecule production, the potential for producing protein ingredients enriched in previously dilute proteins or peptides on an industrial scale is becoming very real. Accordingly, these fractions could have very specialized and or unique functional properties.

Protein Functionality - General

Functionality in the current context is defined as the capacity of a protein to impart specific desirable attributes to a food product. For example, gel formation is an important

functional property with significant consequences to the final texture of various foods. Solution conditions can be “tuned” to deliver gels with a variety of rheological properties and subsequently a variety of textures, from firm to soft, brittle, mushy, etc [18, 19]. Other important examples of protein functionality include solubilization, thermal stability, emulsification, foam formation and foam stability. The reader is directed to appendix 1, as it thoroughly covers recent advances in modifying and understanding why protein functionality. What now follows is a general overview of foams and some of the key physical properties that make these materials unique.

Foam – Introduction

Foam is a dispersion of gas bubbles within a continuous solid or liquid phase. As a two phase system, foams are thermodynamically unstable, meaning the two phases will completely separate if given enough time; however, the kinetics of foam breakdown can vary tremendously, from seconds for certain liquid foams to years for certain solid foams [20, 21]. Foams are encountered in a range of products and technologies, including personal care products, fire fighting substances, fermentation broths, enhanced oil recovery, and perhaps most commonly as a component of foods [22]. Foamed food products often represent the height of culinary art and include such consumer favorites as meringues, soufflés, cappuccinos, and various confections just to name a few [23]. These products are primarily valued for their unique textures, which are often described with words such as light, delicate, smooth, etc. There have been a number of excellent reviews on foams and a few examples with a food science emphasis include [20, 22, 23] Indeed, appendix 2 of the current document contains several detailed sections relating to

various aspects of food foams. Accordingly, all efforts have been made to reduce redundancies across this document.

Foam – Creation & Stability

There are a variety of means to create foams, and perhaps the most important method in the food industry is whipping [24]. During whipping, blades of various geometries rapidly pass through the pre-foam solution, and in doing so disperse gas bubbles, which are subsequently further reduced in size by the shearing forces of the blades. Another example of aeration is sparging, in which gas is forced through small orifices into the pre-foam solution. This mechanism is perhaps never used in the food industry; however, it is often used in the creation of model foams, as the resulting bubble size distribution is more controllable [22, 25, 26]. Pressure changes during processing can also result in bubble formation, i.e. carbonation in drinks.

An important physical property of foams is the amount of gas which has been incorporated into the structure, or its air phase fraction (ϕ). Note that ϕ can vary theoretically from 0 (no dispersed air) to 1 (completely gas). Foams transition from viscous fluids to semi-solid like structures as ϕ increases from zero above the random close pack volume, $\phi_{rcp}=0.64$ [27]. Above ϕ_{rcp} , the formerly spherical bubbles begin contacting one another, forming so called “polyhedral” or “dry” foams. Note the terms “bubbly” and “polyhedral” will be used in the remainder of this document to differentiate these two foam classes. Bubbly foam stability is primarily a function of Stoke’s law, which states that the creaming velocity (v) of an isolated particle (gas bubble) in a

solution depends on the radius of the particle (r), the viscosity of the continuous phase (η_0), and the difference in density between the particle (ρ) and continuous phase (ρ_0) by the following relationship [21]:

$$v = 2r^2(\rho_0 - \rho)/9\eta_0 \quad (1)$$

It is seen that unless the continuous phase is quite viscous, the bubbles in a bubbly foam rapidly rise and congregate at the top, forming the early stages of the polyhedral foam [28]. While the mechanisms responsible for bubbly foam stability are well understood, the stability of polyhedral foams is more complicated. Many foams encountered in a food setting are polyhedral, hence they will be the primary focus of this review.

There are three primary destabilization mechanisms in liquid foams: disproportionation, drainage and coalescence [21]. Disproportionation is the diffusion of gas from smaller bubbles, through the dispersed phase and into adjacent bubbles of larger size. The driving force for this mechanism is the pressure disparity between bubbles of different sizes as defined mathematically by the Laplace equation:

$$\gamma(1/R_1 + 1/R_2) = \Delta P \quad (2)$$

where γ is the interfacial tension, of which R_1 and R_2 are the two principal radii of curvature and ΔP is the pressure difference [21]. According to equation 2, bubbles of smaller size have higher internal pressures, leading to the driving force for this gas escape. Hence, one means of controlling this form of instability is to create a distribution of bubble sizes that is as uniform as possible. Furthermore, selection of a gas with decreased solubility in the continuous phase will also retard this phenomenon.

Coalescence is the rupture of the thin film separating two gas bubbles, resulting in the

formation of one larger bubble. This is due to thinning of the film, often as a result of gravity induced drainage. Drainage is simply the downward flow of liquid through a polyhedral foam. All three destabilization mechanisms depend heavily on the behavior of the aqueous/gas interface of which there is a tremendous amount in a typical foam, i.e. 10^5 m^2 per m^3 of foam [28]. Accordingly, the properties of the aqueous/gas interface are easily argued to be most important in determining foam properties as a whole. Interfacial properties are in turn dictated by the presence and behavior of surfactants, the subject of a following section.

Foam – Rheology

The most important physical factor governing foam rheology is the volume of gas (ϕ) incorporated into the continuous phase. The rheology of bubbly and polyhedral foams is quite different, as the former behave essentially as viscous suspensions, with viscosity being dependent on the size, compressibility and stability of the bubbles [27]. Above ϕ_{rcp} , polyhedral foams begin taking on more solid-like characteristics. There is an ever developing quantitative framework to describe the various rheological behaviors of polyhedral foams and concentrated emulsions, as the two systems share many similarities [27, 29]. Elastic behaviors occur in these colloidal systems as the dispersed phase particles are repulsive and they can not move freely upon perturbation when $\phi \geq \phi_{\text{rcp}}$. [27, 30]. Due to their repulsive nature the dispersed droplets (bubbles or air) will deform to minimize contacts with their neighbors, and it is the work done against the interfacial tension to create additional droplet surface area, coupled with excluded volume effects, that ultimately gives rise to their elasticity [27]. New experimental techniques that allow

the production of nearly monodisperse concentrated emulsions have conclusively revealed positive correlations between increases in ϕ and the elastic shear modulus (G') [27, 31], in agreement with the theory of this subject [30]. Early theoretical work with polyhedral foams/ concentrated emulsions assumed perfectly ordered structures that were all deformed uniformly [32]. Later work with Monte Carlo simulations assumed disordered structure and “non-affine” motion among particles [33], which is now thought to better represent the actual systems [31]. Indeed, the data produced from this work better agreed with experimentally observed relationships between G' and ϕ .

A notable solid like behavior of polyhedral foams is their exhibition of a yield stress (τ_0), and a simple and effective method has been developed to measure τ_0 of whipped protein foams (considered polyhedral) via vane rheometry [34]. Initial work with this method established that it takes less protein and less whipping time for egg white protein (EWP) to produce foams with significantly improved τ_0 as compared to those prepared from whey protein isolate (WPI) [35]. The following equation, originally derived from theoretical analyses of ideal, two-dimensional foams and partially verified via stable oil/water emulsions, was used to analyze the data [30]:

$$\tau_0 = \frac{\gamma}{R_{32}} \phi^{1/3} Y(\phi) \quad (3)$$

where R_{32} equals the Saunter mean bubble radius, γ is the equilibrium surface tension, and $Y(\phi)$ is an empirically derived function of the system. Experimental measurements of ϕ and γ are readily accessible, whereas experimental determination of R_{32} is more difficult. Confocal microscopy was applied to the previously mentioned EWP and WPI foams to quantify their bubble size distributions; however, essentially no differences were

detected between the two protein foams based on protein type or as a function of whip time [35]. It seems this may reflect a limitation of the method and not an actual physical phenomenon, as Dickinson et al were recently able to qualitatively discern changes in bubble sizes upon whip time for EWP solubilized in water with high contents of invert sugar [24]. Regardless, an important assumption in equation 3 is a monodisperse distribution of bubbles which is unrealistic for whipped protein foams. However, assuming minimal differences in bubble size and applying equation 3 to the initial data for EWP and WPI foams revealed a correlation between τ_0 and $\phi^{1/3}$ within specific protein types and concentrations, where $\phi^{1/3}$ was varied by whip time [35]. Later, work with whey protein solutions (15% w/v protein), including WPI, β -lactoglobulin, α -lactalbumin, and hydrolyzed whey proteins revealed a correlation between τ_0 and $\phi^{1/3}$ [36]. This data is supported with experimental work with concentrated emulsions, in which increases in ϕ (dispersed oil) generate increases in the elastic shear modulus (G') [29, 37]. However, foams are notoriously complicated systems and later work from our group involving WPI did not produced expected results between τ_0 and $\phi^{1/3}$ [38, 39]. There are several explanations for these discrepancies including: 1) As discussed previously, we do not have a method for accurately describing the bubble size distribution as represented by R_{32} in equation 3. 2) Equation 3 is unsuitable for describing the complicated rheology of foams because of its assumptions, including a monodisperse distribution of bubbles, which is impossible for whipped protein foams. 3) Another limitation of equation 3 (and most equations pertaining to polyhedral foam and concentrated emulsion rheology) is that they assume a constant and unchanging interfacial tension throughout the system. This assumption may be more closely

approached for these colloidal systems when stabilized by small molecular weight surfactants, such as sodium dodecyl sulfate. This is because such SMWS's rapidly regain their interfacial equilibrium upon perturbation, i.e. rheological measurements [31].

Within whey protein based foams, recent work suggests a link between the dilatational rheological properties of the air/water interface and foam τ_0 . Specifically, proteins and/or peptides which induce high values of dilatational elasticity (E') and/or a low viscous modulus at a model air/water interface seem to promote high values of τ_0 when used to produce foams [38-40]. However, these direct interfacial and foaming measurements have not been extended to whipped foams prepared from other proteins, specifically egg white protein (EWP), which is the traditional foaming agent of choice in the food industry. Direct foaming comparisons have shown it takes less protein and less whipping times for EWP to produce foams with significantly improved τ_0 as compared to WPI [35]. Yet again, it remains unclear whether there is an interfacial basis for this improvement. This is a primary objective of the research presented in chapter 4 of this document.

Surfactants & Adsorption

There is an excess of free energy at all phase boundaries, with units typically expressed as J/m^2 or N/m [28]. At liquid/liquid or liquid/gas interfaces this free energy is manifest as a two dimensional tension, commonly referred to as the interfacial tension (γ). Note the interfacial tension for pure water and air is ~ 72 mN/m at room temperature. When considering the aqueous/gas interface, the gas phase is more hydrophobic than the

surrounding liquid, which for the vast majority of foamed foods will be water.

Surfactants are amphipathic molecules, i.e. contain both hydrophobic and hydrophilic chemical groups, hence they spontaneously adsorb at phase boundaries [28]. Surfactants are typically dispersed in the continuous phase, and the surfactants' hydrophilic groups allow for their solubilization, whereas their hydrophobic groups promote their attraction with the gas phase [21]. Upon surfactant contact with the interface, structured water molecules that were complexed to both the hydrophobic surfactant groups and the hydrophobic air phase are released, leading to a large entropic gain for the system [41]. Note a similar phenomenon is responsible for the spontaneous folding of globular proteins in aqueous solutions, during which hydrophobic amino acids are concentrated within the interior of the protein. [42]

There are two general types of foam surfactants: 1) small soap-like molecules or small MW surfactants (SMWS) and 2) proteins [43]. In foods, the first class consists of natural molecules such as monoglycerides and lecithin and synthetic molecules such as Tweens and Spans [28]. For protein surfactants, egg white has traditionally been the surfactant of choice in foods; however substitution with other proteins including those derived from bovine whey is becoming more prevalent [44].

The most obvious outcome of adsorption for either surfactant type is a reduction in the interfacial tension. This dynamic process depends on numerous factors including, the concentration of the surfactant solution, the chemistry of the surfactant, the type of interface, temperature, the presence of cosolutes, etc . Eventually an equilibrium

interfacial tension is reached, which for concentrated solutions, will typically be lower for SMWS's than proteins [45]. Maximum equilibrium surface tension values for proteins at the air/water interface commonly approach 45 mN/m, whereas for SMWS's this value is closer to 30 mN/m [43]. Note for proteins the times involved at achieving an equilibrium interfacial tension can be many hours up to a day.

Mathematical models of surfactant adsorption are well suited to explain the behavior of SMWS's, especially at low concentrations. Perhaps the most frequently used adsorption equation is that of Gibbs [46]:

$$\Gamma = -\frac{C}{RT} \frac{d\gamma}{dC} = -\frac{1}{RT} \frac{d\gamma}{d \ln C} \quad (4)$$

where Γ is the surface load (units of mg/m²), C is the bulk surfactant concentration, and R is the universal gas constant. This equation is often suitable for dilute solutions of SMWS's; however, it's inappropriate for more concentrated solutions. Expansions of this model for surfactant adsorption, as well as the application of other models have been very successful for describing/predicting the adsorption of SMWS's under most conditions [46]. However, mathematical models are not yet completely sufficient to describe protein adsorption, due to the complexities of this phenomena. Nonetheless, there is considerable activity in this area, due to importance of protein adsorption in various technologies [47, 48].

The most notable difference in the adsorption of SMWS's and proteins, is that proteins can undergo internal rearrangements at phase boundaries, further exposing their hydrophobic groups to the more hydrophobic phase (air in foams) [49]. SMSW's are

typically much simpler structures and hence undergo little to any rearrangements at the interface. The degree of interfacial protein rearrangement depends on numerous factors, including the intrinsic flexibility of the molecules, solutions conditions, etc. Also note the timescale of these rearrangements is on the order of hours with slight decreases in γ being detectable. In a comparative study of ~ 30 different proteins of various types it was found that the capacity to lower interfacial tension was correlated to the adiabatic compressibility of the proteins, suggesting those molecules with less structure were more effective at lowering interfacial tension [50]. This was in agreement with earlier work suggesting a link between molecular flexibility and the capacity to lower γ [51-53].

The degree of rearrangement also depends on the surface pressure (Π) of the interface, where Π is simply defined as the net reduction in interfacial tension ($\gamma_0 - \gamma$).

If the surface pressure is high, i.e. there are many surfactants already at the interface, a newly arriving protein should unfold to a lesser degree than a protein that adsorbs at an empty interface [54]. This has been directly confirmed for β -Ig adsorbed at the oil/water interface [55]. These researchers utilized Fourier Transform Infrared Spectroscopy (FTIR) to measure secondary structure changes both at the interface and after heating at pH 6.0 and 7.0. Denaturation via heating and adsorption seem to share a similar intermediate, although the induced changes are ultimately different for the two processes. Both processes begin with the loss of β -sheet structure; however, adsorption induces more unordered structure and less intermolecular β -sheet and was generally less profound than heat induced denaturation. Some changes in secondary structure were evident immediately upon adsorption, however changes continued up to 72 hr later. Furthermore,

there were fewer changes in the IR spectra when there was an excess of the protein in the bulk phase, due to reasons discussed previously. Interestingly, β -lg was more readily heat denatured at pH 6.0 and was more deformable at the interface at this lower pH.

Surfactant adsorption is typically described in three different stages: 1) diffusion/convection to the interface, 2) initial adsorption at the interface and 3) rearrangements of adsorbed molecules at the interface [51]. Note the third stage is most important for proteins as discussed earlier. Clearly, the concentration of the surfactant is important, as higher bulk concentrations promotes a more rapid adsorption, due to diffusion considerations. The initial anchoring at the interface is primarily a function of the surfactant's surface hydrophobicity [56, 57]. Indeed, surface hydrophobicity has been correlated with improved foaming properties by a number of independent researchers [20, 25, 58]. Finally, the capacity to rearrange at the interface also results in further decreases in interfacial tension.

Once adsorbed at an interface, proteins are much less likely to desorb than SMWS's. This is because proteins tend to make more contacts with the interface per molecule [59]. The degree of protein desorption depends heavily on the surface pressure of the interface, with lower Π 's promoting less desorption. This is because proteins adsorbing at lower Π 's can potentially rearrange to a greater extent, inducing more contacts with the interface. The reversibility of protein adsorption has been discussed in detail [59]. The adsorbed amount, Γ , of proteins and SMWS's are similar and typically on the order of 2-3 mg/m² [43]. However, the number of molecules adsorbed per interfacial area is

much larger for surfactants, meaning these molecules are able to pack in at the interface more efficiently, primarily due to their smaller size and simpler structures as compared to proteins. Perhaps the most important difference between these two surfactant classes is the different interfacial rheological states that they impart, which is the subject of the next section.

Interfacial Rheology

While the most apparent outcome of surfactant adsorption is the reduction in interfacial tension, the response of a surfactant covered interface to deformation is more relevant to understanding foam functional properties than equilibrium interfacial tension values [60]. This dynamic response depends on the particular chemical properties of a given surfactant(s), and can be quantified experimentally via interfacial rheological measurements. Interfacial rheology has long been thought important to the understanding of foams and many other colloidal systems; hence the scientific literature devoted to this subject is quite extensive [49, 61]. A brief summary of some key aspects of interfacial rheology follows with an emphasis on protein interfacial rheology and more specifically, protein interfacial rheology under dilatational deformations.

There are two primary types of interfacial rheology: 1) shear and 2) dilatation. Shear involves perturbing a 2-dimensional interface in any direction parallel to the interfacial plane [49]. This results in a shape change of the interface but the area perturbed remains constant. Practically, this is often achieved by placing some type of probe, i.e. a DuNouoy Ring, at the interface and then monitoring the encountered resistance as the probe shears the interfacial plane. This is actually a 3-dimensional perturbation with the

third dimension (z-direction) being on the scale of the adsorbed surfactant. Sinusoidal shear oscillations at an interface lying perpendicular to the z-direction result in the shear modulus G and the surface shear viscosity η and are defined by

$$p_{xy} = G e_{xy}, \quad p_{xy} = \eta (de_{xy}/dt) \quad (5)$$

where p_{xy} and e_{xy} are the surface shear stress and strain components, and t is the time [62]. Note these terms are directly analogous in 2-dimensions to the more common 3 dimensional bulk elasticity and viscosity.

Dilatational rheology involves changes in interfacial area while maintaining a constant shape [60]. A linear, viscoelastic modulus can be experimentally determined by subjecting a surfactant covered interface to infinitesimally small periodic compressions and expansions. This modulus (E) was originally defined by Gibbs and is mathematically represented as:

$$E = \frac{d\lambda}{d \ln A} \quad (6)$$

where λ is the interfacial tension and A is the interfacial area [63]. It turns out E contains both an elastic contribution (E') corresponding to the energy recovered in the interfacial perturbation and a viscous component (E'') corresponding to the energy lost to relaxations [60]. The viscoelastic modulus is a complex number, E^* , and the interfacial storage and loss moduli correspond to the real and imaginary components of the Gibbs elasticity:

$$E^* = E' + iE'' = E_d + \omega\eta_d \quad (7)$$

Note that E' is the real part of the complex number and corresponds directly to the elasticity, E_d , whereas the imaginary part, E'' , is equal to the viscous modulus when

multiplied by the angular frequency, ω . Experimentally, E'' is reflected in the phase angle (ϕ), or the lag in stress ($d\gamma$) with the strain (dA), and the elastic and viscous contributions are given as [61]:

$$E' = |E| \cos (\phi) ; E'' = |E| \sin (\phi) \quad (8)$$

where $|E|$ is the absolute value of the complex modulus.

Traditionally, interfacial shear rheology was more popular among experimenters, primarily because it was more accessible than dilatational rheology. The advantages and disadvantages of each approach have been discussed [64]. It generally seems that shear rheology is more sensitive to surfactant-surfactant interfacial interactions, because Γ is essentially constant during these measurements. During the expansions and compressions of dilatational rheology, surfactant adsorption/desorption phenomena complicate interpretation of the results, as these effects must be considered along with surfactant-surfactant interfacial interactions. However, these adsorption/desorption phenomena do occur in actual foams and emulsions; hence it is more realistic to consider their effects. Furthermore, interfacial compressions and expansions are considered much more relevant to understanding the formation and breakdown of foams as compared to shearing deformations, as the magnitude of these forces are much greater than those encountered in shear. Note that in actual foams or emulsions the interfaces would simultaneously experience both shearing and dilatational deformation. Therefore, it is often argued that dilatational rheology is more relevant to understanding the science of foams [63].

There are several common means of measuring interfacial dilatational rheology, with the basic requirements being 1) a means to accurately expand or compress an interface and 2) a means to accurately measure the tension of the interface. Typically, these expansions are periodic in nature, and most often sinusoidal; however, there are means of measuring dilatational interfacial rheology under continuous expansion, with a notable example being the overflowing cylinder method described by Prins [65]. The classic means of measuring periodic interfacial expansions and compressions is the implementation of a trough (Langmuir) with a moveable barrier that generates the deformations, and a probe (often a Wilhelmy Plate) at some distance from the barrier to measure the change in tension. These methods are described in more detail by [49].

A more recent and ever increasingly popular instrument for measuring interfacial dilatational rheology is the modified drop tensiometer [46, 66, 67]. The basic premise of these instruments is the application of the Young-Laplace equation of capillarity to determine the interfacial tension of a drop (or bubble) dangling from a capillary (vertical or inverted) by accurately determining the shape of the drop [67]. Drop or bubble images are captured via a digital camera and automatically analyzed via a computer to back out the interfacial tension. The interfacial area of the drop or bubble can be compressed or expanded via a stepper motor and the subsequent change in interfacial tension can be monitored during these area changes. From these changes in γ during changes in A , dilatational rheological constants can be calculated. The details for this method will not be discussed here, but the interested reader is directed to the following references [66,

67]. The basic approach is also outlined in the methods section of chapter's 1, 2 and 3 of the current document.

There is an abundance of data relating to the adsorption and rheological properties of proteins at model interfaces, with an ever increasing amount of data being reported [45, 49, 64]. This reflects both the importance of these phenomena to numerous fields, including food science, and the growing commercial availability of instruments devoted to studying interfacial phenomena, with the previously mentioned drop and bubble tensiometers being a notable example. Chapters 1, 2, and 3, as well as appendices 1 & 2 all review recent advance in protein interfacial rheology, hence this subject will not be covered here.

Peptides at Interfaces

Investigations into the behavior of peptides are an active area of research due to their technological importance in a number of fields including food science. A particular interest seems to be in the capacity of these molecules to self assemble at phase boundaries, with various nanotechnological applications in mind [68].

The adsorption of β -casein (β -CN) and several distinct peptides derived from this molecule were investigated for secondary structure changes at a Teflon/water interface using far-ultraviolet CD [69]. Unordered random coil was primarily detected in solution for all species investigated; however, upon adsorption, secondary structure, primarily α -helix, was detected. An approximately 28 amino acid long residue consisting of the highly charged N terminal region of β -CN was more apt to form α -helix upon adsorption

as compared to the more hydrophobic portions of the molecule found at its C terminal end. Higher surface loads were detected for the highly hydrophobic C terminal peptides. Correlations were not found between secondary structure at the interface and foaming properties; however, higher surface loads did seem to correlate with improved foaming properties. Secondary structure induction was also found to be sensitive to both pH and ionic strength, with secondary structure increasing at lower pH levels. This was attributed potentially to a reduction in the charge of the protein/peptides as they contain higher amounts of negatively charged amino acids.

There have also been investigations into the interfacial behaviors of synthesized peptides. An example is the application of dynamic interfacial tension measurements to monitor adsorption rates of 2 distinct synthesized peptides: Lac21 and Lac28 at the octane/water interface [70]. Both peptides were helical; however, Lac21 primarily exists as a monomer in solution, whereas Lac28 primarily exists as a tetramer. These measurements showed that Lac21 adsorption was much more rapid than Lac28, due to the tetrameric association of Lac28. Furthermore, the adsorption of Lac28 is shown to be much slower than would be expected if limited by diffusion. The energy barrier for Lac28 adsorption is estimated and found comparable to the expected free energy barrier for tetramer dissociation.

Protein/Sugar Interactions in Foams & Interfaces

Foamed food products often contain high contents of sugars and proteins. An understanding of the behaviors of these compounds both within solution and at the

air/water interface is desired to better predict/control bulk foaming properties. Sugars have been long observed to increase the denaturation temperature of proteins dissolved in aqueous solutions. It has been shown that the enthalpy of unfolding is minimally affected by the presence of sucrose; rather the activation energy to this unfolding event is increased [71]. The suggested mechanism for this stabilization is that sugar molecules are "preferentially excluded" from the surface of proteins. This leads to an unfavorable thermodynamic situation, meaning the unfolding of the proteins, which increases the protein surface area, leads to an even more thermodynamically unfavorable situation and is hence restricted [71]. This stabilization effect has been shown for a range of proteins, including β -lg [72]. The addition of 10% sucrose decreased foam overrun of whipped WPI solutions and improved foam stability against drainage [73]. Overrun of whipped solutions of isolated β -lg was also found to decrease upon sugar addition, whereas the drainage rate slightly increase. Surprisingly, the specific viscosity of the purified β -lg solution increased upon sucrose addition. Drainage stability of WPI foams increased upon sucrose addition, although no specific viscosity data was provided for WPI [73].

The addition of up to 20% lactose increased the foam stability (as assessed in a specialized sealed column apparatus) of WPI foams, whereas foamability was minimally affected upon lactose addition at all concentrations [74]. Corresponding increases in specific viscosity upon lactose addition seemed to explain this increase in foam stability. The addition of 12.8% powdered sugar (typical for a confection application) was found to increase foam stability (assessed by drainage rates) of whipped WPI solutions [38]. Powdered sugar addition had differing affects on foam drainage as the ratio of

polymerized WPI (soluble, covalently linked aggregates of whey protein) to native WPI was increased, such that there was no significant ($P \leq 0.05$) effect for the 50/50 native to polymerized WPI foam, and foam drainage decreased for the 100% pWPI foam. The apparent viscosity of the various native WPI/pWPI mixtures reflected these changes in drainage, further suggesting that the viscosity of the continuous phase strongly influences foam drainage rates. Indeed, an association with solution viscosity and foam drainage stability has been noted for some time [20].

Pepsin enzymatic activity after foaming and recovery was better preserved in the presence of 0.5-M trehalose [75]. Equivalent additions of this sugar also decreased the detected denaturation after foaming and recovery of the protein IgG. This again, suggested the sugar was limiting the denaturation of proteins at the air/water interface. Trehalose, when added to WPI prior to spray drying, improved the foaming properties of this protein ingredient [76].

Less work has been done at measuring protein interfacial properties of proteins in the presence of sugars. The short-time adsorption rates of BSA were found to increase in the presence of increasing concentrations (1 M) of sucrose [77]. A potential explanation was that the protein molecule would be more compact in sugar solutions, due to preferential hydration, and hence adsorb more rapidly. It was also noted that the increased viscosity of concentration sugar solutions should limit diffusion to the interface, meaning protein adsorption in sugar solutions would be a balance of these two phenomena.

In contrast, the adsorption of ovalbumin (0.001 %) was found to decrease in the presence of sucrose, with the maximum reduction occurring at 0.025 % sucrose, while increasing concentrations of sucrose, up to 0.05 %, restored the adsorption pattern to more closely resemble control ovalbumin [78]. This sucrose effect was more dominant near the isoelectric point of ovalbumin. Light scattering data suggested that ovalbumin at pH 7.0 had a greater thermodynamic affinity for a 25% sucrose solution than buffer alone. Light scattering data also suggested the presence of 25% sucrose increased the aggregation of ovalbumin at pH 5.5, which is near the molecule's pI. Mixing calorimetry data suggested that ovalbumin participated in hydrogen bonding with the sucrose molecule, which increases its hydrophilicity and decreases its surface activity. Equivalent additions of sucrose were found to increase the surface activity of the micellar sodium caseinate. The data suggested the sugar aids in the dissociation of the micelles, promoting more adsorption.

Impetus for Further Study

A review of the literature prior to beginning this dissertation revealed a fair amount of work in the area of protein-foaming functionality. Generally two types of approaches were evident: 1) Studies where actual foams were made on the bench top using a relatively high concentration ($\geq 5\%$ w/v) of a commercially available protein ingredient. Common measurements included foam overrun and drainage stability. 2) Studies at model air/water interfaces using very dilute protein solutions ($\leq 0.01\%$ w/v) and typically using an isolated protein species. Typical measurements include measuring changes in interfacial tension, changes in surface load, interfacial rheological measurements, etc. Both approaches have their advantages and disadvantages. Clearly, with the 1st approach,

one can directly evaluate whether or not a protein ingredient is effective as a foaming agent under a given set of conditions. However, the mechanisms for improvements in foaming functionality typically aren't readily evident from such studies. In the 2nd approach, a great deal has been learned about the kinetics and thermodynamics of protein adsorption, and this data has spawned numerous mathematical models to describe these phenomena. However, the practical implication of this work as it relates to actual food foams is often lost, again, as these studies typically involve very dilute solutions (to slow protein adsorption sufficiently to allow measurement) of isolated proteins. Accordingly, it was hoped with the current work to combine these two approaches by taking both foaming measurements and interfacial measurements with the *same* material. Furthermore, a deliberate effort was made to make interfacial measurements at concentrations approaching those actually found in food foams. Finally, an emphasis was placed on understanding foam yield stress due to its practical significance in the food industry (as discussed earlier) coupled with the fact, that there was far less information regarding the rheology of foams, as compared to studies examining either overrun or stability.

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CHAPTER 1.

Electrostatic Effects on the Yield Stress of Whey Protein Isolate Foams

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Electrostatic effects on the yield stress of whey protein isolate foams

J.P. Davis^a, E.A. Foegeding^{a,*}, F.K. Hansen^b

^a Department of Food Science, North Carolina State University, Box 7624, Raleigh, NC 27695-7624, USA

^b Department of Chemistry, University of Oslo, N-0315 Oslo, Norway

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Abstract

The mechanisms responsible for foam structure are of practical interest within the food industry. The yield stress (τ) of whey protein isolate (WPI) foams as affected by electrostatic forces was investigated by whipping 10% (w/v) protein solutions prepared over a range of pH levels and salt concentrations. Measurements of foam overrun and model WPI interfaces, i.e. adsorption kinetics as determined via dynamic surface tension and dilatational rheological characterization, aided data interpretation. Interfacial measurements were also made with the primary whey proteins, β -lactoglobulin (β -lg) and α -lactalbumin (α -la). Yield stress of WPI foams was dependent on pH, salt type and salt concentration. In the absence of salt, τ was highest at pH 5.0 and lowest at pH 3.0. The addition of NaCl and CaCl₂ up to 400 mM significantly increased τ at pH 7.0 but not at pH 3.0. Furthermore, at pH 7.0, equivalent molar concentrations of CaCl₂ as compared to NaCl increased τ to greater extents. Salts had minimal effects on τ at pH 5.0. Comparisons with interfacial rheological data suggested the protein's capacity to contribute towards τ was related to the protein's potential at forming strong, elastic interfaces throughout the structure. The dynamic surface tension data for β -lg and α -la were similar to WPI, while the interfacial rheological data displayed several noticeable differences.

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1. Introduction

Foams prepared from protein solutions function as structuring materials in many food products, such as angel food cake, confections and whipped toppings. Frequently these foams are further processed, i.e. heating, mixing, etc., meaning that structurally robust foams are often desirable. As such, the rheological characterization of foams and the mechanisms responsible for these structures are of practical interest within the food industry.

Protein-based liquid foams have been extensively studied for many years because of their importance in numerous fields including food science, with the majority of these investigations having focused on foam formation and stabilization mechanisms [1,2]. A significant amount of data has also been collected on the interfacial properties of some common food proteins (whey, egg white and soy primarily), leading to important insights on how these properties, such

as surface pressure, surface concentration and interfacial rheology contribute towards foam formation and stability [3–5]. The rheological characterization of foams has received far less attention and this seems partially attributable to the inherent difficulty of these studies [6]. Traditional rheometer geometries such as the parallel plate, or cup and bob systems are problematic for foams due to wall slip and sample destruction [6]. A viable alternative is the vane geometry, which overcomes the problem of wall-slip by placing the shear surface within the material being tested [7].

Vane-based methods have been successfully applied to characterize a variety of non-Newtonian fluids that would otherwise exhibit significant slip effects at smooth walls [7]. Accordingly, a method has been developed for measuring the yield stress (τ) of protein-based liquid foams via vane rheometry [8]. These researchers examined the effects of whip time and protein concentration on τ of foams consisting of either egg white protein (EWP) or whey protein isolate (WPI). EWP foams had significantly higher values of τ at equivalent protein concentrations and whip times; however, the reasons behind these differences were not determined.

* Corresponding author. Tel.: +1-919-513-2244;

fax: +1-919-515-7124.

E-mail address: allen.foegeding@ncsu.edu (E.A. Foegeding).

Factors contributing to the rheological behavior of liquid foams are not completely clear. Below an air phase volume (ϕ) of approximately 70%, bubbles maintain their spherical shape and the foam essentially behaves as a viscous fluid [6]. At higher ϕ 's, the bubbles pack together, forming compressed polyhedra, and these so called polyhedral foams display solid-like behaviors, including a yield stress [6]. Most theoretical models addressing polyhedral foam rheology predict that interfacial tension, air phase volume and bubble size are the dominant variables [6]. These three parameters were directly and indirectly measured for EWP and WPI foams to better understand the large discrepancy in τ between these two systems [9]. The data was evaluated according to the following model which predicts τ of monodisperse, polyhedral foams [10]:

$$\tau = \frac{\gamma}{R_{32}} \phi^{1/3} Y(\phi) \quad (1)$$

where γ is the surface tension, R_{32} the Sauter mean bubble radius (≈ 3 times the volume per surface area; $3V/S$) and $Y(\phi)$ an empirically derived function of the system. While knowingly oversimplified for real foams, aspects of this model have been experimentally verified using concentrated emulsions as experimental systems [10]. In contrast to Eq. (1) predictions, EWP foams had yield stress values that were significantly greater, despite having similar ϕ 's, bubble sizes, and lower values of γ . It was suggested that a protein's capacity to contribute towards a foam's yield stress was related to factors other than the equilibrium surface tension it induced.

Most theoretical models pertaining to foam rheology assume an unchanging surface tension that is constant throughout the foam. However, actual foam interfaces undergo rapid fluctuations in γ which are expected to significantly influence foam rheology [11]. This dynamic behavior is reflected in the dilatational modulus, ε , which can be experimentally determined via small, periodic compressions/expansions of the interface, and is mathematically represented as [12]:

$$\varepsilon = \frac{d\gamma}{d \ln A} \quad (2)$$

where A is the interfacial area. The term dilatational modulus implies that this magnitude contains both a pure elastic component, as symbolized by the Gibbs' elasticity, E , and a viscous component, often denoted by the surface dilatational viscosity, η_d . The dilatational modulus may in this case be equated to the complex surface elasticity, E^* . There is a limited amount of theoretical work which suggests a significant influence from dilatational interfacial rheology on foam rheology; however, there is little (if any) experimental evidence available to relate these effects [11].

Electrostatic forces among whey proteins (all proteins) significantly influence both bulk foaming properties, such as overrun and stability [13–16], and interfacial properties, such as adsorption rates and interfacial rheology [17–19]. It has been found that the yield stress of 15% protein (WPI) foams

was significantly increased by the addition of either 0.4 M NaCl or CaCl₂ at pH 7.0 [20]. However, no physical basis was noted that could explain this difference. The current study was designed to systematically investigate electrostatic effects on τ of WPI foams. Special interest was given to the dilatational interfacial rheological behavior of the proteins as related to foam yield stress.

2. Materials

Calcium chloride dihydrate (SigmaUltra, minimum 99.0%) was purchased from Sigma Chemical Co. (St. Louis, MO). Certified A.C.S. sodium chloride and sodium sulfate were purchased from Fisher Scientific (Fair Lawn, New Jersey). WPI (BiPro, 94.6% protein, lot JE 111-9-420) and α -la (BioPURE, lot JE 001-2-921) were supplied by Davisco Foods International (Le Sueur, MN). The protein content in a typical lot of BiPro is 66% β -lg, 22% α -la and 4% bovine serum albumin as reported by the manufacturer. The α -la sample was greater than 90% purity as determined by capillary electrophoresis. Mineral analysis revealed the molar ratio of calcium to α -lactalbumin was ~ 0.2 . Two lots (101K7031 and 052K7017) of high purity (approx. 90%) β -lg was purchased from Sigma Chemical Co (product # L-3908). Lot 101K7031 was used for all measurements except for the mixed layers plot (Fig. 5). Deionized water was obtained using a Dracor Water Systems (Durham, NC) purification system. The resistivity was a minimum of 17.2 M Ω cm.

3. Methods

3.1. Foaming solution preparation

WPI foaming solutions (250 ml) were prepared from stock solutions of 2 M NaCl and CaCl₂, and 15% (w/v) protein as such: (1) Deionized water was added to a beaker with magnetic stirrer such that ~ 30 ml would remain for pH adjustment. (2) The appropriate amount of salt stock was added and equilibrated for a minimum of 5 min. (3) The appropriate amount of protein stock was added and equilibrated for a minimum of 30 min. (4) The pH was adjusted to 3.0, 5.0 or 7.0 using 1–2 M NaOH or HCl. (5) The volume was adjusted to 250 ml. The final protein concentration for each solution was 10% (w/v). Solutions were stored overnight at 4 °C. The next day, solutions were equilibrated at room temperature for a minimum of 1 h under mild stirring, prior to foam generation. Ionic strength of the solutions was calculated as follows:

$$m = \frac{1}{2} \sum C_i Z_i^2 \quad (3)$$

where C_i represents molar concentrations of the various ions in solution and Z_i represents their charges.

3.2. Foam generation

A Kitchen Aid Ultra Power Mixer (Kitchen Aid, St. Joseph's, MI) with a 4.3 L stationary bowl and rotating beaters was used for foam formation. Solutions (225 ml) were whipped at speed setting 8 (planetary rpm of 225 and beater rpm of 737) for 20 min.

3.3. Yield stress measurements

Yield stress was measured using vane rheometry [8]. A Brookfield 25xLVTDV-ICP (Brookfield Engineering Laboratories, Inc. Middleboro, MA) viscometer was used at a speed of 0.3 rpm. After whipping, the beaters were gently removed from the foam prior to yield stress measurement. The vane (10 mm diameter and 40 mm length) was lowered into the foam until the foam surface was level with the top of the vane. The viscometer was set to zero and rotation was started within 4 min of foam formation. Maximum torque response (M_0) was recorded for each of three measurements taken per foam. Torque readings were used to calculate yield stress values according to the methods of [21–23]:

$$\tau = \frac{M_0}{[(h/d) + (1/6)](\pi d^3/2)} \quad (4)$$

where τ is the yield stress, and h and d the height and diameter of the vane. The average of three measurements per treatment served as one data point. Each treatment (specific pH and salt concentration) was replicated a minimum of three times. Yield stress generally decreased over progressive measurements within the same treatment, suggesting that factors contributing to this measurement were not completely stable over the measurement time (4 min maximum) [8].

3.4. Overrun

Overrun measurements were begun immediately after the final yield stress measurement. Foam was gently scooped from the bowl in a circular pattern with a rubber spatula, filling a standard weigh boat (100 ml) 4 times. The mean value was used to calculate overrun and air phase fraction according to [24]:

$$\% \text{Overrun} = \frac{\text{wt. 100 ml solution} - \text{wt. 100 ml foam}}{\text{wt. 100 ml foam}} \times 100 \quad (5)$$

$$\text{Air phase fraction } (\phi) = \frac{\% \text{overrun}}{\% \text{overrun} + 100} \quad (6)$$

Each treatment (specific pH and salt concentration) was replicated a minimum of three times to determine the average overrun.

3.4.1. Interfacial measurements—solution preparation

All glassware and magnets used for interfacial measurements were cleaned in a solution of concentrated sulfuric acid and nochromix® cleaning solution for at least 12 h, followed by a thorough rinsing with deionized water. The various salt stock solutions were prepared at a concentration of 1 M. WPI and β -lg stock solutions were exhaustively dialyzed (MW cut off, 6000–8000 Da) against deionized water at 4 °C to remove residual ions. Stock solutions of α -la were not dialyzed; however, the CaCl_2 to α -la ratio was established (see methods), which is known to significantly affect the adsorption behavior of α -la [25]. Protein concentration was determined spectrophotometrically (Shimadzu UV160U) at 280 nm using an experimentally determined extinction coefficient for WPI and extinction coefficients of 0.96 and $1.951 \text{ g}^{-1} \text{ cm}^{-1}$ for β -lg [26] and α -la, respectively [27]. A charcoal treatment for removing residual fatty acids from protein as described by [28] was not applied, since residual fatty acids are expected to be present in most commercial whey protein ingredients.

Interfacial treatments (10 ml) were prepared from the above stock solutions as such: (1) Deionized water was added to a beaker with magnetic stirrer such that ~0.5 ml would remain for pH adjustment. (2) The appropriate amount of salt stock was added and equilibrated for a minimum of 5 min. (3) The appropriate amount of protein stock was added and equilibrated for a minimum of 20 min. (4) The pH was adjusted to 3.0, 5.0 or 7.0 as needed, using 1 to 100 mN NaOH or HCl. (5) The volume of each solution was adjusted to 10 ml. The pH after volume adjustment never differed more than 0.03 units from the desired value.

3.4.2. Interfacial measurements—methodology

Methods that determine the dynamic interfacial tension of adsorbing/desorbing surfactants via the shape analysis of drops and bubbles are well-established [29,30]. A commercially available automated contact angle goniometer (Rame-Hart Inc., Mountain Lakes, NJ) was used for data collection. The DROPimage computer program [31] was used to control the instrument, measurement sequences and to perform the calculations. The surface tension of a 16 μ l capillary drop was monitored for a total of 5 min with a 1 s resolution. The first measurement was recorded immediately after drop formation (≤ 0.5 s). The drop volume was kept constant over this 5 min period by a feedback system provided in the program, minimizing evaporation effects.

Modifications of drop shape analysis methods that allow for dilatational rheological characterization are also well-established [12,32,31]. After aging for 5 min (see above), oscillation was begun with a volume amplitude of 1 μ l. The minimum volume increment provided by the syringe was 0.0167 μ l and the pump timing was adjusted according to the desired frequency. Each measurement was taken over five periods while collecting 12 data points per period. The measured surface tension response during a surface area change was used to determine the dilatational

modulus. From this modulus and from the phase angle between the surface area change and surface tension response, the DROPimage software calculates E' and E'' , which are equivalent to and proportional to the elastic and viscous components of the interface, respectively. The detailed procedure for these calculations has been described elsewhere [31].

3.5. Density determination

The densities of the component phases are required inputs for the determination of interfacial tension from the shape analysis of drops and bubbles [33]. Accordingly, a Mettler-Toledo DE 40 density meter equipped with a viscosity correction card was used to determine the density of each solution at 23 °C. The accuracy of the instrument was $1 \times 10^{-4} \text{ g/cm}^3$ and every solution was measured in duplicate and averaged prior to interfacial measurements.

3.6. Data analysis

The overrun and yield stress data were analyzed by analysis of variance using the general linear model procedure of the SAS statistical software package (Version 8.2, SAS Institute, Inc., Cary, NC). An LSD t -test was used to compare means. Significance of difference was established at $P \leq 0.05$.

4. Results

Yield stress and overrun measurements are summarized in Fig. 1. In the absence of salts, τ was lowest at pH 3.0 (~40 Pa), while at pH 5.0 and 7.0, τ was significantly higher with approximate values of 60 and 55 Pa, respectively. No concentration of either salt significantly affected τ at pH 3.0. Increasing salt concentrations progressively increased τ at pH 7.0, with CaCl_2 increasing this magnitude more effectively than equivalent molar concentrations of NaCl. For example, 0.4 M NaCl increased τ approximately 70%, while 0.4 M CaCl_2 increased τ almost 100%. It is noted that μ of a CaCl_2 solution is three times that of an equivalent molar concentration of NaCl. At pH 5.0, the only salt concentration significantly increasing τ was 0.4 M CaCl_2 .

In the absence of salts, overrun was highest at pH 3.0, intermediate at pH 7.0 and lowest at pH 5.0, with approximate values of 1200% ($\phi = 0.92$), 1050% ($\phi = 0.91$) and 900% ($\phi = 0.90$), respectively. The effects of NaCl were minimal on foam overrun. Most notable was at pH 3.0, where 0.4 M NaCl significantly decreased (~35%) this property. Increasing concentrations of CaCl_2 decreased foam overrun at pH 3.0, while the effects of this salt at pH 5.0 and 7.0 were minimal.

The interfacial behavior of WPI (0.184% (w/v) protein) as affected by select electrostatic treatments was also investigated. The kinetics of WPI adsorption at the air/water

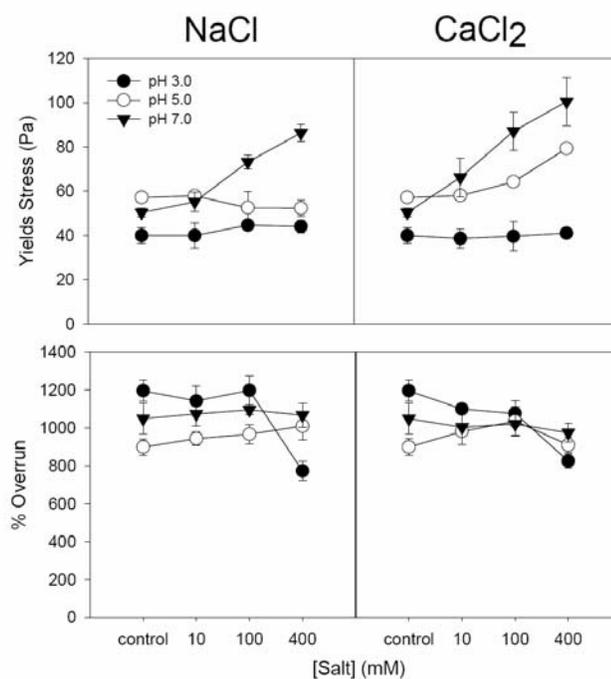


Fig. 1. Yield stress and overrun values of WPI foams as affected by pH and/or NaCl and CaCl_2 . (●) pH 3.0, (○) pH 5.0, (▼) pH 7.0. Error bars are standard deviations of mean values.

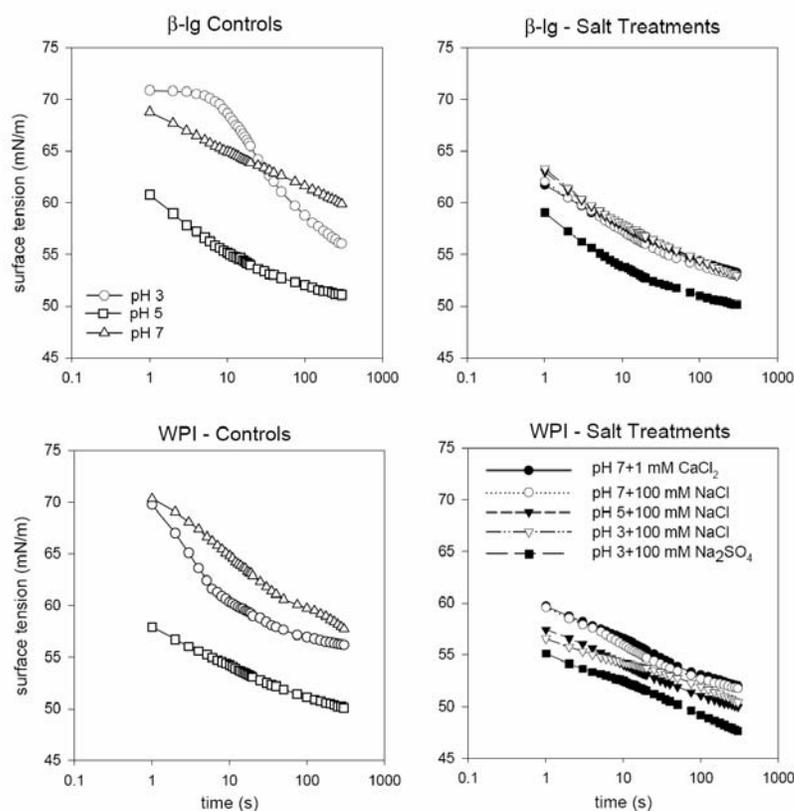


Fig. 2. Typical dynamic surface tension measurements of β -Ig and WPI as affected by salts at different pH levels. Controls: (○) pH 3.0, (□) pH 5.0, (▽) pH 7.0. Salts: (○) pH 7.0 + 100 mM NaCl, (●) pH 7.0 + 1 mM CaCl_2 , (▼) pH 5.0 + 100 mM NaCl, (▽) pH 3 + 100 mM NaCl, (■) pH 3.0 + 100 mM Na_2SO_4 .

interface were indirectly monitored via dynamic surface tension measurements of freshly formed pendant drops (Fig. 2). Surface tension decline was notably most rapid at pH 5.0 in the absence of salts. In fact, the initial decline in γ happened so quickly at pH 5.0, it was beyond the resolution of this technique (≤ 0.5 s). Salt addition at pH 3.0 or 7.0 increased the rate of γ decline, whereas salt addition minimally affected this parameter at pH 5.0 (Fig. 2). Dynamic surface tension responses were basically indistinguishable at pH 3.0, 5.0 or 7.0 in the presence of 100 mM NaCl (Fig. 2). No treatment had reached an equilibrium surface tension by 5 min, at which time interfacial rheological measurements were begun.

The bulk of the interfacial rheological data was collected at 0.04 Hz as treatment effects were initially found to be well differentiated and repeatable at this frequency. (The frequency dependence of select samples will be discussed later.) Interfacial elasticity slightly increased over time for most treatments, while the viscous response of the interface, which is proportional to E'' , was stable over time for

most treatments. This is illustrated in Fig. 3, which compares E' and E'' for the WPI controls (no salt). The average surface tension declined approximately 2 mN/m for most treatments over the entire measurement time of 625 s (data not shown). E' was greater than E'' for all treatments and therefore the dominant contributor to ϵ , which is considered a measure of the total viscoelastic resistance of the interface to dilatational deformations. Moreover, E' differentiated treatment effects more effectively than E'' , although treatment differences generally coincided for both E' and E'' .

Fig. 4 summarizes the interfacial rheological data for WPI (and pure β -Ig) treatments at the three different pH values with and without salt. Differences in E' at similar surface pressures ($\Pi = \gamma_0 - \gamma$, where γ_0 is the surface tension of the solvent, and γ is the surface tension of the solvent upon surfactant adsorption) indicate differences in solubility at the interfacial layer [34], and this will be discussed in more detail later. In the absence of salt, WPI interfacial elasticity was greatest at pH 5.0, somewhat lower at pH 7.0 and very

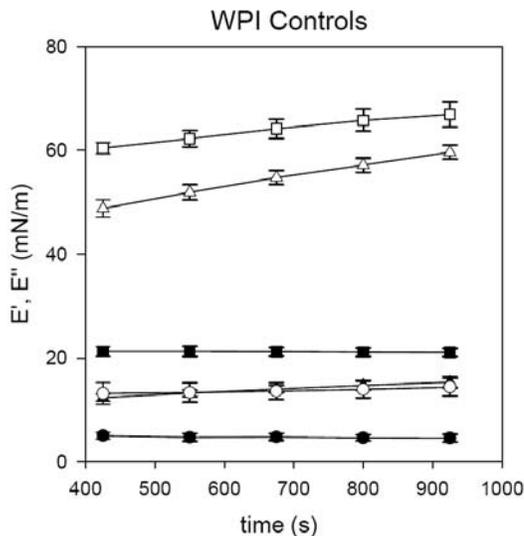


Fig. 3. Time dependence of dilatational interfacial rheological measurements for WPI in the absence of salts. Frequency was 0.04 Hz, all samples aged 5 min prior to first measurement. Open symbols are E' , closed symbols are E'' . (○) pH 3.0, (□) pH 5.0, (△) pH 7.0. Error bars are standard deviations of mean values.

low (~ 15 mN/m) at pH 3.0 (Fig. 4A). Furthermore, Π was significantly greater at pH 5.0 (Fig. 4A). The addition of 100 mM NaCl at pH 7.0 increased E' over the pH 5.0 control. The equivalent salt addition at pH 3.0 increased both Π and E' as compared to the pH 3.0 control; however, E' of this sample was still noticeably lower than any sample at pH 5.0 or 7.0. In fact, the addition of 100 mM NaCl, CaCl₂ or Na₂SO₄ at pH 3.0 all increased E' to similar magnitudes as compared to the pH 3.0 control, even if the Π values were slightly different. Smaller amounts of CaCl₂ (10 mM) had the same effect on E' as 100 mM NaCl at pH 7.0. Note that salt addition increased Π as compared to the controls for all samples. E'' values were generally more similar among treatments than E' values (Fig. 4A and B). However, it can be seen that E'' of all treatments at pH 3.0 were lower than treatments at pH 5.0 or 7.0.

Figs. 2 and 4 also provide a direct comparison between the interfacial behavior of WPI and pure β -lg at an equivalent protein concentration. Trends in dynamic surface tension were similar for β -lg to those observed for WPI, i.e. an increased rate of surface tension decline at pH 5.0 or upon the addition of salt at pH 3.0 or 7.0 (Fig. 2). Most noticeable in the rheological data was the shift to significantly higher values of elasticity for all treatments with pure β -lg when compared to WPI. Furthermore, unlike WPI, the elasticity of β -lg interfacial layers at pH 7.0 never surpassed the pH 5.0 control, regardless of the salt concentration. Finally, the difference in elasticity at pH 3.0 or 7.0 for the β -lg controls was negligible, whereas for WPI, elasticity of the pH 3.0

control was noticeably lower than the pH 7.0 control (Fig. 4A and C).

Fig. 5 compares the interfacial rheological behavior of WPI, β -lg and α -la (the second most prevalent protein in WPI). The surface tension/time dependence of α -la in the absence of salt closely resembled that of WPI and/or β -lg, and is not shown, as the most interesting results were the final values of surface pressure. Both E' and E'' values were much lower for α -la than those of β -lg, while the data for WPI generally fell in between the pure proteins.

5. Discussion

Fig. 1 provides a direct comparison between the yield stress and overrun of WPI foams. Eq. (1) predicts that τ will increase proportionally with $\phi^{1/3}$ and this relationship has been verified in similar foaming systems [20,9]. However, this dependence was clearly not observed in the present work. This is well illustrated at pH 3.0, where overrun, which is directly proportional to ϕ , was generally greatest and τ generally lowest (Fig. 1). Overrun of whipped WPI foam (5% (w/w) protein) was previously reported to be greatest at pH 5.0, minimum at pH 4.0 and intermediate at pH 7.0 [13]. While this data is different than the current results, a possible explanation lies in the different protein concentrations and methods of overrun measurement between the two studies.

It has been estimated that for whipped foam, approximately 10^5 m² of surface area is created per m³ of foaming solution [35]. Therefore, if one assumes a surface concentration (Γ) of roughly 3 mg/m², a bulk concentration of 1 mg/ml would be more than sufficient to supply this surface area. For most protein foaming applications including this one, the bulk protein concentration is at least an order of magnitude greater, meaning there is a substantial surplus of unadsorbed protein remaining in solution after foam formation. However, the protein concentration in model interfacial systems is often quite dilute, such that most of the protein is adsorbed, or the protein is spread at the interface to create monolayers. While these approaches have several advantages [36], it may be beneficial to study a model protein system at concentrations such that the adsorbed protein structure and potential adsorption/desorption mechanisms more closely mimic actual food foams. The interfacial concentration of β -lg rapidly approaches saturation (~ 2 mg/m²) at bulk concentrations greater than 1 mg/ml [37]. Therefore, the protein concentration selected for interfacial measurements was 0.184%, which is almost twice this critical concentration and equates to 10^{-4} M of β -lg.

Surface tension decline essentially results from two processes: (1) new proteins inserting at the interface, i.e. an increase in Γ and (2) rearrangements of adsorbed proteins which further position their hydrophobic portions in the hydrophobic air [35]. These rearrangement processes are relatively slow compared to the first process and decrease with

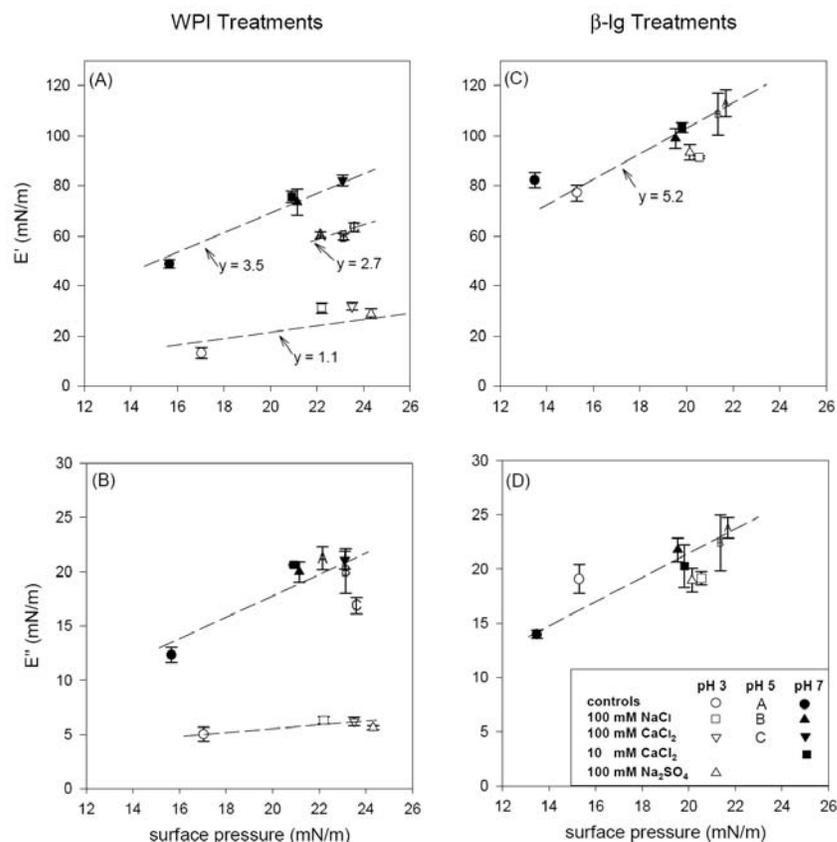


Fig. 4. Relationship of E' and E'' to Π for β -Ilg and WPI interfaces as affected by salts at different pH levels. Frequency was 0.04 Hz, all samples aged 5 min (○) pH 3.0, (□) pH 3.0 + 100 mM NaCl, (△) pH 3.0 + 100 mM Na_2SO_4 , (▽) pH 3.0 + 100 mM CaCl_2 , (A) pH 5, (B) pH 5 + 100 mM NaCl, (C) pH 5 + 100 mM CaCl_2 , (●) pH 7.0, (■) pH 7.0 + 10 mM CaCl_2 , (▲) pH 7.0 + 100 mM NaCl, (▼) pH 7.0 + 100 mM CaCl_2 . Error bars are standard deviations of mean values.

increasing surface pressure [38]. Therefore, development of Π for all treatments was suspected to be primarily a function of increased Γ , as protein rearrangements were expected to be minimal due to (1) the relatively short measurement time employed in this study and (2) the relatively rapid Π development observed for all treatments.

The very rapid surface tension decline observed for WPI at pH 5.0 was expected (Fig. 2), since all the major whey proteins have pI's near 5.0 [39]. The protein's net charge is neutral at this pH, which facilitates adsorption to the air/water interface due to a reduction in electrostatic repulsion [4]. The proteins carry net positive and net negative charges at pH 3.0 and 7.0, respectively, thus electrostatic repulsion is expected to be more prominent during adsorption, which explains the reduced rates of surface tension decline at these pH's as compared to pH 5.0. Salt addition at pH 3.0 or 7.0 increased the rate of surface tension decline for WPI, and this likely resulted from the free ions screening the charged

proteins that were concentrating at the interface, thus reducing interfacial repulsion (Fig. 2) [40].

The trends in surface tension decline were similar for pure β -Ilg when compared to WPI (Fig. 2). Note the initial period in which surface tension remained virtually constant for β -Ilg at pH 3.0. This phenomenon was consistently observed and usually lasted 6–8 s prior to a very rapid surface tension decline that surpassed the pH 7.0 control. It appears Γ_0 (the minimal surface coverage necessary to induce γ decline) was not immediately realized at pH 3.0. This observation may be explained by the net charge of β -Ilg at pH 3.0, which is twice the charge at pH 7.0 (+20 versus -8, calculated from amino acid sequence). Interfacial repulsion is therefore expected to be more substantial for the acidified protein, which seems to explain this brief time lag prior to surface tension decline. However, the relative hydrophobicity of β -Ilg (determined chromatographically) reportedly increases ~200 fold with a pH shift from 7.0 to 3.0 [41] and this seems to explain the

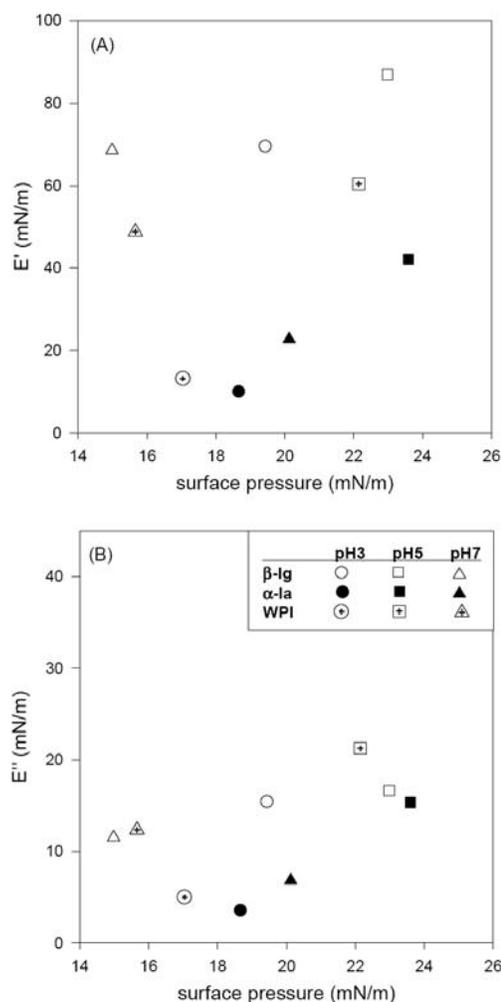


Fig. 5. Relationship of E' and E'' to Π for β -lg, α -la, and WPI at different pH levels in the absence of salt. Frequency was 0.04 Hz, all samples aged 5 min (○) β -lg-pH 3.0, (□) β -lg-pH 5.0, (△) β -lg-pH 7.0, (●) α -la-pH 3.0, (■) α -la-pH 5.0, (▲) α -la-pH 7.0, (See figure for WPI symbols).

rapid surface tension decline induced by the acidic protein. That is, anchoring at the interface is hindered by increased electrostatic repulsion, but once anchored, the acidified protein decreases surface tension more effectively (compared to pH 7.0) due to its increased hydrophobicity. These results are generally comparable to [42], although solution conditions (pH and ionic strength) and time references were not identical between the two studies.

Figs. 4 and 5, where the modules are plotted against surface pressure, illustrate the effects of composition, pH and salt on Π , E' and E'' , as well as the correlation between these. By taking the slope, y , of E' as a function of Π (i.e. $y = E'/\Pi$), one can obtain a measure for the interaction

of the protein in the surface layer [34]. This slope has been shown to be equal to the scaling coefficient in the equation

$$\Pi \sim \Gamma^y \quad (7)$$

In the case of good solvent conditions in the surface layer, the value of y is predicted to be 2.85, and will increase up to high values (ca. 50) for θ conditions [34]. The derivation of this dependency supposes that the elastic module is equal to the Gibbs elasticity, E_0 , that is equal to E' only in the case where $E'' = 0$. By using E' instead of E_0 , the theoretical y will be underestimated, with the error depending on the magnitude of E'' . However, the linear relationship between E' and Π can be used to evaluate the effect of salt on the viscoelastic properties of the surface layer. During adsorption, this slope is constant if the solvent-surface interactions (here, protein surface conformation) do not change and there is no substantial desorption, i.e. E' as a function of Π will plot as a straight line through the origin. This looks to be the case with many of the systems investigated here.

Protein-water and protein-protein interactions at the interface yield a surface with viscoelastic rheological properties. These interactions, such as hydrogen bonding and hydrophobic interactions, generally increase near a protein's pI, as the reduced net charge favors non-electrostatic attractions [19,44,43]. This, and the increase in Γ as discussed above, explains the higher E' values observed at pH 5.0 for the WPI controls (Fig. 4A). The magnitude of the surface dilatational modules are thought to be a function of both surface coverage and surface interactions [43]. Accordingly, the increased modules observed upon salt addition at pH 3.0 or 7.0 seems attributable to a reduction in electrostatic repulsion for proteins arriving to the interface and a reduction in electrostatic repulsion among proteins interacting at the interface (Figs. 4 and 6).

Clearly, WPI is more effective at forming a strong, viscoelastic network at pH 7.0 than at pH 3.0, and upon salt addition, the protein forms stronger networks at pH 7.0 than at pH 5.0 (Figs. 4A and 6). The value of y for WPI at pH 7.0 is ca. 3.5 and is fairly independent of salt type or concentration. From this, it may be deduced that the protein conformation at pH 7.0 leads to higher surface elasticity at comparable surface pressures, and the main effect of Na^+ and Ca^{2+} ions at this pH is to increase adsorption and thus both surface pressure and elasticity. Some changes in conformation due to the presence of the larger cations may also take place. Furthermore, the potential for sulfhydryl-disulfide interchange reactions at the interface becomes more prevalent around pH 7.0 [44]. The value of y for WPI at pH 5.0 is seen to be slightly less than at pH 7.0, but it may be dangerous to interpret this as an effect of the surface conformation of a single protein, for reasons given below. Low concentrations (10 mM) of CaCl_2 were especially effective at increasing E' for both WPI and β -lg at neutral pH. This was attributed to the divalent cation, Ca^{2+} , promoting interfacial interactions between the negatively

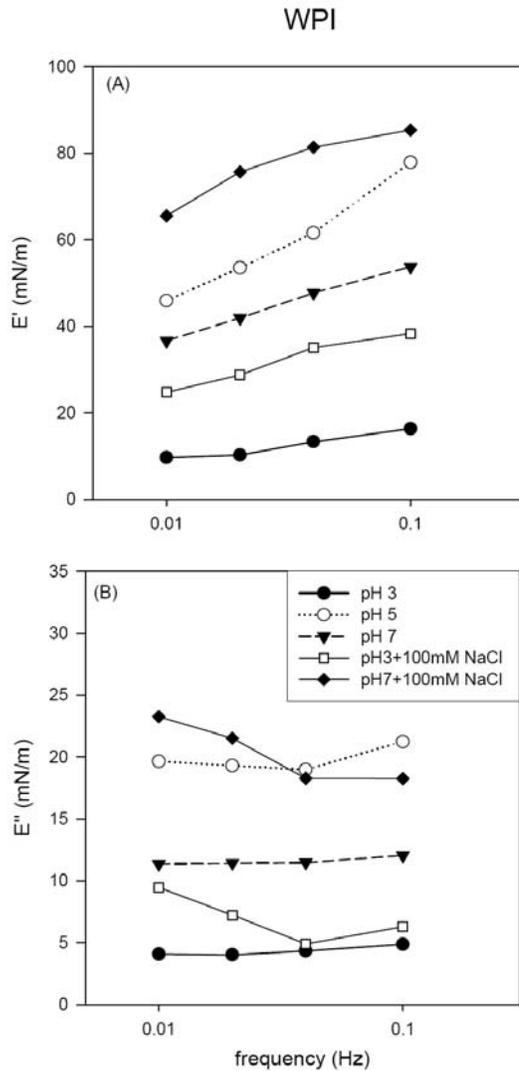


Fig. 6. Typical frequency dependence of E' and E'' of adsorbed WPI interfaces. Total age at the end of each measurement was 12.5 min for all samples. (●) pH 3, (○) pH 5, (▼) pH 7, (□) pH 3.0 + 100 mM NaCl, (◆) pH 7 + 100 mM NaCl.

charged proteins. Similarly, low levels (4–5 μM) of aluminum ions (Al^{3+}) were shown to substantially increase ε of β -lg layers at the air/water interface, in addition to improving the stability of foams consisting of this protein [45].

The addition of 100 mM Na_2SO_4 at pH 3.0 induced the most rapid decrease in surface tension for all treatments, while E' was only slightly increased at pH 3.0, even though the SO_4 ion is divalent at pH 3.0 (Figs. 2 and 4A). Therefore, the increase in adsorption rate and slight increase in E' imparted by Na_2SO_4 seems to result only from electrostatic

shielding, not specific ion mediated protein–protein interfacial interactions.

When considering the pure β -lactoglobulin samples, the values of γ are more independent of both pH and salt concentrations. Even if there are some differences, the value of γ is close to five for all samples (Fig. 4C). This may indicate that the effect of pH and salt is mostly electrostatic and influences the repulsion between proteins both between surface and bulk and in the surface layer itself. It does not, however, have much effect on the protein conformation in the surface layer.

Interfacial elasticity for WPI at pH 3.0 (no salt) was extremely low (~ 15 mN/m), and constant over the time frame tested, unlike all other samples for which E' slightly increased with time (Fig. 3). The values of γ are accordingly low (< 1 to ca. 1.4). Whey protein mixtures have been shown to selectively adsorb to emulsified coconut oil in a pH dependent manner [46]. Specifically, the percent α -la recovered from the interface increased from 11% at pH 7.0 to 48% at pH 3.0, whereas the percent β -lg decreased from 46% at pH 7.0 to 13% at pH 3.0. When this is considered in the context of the current data, it suggests that higher contents of α -la at the interface (assuming similarities between the air/water and oil/water interfaces) significantly weaken whey protein interfacial films. Indeed, interfacial shear measurements suggest that β -lg films (oil-aqueous interface) are weakened by the presence of α -la [47]. Interestingly, the yield stress of WPI foams was shown to significantly increase as the ratio of β -lg to α -lactalbumin was increased [20]. The measurements of α -la as plotted in Fig. 5 were conducted to further corroborate these suggestions. It was indeed observed that the modulus for α -la were considerably lower than those of β -lg, and the corresponding data for WPI fell somewhere between the two pure proteins. The elasticity of the WPI sample at pH 3.0 was more similar to α -la than at pH 5.0 or 7.0, supporting the hypothesis that α -la is preferentially adsorbed from a whey protein mixture at this acidic pH.

Purely elastic responses at frequencies $\leq \sim 0.1$ Hz (dilational deformations) were reported for a range of proteins at surface pressures less than ~ 10 mN/m [48]. Above this surface pressure, a viscous response was measured, which indicated relaxation processes occurring within the interfacial layer. Accordingly, a viscous response was measured in all current treatments at 0.04 Hz, as the lowest surface pressure measured prior to oscillation was ~ 14 mN/m, while most surface pressures were closer to 20 mN/m (Fig. 4). Diffusional interchange with the bulk solution is a possible relaxation process which should become more prevalent with increasing surface pressures, as higher Π 's increase the likelihood of protein desorption [49]. The dilatational rheological behavior of select WPI samples was measured over a range of frequencies to test for diffusional interchange, as a dependence of E' with frequency is indicative of this process [48]. This frequency dependence was indeed noted for all samples (Fig. 6), indicating that the measured E'' values were partially attributable to diffusional

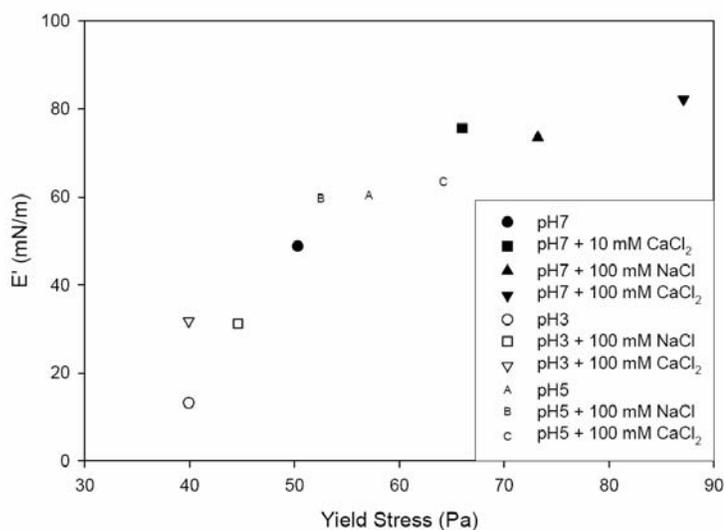


Fig. 7. Average E' of WPI interfaces vs. average value of yield stress for WPI foams. (○) pH 3.0, (□) pH 3.0 + 100 mM NaCl, (▽) pH 3 + 100 mM CaCl₂, (A) pH 5, (B) pH 5 + 100 mM NaCl, (C) pH 5 + 100 mM CaCl₂, (●) pH 7.0, (■) pH 7.0 + 10 mM CaCl₂, (▲) pH 7.0 + 100 mM NaCl, (▼) pH 7.0 + 100 mM CaCl₂.

exchange. The theory that correlates surface dilatational properties to diffusional transport [50,51] predicts that E'' versus frequency should go through a maximum when the slope of E' ($dE'/d\omega$) is at its maximum. This also seems to be the case here, even if the data does not fit this theory exactly. However, it has been shown that dilatational viscosity cannot be explained solely by diffusion in time scales ranging from 1 to 1000 s, meaning relaxation methods *within* the interfacial layer were also present in the current samples [48].

Eq. (1) predicts that foam yield stress is directly proportional to surface tension. However, as discussed in the introduction, this model assumes interfacial tension is at an equilibrium and homogenous throughout the foam, which is likely to be untrue in actual foams. Therefore, it was not surprising that there was no observable correlation between τ and the value of γ at 5 min. Average yield stress values for a range of treatments were compared to measurements of E' obtained from the model WPI interfaces at 0.04 Hz (Fig. 7). Examination of the data suggests a positive, curvilinear correlation between the two variables; however, specific mechanisms to explain this correlation are not established. Clearly the relationship between foam stability and interfacial rheology should indirectly improve foam τ . The three primary mechanisms of foam instability, drainage, disproportionation and film coalescence, are all strongly dependent on the dilatational rheology of foam interfaces; essentially the rate of each mechanism is reduced by a more elastic interface [52]. Therefore, increases in E' should promote smaller, more stable bubbles, which by nature increase foam τ . Future work will expand these measurements to other systems, i.e. egg white, to see if this

positive relationship between E' and τ is a true physical relationship.

6. Conclusion

The yield stress of whey protein isolate foams was found to be dependent on the pH, salt type, and ionic strength within the pre-foam solutions. Relationships between τ and overrun, and τ and γ as predicted by a previously described theoretical model were not validated in these foaming systems. Foam yield stress as measured over a range of electrostatic environments (specific pH and salt concentration) did positively correlate with the dilatational elasticity (E') of equivalent model WPI interfaces. Therefore, interfacial dilatational measurements may provide a valuable tool in predicting/improving the yield stress of protein foams. The substitution of purified β -lg (the primary protein in WPI) for WPI in model foam interfaces produced similar surface tension/time dependencies under similar treatment conditions; however, there were large discrepancies in the interfacial rheological data, underscoring the hazards of oversimplifying model foam systems. Comparisons of WPI interfacial rheological data with that of purified α -la and β -lg suggest that the presence of α -la in WPI may weaken WPI interfacial films as compared to β -lg alone.

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CHAPTER 2.

Foaming and Interfacial Properties of Polymerized Whey Protein Isolate

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Foaming and Interfacial Properties of Polymerized Whey Protein Isolate

J.P. DAVIS AND E.A. FOEGEDING

ABSTRACT: Yield stresses (τ) of whipped foams prepared from various ratios of native whey protein isolate (WPI) and polymerized whey protein isolate (pWPI) were characterized by means of vane rheometry. Yield stress displayed a parabolic response to increasing concentrations of pWPI, peaking at 50%. Foam air phase volume steadily decreased with increasing pWPI content, whereas equilibrium surface tension steadily increased. Dynamic surface tension measurements revealed that native WPI adsorbed much more rapidly than pWPI, presumably because of the latter's larger size. Interfacial dilatational elasticity (E') displayed a parabolic trend with increasing pWPI content, peaking at 50%. This suggested that pWPI coadsorbs with native WPI, bolstering E' of native WPI interfaces. However, too much pWPI caused a weakening of the network. A positive, curvilinear relationship between E' and τ was observed, consistent with a previous observation for WPI foams formed at various pH levels and salt concentrations, further suggesting a general link between these parameters.

Keywords: whey proteins, foam, yield stress, interfacial rheology

Introduction

Whey protein ingredients combine a well-balanced nutritional profile with a range of valuable functional properties, including the capacity to form and stabilize gels, emulsions, thermally stable sols, and foams (Kinsella and Whitehead 1989). Numerous treatments are available to improve whey protein functionality, including enzymatic hydrolysis, fractionations, and heat treatments (Foegeding and others 2002). Mild heat treatments have been shown to improve foaming properties, whereas more extensive heating conditions can be detrimental (Zhu and Damodaran 1994). Heating of whey proteins at pH values greater than 6.0, low ionic strengths, and at a concentration below that necessary for gelation, leads to the formation of soluble polymers of disulfide-linked protein monomers (Vardhanabhuti and others 2001). The structure and resulting functional properties of whey protein polymers (WP polymers) depends on numerous conditions, including protein concentration, heating temperature, and the presence of cosolutes, among others (de la Fuente and others 2002). Optimal ratios of native whey protein isolate (WPI) and WP polymers were determined for foamability (the efficiency of foam formation) and foam stability (Zhu and Damodaran 1994). Changes in protein adsorption to the air/water interface and interfacial rheological properties as altered by the WP polymers were hypothesized to cause differences in foaming properties; however, no interfacial measurements were presented.

The surfactant behavior of proteins at model interfaces has been intensively studied for many years and is the subject of recent reviews (Dickinson 1999; Wilde 2000; Bos and van Vliet 2001). Protein adsorption to most interfaces is a spontaneous process and, once adsorbed, the potential for protein-protein interactions depends on the intrinsic nature of the protein, environmental conditions, the

type of interface, bulk protein concentration, and so on (Murray 1998). These protein-protein interactions can lead to measurable interfacial rheological properties, which in turn have primarily been related to foam stability measurements (Hammershoj and others 1999; Murray 2002; van der Ven and others 2002). However, the role of interfacial rheology as related to foam rheology is relatively unexplored (Edwards and Wasan 1996).

Foams provide unique and desirable textures to a range of food products, including various cakes, confections, and toppings. Understanding the factors governing foam rheology is therefore important from a product development perspective because it should promote a more efficient delivery of new and existing foam textures from the producer to the consumer. Understanding the factors governing foam rheology is also important from a processing perspective, as food foams are often extensively processed after formation, such as pumping, coating, heating, and so on, meaning their successful manufacture primarily depends on their rheological properties. Although traditional geometries for determining foam rheology can be problematic, the vane method is established as a simple and accurate means of determining foam yield stress (τ), which relates to the empirical concept of foam robustness (Pernell and others 2000). A theoretical analysis of foam rheology suggests that air phase volume (ϕ), equilibrium interfacial tension (γ), and bubble size are critical factors in determining foam τ (Princen and Kiss 1989). However, whipped foams prepared from WPI solutions over a range of pH levels and salt concentrations did not follow predicted relationships between τ and ϕ , or τ and γ (Davis and others 2004). Interestingly, a positive, curvilinear relationship between the dilatational elasticity of model interfaces and foam τ was noted, suggesting a potential dependence between these parameters.

The primary goals of the current work were to (1) investigate the effects of WP polymers on foam τ , (2) investigate interfacial properties of WP polymers compared with native WPI, and (3) determine whether any correlation was present between foam τ and interfacial rheological measurements.

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Materials and Methods

Materials

A commercial sample of WPI (BiPro, 92.3% protein, lot LE 007-1-4220) was supplied by Davisco Foods Intl. (Le Sueur, Minn., U.S.A.). Extra-fine granulated sugar (Dixie Crystals, Savannah, Ga., U.S.A.) was purchased from a local store and stored at room temperature. Deionized water was obtained using a Dracor Water Systems (Durham, N.C., U.S.A.) purification system. The resistivity was a minimum of 17.2 MΩ-cm. All other chemicals were reagent grade.

Protein hydration and heat polymerization

WPI was hydrated in deionized water under mild stirring to a protein concentration of 10% w/v. All solutions were adjusted to pH 7.00 with 1.0 N HCl, before volume adjustment. Native WPI samples were equilibrated at room temperature for about 1 h, before overnight storage at 4 °C. The polymerization process was accomplished by heating native WPI in closed polycarbonate tubes (18 cm × 1.9 cm internal dia) in a water bath at 80 °C for 30 min. Polymerized samples were cooled at room temperature for about 1 h, before overnight storage at 4 °C. All solutions were equilibrated at room temperature the next day, before usage.

Foaming solution preparation

Various ratios of polymerized and native WPI were created: (1) Density measurements at room temperature (23 °C) of the 2 solutions were determined (discussed subsequently). (2) Volumetric ratios of the 2 solutions were prepared by mass measurement (Denver Instrument Co., CO; XE Series, model 400, Denver, Colo., U.S.A.), followed by a volume conversion using the density measurements. When required, 12.8% w/w sugar was added to the various foaming solutions, a typical sugar content for a cake application (Pernell and others 2002).

Density determination

Density measurements were used for solution preparation as described previously. Densities of the component phases are also required inputs for the determination of interfacial tension from the shape analysis of drops and bubbles (Kwok and others 1995). Accordingly, a Mettler-Toledo DE40 density meter (Mettler-Toledo, Columbus, Ohio, U.S.A.) equipped with a viscosity correction card was used to determine the density of each solution at 23 °C. The accuracy of the instrument was 1×10^{-4} g/cm³ and every solution was measured in duplicate and averaged before interfacial measurements.

Foam generation

A Kitchen Aid Ultra Power Mixer (Kitchen Aid, St. Joseph's, Mich., U.S.A.) with a 4.5-qt (4.3 L) stationary bowl and rotating beaters was used for foam formation. Solutions (225 mL) were whipped at speed setting 8 (planetary rpm of 225 and beater rpm of 737) for 20 min.

Yield stress measurements

Foam yield stress was determined by vane rheometry (Pernell and others 2000; Davis and others (2004). A Brookfield 25xLVTDV-ICP (Brookfield Engineering Laboratories, Inc., Middleboro, Mass., U.S.A.) viscometer was used at a speed of 0.3 rpm. After foam formation, the beaters were carefully removed from the foam before yield stress measurement. The vane (10-mm dia and 40-mm length) was lowered into the foam until level with the foam surface. The viscometer was set to zero and rotation was begun. Maximum torque response (M_0) was documented for each of 3 measurements taken per foam and used to calculate yield stress according to published information (Dzuy and Boger 1983, 1985; Steffe 1992):

$$\tau = \frac{M_0}{\left[\frac{h}{d} + \frac{1}{6} \right] \left(\frac{\pi d^3}{2} \right)} \quad (1)$$

where τ is the yield stress, and h and d are the height and diameter of the vane. Three consecutive measurements (4 min/max) were taken per treatment, and each treatment was replicated a minimum of 3 times.

Overrun

Overrun measurements were begun immediately after the final yield stress measurement. Foam was carefully scooped from the bowl in a circular pattern with a rubber spatula, filling a standard weigh boat (100 mL) 3 times. The mean value was used to calculate overrun and air phase fraction according to Campbell and Mougeot (1999):

$$\% \text{ Overrun} = \frac{(\text{wt } 100 \text{ mL solution}) - (\text{wt } 100 \text{ mL foam})}{\text{wt } 100 \text{ mL foam}} \times 100 \quad (2)$$

$$\text{Air phase fraction} = \phi = \frac{\% \text{ overrun}}{(\% \text{ overrun} + 100)} \quad (3)$$

Overrun measurements were found to be stable over the entire measurement time (3 min/max). Each treatment was replicated a minimum of 3 times to determine the average overrun.

Drainage

Foam drainage was measured according to the method of Phillips and others (1990). The time for 1/2 of the pre-foam mass to drain through a hole in a whipping bowl was taken as a measurement of foam stability. Two drainage samples per foaming treatment were collected, 0 to about 10 g and 110 to 120 g for examination via electrophoresis (discussed subsequently). Protein content of the pre-foam solutions and the drainage material was measured via a bicinchoninic acid (BCA) protein assay kit (nr 23225; Pierce, Rockford, Ill., U.S.A.).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Foaming solutions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970) using a Mini-PROTEAN 3 Cell system (Bio-Rad, Hercules, Calif.) both before and after foaming (drainage material). Samples were diluted in Laemmli buffer and Laemmli buffer containing 710 mM 2-mercaptoethanol for nonreducing and reducing conditions, respectively. The stacking and resolving gels contained 4% and 12% polyacrylamide, respectively. Proteins were stained with Coomassie brilliant blue R250 staining solution (Bio-Rad) and destained with deionized water.

Viscosity measurements

A controlled stress rheometer (StressTech; Reologica Instruments AB, Lund, Sweden) was used for viscosity determination. The measuring system, CCE25, consisted of a cup and bob geometry, and a range of shear rates (0.5/s to 225/s) was tested after a preshear at 50/s for 30 s. All measurements were carried out at room temperature (23 °C).

Interfacial measurements: solution preparation and methodology

The foaming solutions or their dilutions were used for interfacial

Polymerized whey protein foam rheology . . .

measurements. Methods that determine the dynamic interfacial tension of adsorbing/desorbing surfactants by means of the shape analysis of drops and bubbles are well established (Hansen and Myrvoid 1995; Chen and others 1998). The following method of interfacial data collection has been described recently (Davis and others (2004) and is briefly reviewed here. An automated contact angle goniometer (Rame-Hart Inc., Mountain Lakes, N.J., U.S.A.) was used for data collection and calculations in combination with the DROPimage computer program (Myrvoid and Hansen 1998). The surface tension of a 16- μ L capillary drop was monitored for 5 min with a 1-s resolution. After aging for 5 min, oscillation was begun with a volume amplitude of 1 μ L. The measured change in surface tension during a surface area change was used to determine the dilatational modulus. From this modulus and from the phase angle between the surface area change and surface tension response, the DROPimage software calculates E' and E'' , which are equivalent to and proportional to the elastic and viscous components of the interface, respectively. The details for these calculations have been described elsewhere (Myrvoid and Hansen 1998).

Data analysis

Yield stress, overrun, density, drainage, E' , E'' , and surface pressure data were all determined using the General Linear Model procedure of the SAS statistical software package (Version 8.2; SAS Inst., Inc., Cary, N.C., U.S.A.). Analysis of variance was conducted with means separation to determine differences around treatments. Significance of difference was established at $P \leq 0.05$.

Results and Discussion

Yield stress measurements as a function of polymerized WPI (pWPI) are summarized in Figure 1. Note the term "pWPI" refers to heat-polymerized whey proteins prepared specifically from whey protein isolate. Yield stress displayed a parabolic trend with increasing pWPI content, peaking at 50% for all samples, regardless of sugar addition. Sugar addition slightly depressed τ for all treatments. The stability of τ decreased with increasing pWPI content, as seen in the difference between the 1st and 3rd readings. These measurements agree with the results reported by Pernell and others (2000) for foams prepared under equivalent conditions. Note the foam volume was insufficient to accurately measure τ for the 100% pWPI samples.

Increasing whip times did not increase the foam volume sufficiently for τ measurement of the 100 pWPI solution.

Air phase volume (ϕ) measurements as a function of pWPI content are summarized in Figure 2. Air phase volume significantly decreased with increasing pWPI content, and sugar addition shifted ϕ to significantly lower values at equivalent pWPI contents, except for the 100% pWPI foam, for which sugar addition had no significant effect. As discussed subsequently, the rate of liquid drainage through the foams decreased with increasing pWPI content (Table 1). It is established that overrun measurement is influenced by drainage rates, that is, decreased liquid drainage increases foam density and hence decreases overrun, whereas increased liquid drainage decreases foam density, making overrun measurements higher (Halling 1981). Note that overrun and ϕ are related by Eq. 3. This phenomenon certainly cannot be ruled out in the current system.

Foam stability was assessed by the rate of liquid drainage from the whipped foams through a hole in the bottom of a whipping bowl (Phillips and others 1990). Foam half-life, which is the time required for 1/2 of the prefoam mass to drain, increased with increasing pWPI content (Table 1). Sugar addition significantly decreased foam drainage for native WPI, minimally affected drainage for the 50% pWPI foams, and actually increased the drainage rate of the 100% pWPI foam. Foam drainage rates are established to decrease with increases in the continuous phase viscosity (Halling 1981). Visual observation of pWPI suggested these solutions were more viscous than native WPI, and this was confirmed by instrumental rheological characterization. The 100% pWPI solutions displayed non-Newtonian shear thinning behavior consistent with previous observations of equivalent materials (Vardhanabhuti and Foegeding 1999). This indicates that the highest viscosity is at very low shear rates, which may be especially relevant to the physics of foam stability (Halling 1981). It is also noted that this shear thinning behavior decreased with decreasing pWPI content, such that native WPI essentially behaved as a Newtonian fluid (data not shown).

Typical shear rates experienced by materials under gravity-induced drainage range from 0.1/s to 10/s (Barnes and others 1989). Apparent viscosity (η) measurements of the various foaming solutions at 8.5/s are summarized in Table 1. This shear rate was chosen

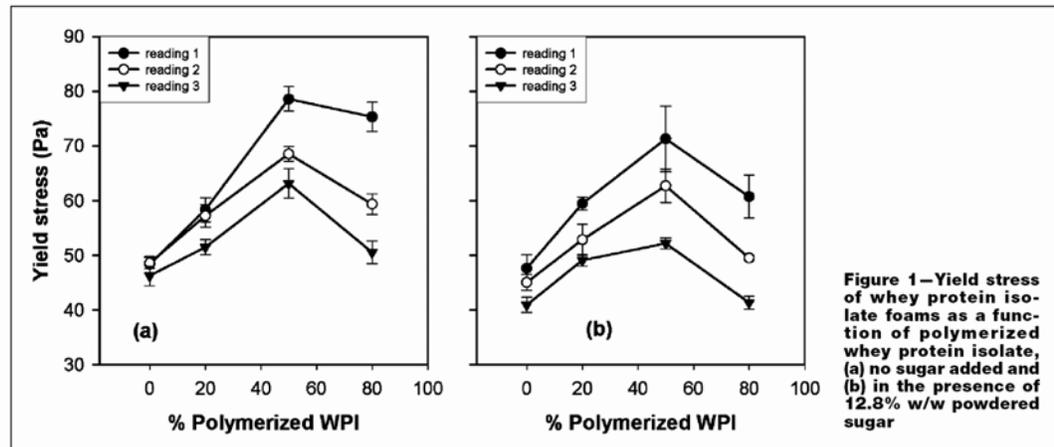


Figure 1—Yield stress of whey protein isolate foams as a function of polymerized whey protein isolate, (a) no sugar added and (b) in the presence of 12.8% w/w powdered sugar

Table 1—Physical and foaming properties of 10% protein solutions^a

Treatment	Density (g/cm ³)	Density ^b (g/cm ³)	η^c (Pa s)	$\eta^{b,c}$ (Pa s)	Drainage 1/2 life (min)	Drainage ^b 1/2 life (min)
Native WPI	1.0247a	1.0745b	0.002	0.004	22.0a	28.3b
20% polymer	1.0248a	—	0.003	—	27.5b	—
50% polymer	1.0248a	1.0747b	0.008	0.010	42.1c	43.8c
80% polymer	1.0248a	—	0.034	—	63.4d	—
Polymerized WPI	1.0249a	1.0747b	0.297	0.144	81.1e	63.8d

^aEach value is the average of at least 3 replications. Same letter per measurement type (not column) indicates no significant difference ($P \leq 0.05$) within that measurement type. WPI = whey protein isolate.

^bIndicates 12.8 w/w powdered sugar in solution.

^cApparent viscosity measured at a shear rate of 8.5/s.

because it was both within the relevant range of shear rates for liquid drainage and within the sensitivity of the instrument. Sugar addition slightly increased η for native WPI and the 50% pWPI solution, while it actually decreased η of the 100% pWPI solutions (Table 1). The mechanism for this observation is unclear. The drainage data when compared with η measurements suggest that increased η primarily contributed toward the reduced drainage rates for foam samples containing higher contents of pWPI and/or sugar.

Qualitative analysis of the proteins within the drainage material was accomplished by SDS-PAGE under reducing and nonreducing conditions (see “Material and Methods” section). Samples were collected at 3 different times: (1) prefoam solution, (2) 1 to about 10 g of drainage material, and (3) about 110 to 120 g of drainage material. Protein content, as determined by a BCA assay, was unchanged for all drainage samples compared with their prefoam solutions. Electrophoretic patterns for the time samples were indistinguishable for each foaming treatment tested (0%, 50%, and 100% pWPI), suggesting no protein was preferentially retained within the foams (data not shown). The vast majority of the 100% polymerized protein did not enter the 4% stacking gel in the absence of reducing agent. However, in the presence of reducing agent, this sample’s electrophoretic pattern was indistinguishable from native WPI, meaning the 100% pWPI solutions primarily consisted of disulfide-linked aggregates with molecular weights above 1 million (Vardhanabhuti and Foegeding 1999).

Zhu and Damodaran (1994) utilized a specialized sealed-column foaming apparatus to assess the effects of pWPI on foamabil-

ity and foam stability. Polymerized whey protein isolate was prepared by heating 9% protein solutions at 90 °C for 20 min. Foamability was determined by the time necessary for the foam to reach a specified height in the column as nitrogen gas was sparged through the protein solution (5%). Foamability peaked at a ratio of 60:40 native to polymerized. The closest measure of foamability in the current study would be overrun; however, these 2 measurements are fundamentally different. In the former measurement, the energy of formation to reach a certain foam height is determined, whereas in overrun measurements, the energy of formation is fixed and the amount of incorporated air is then determined. Therefore, our conclusion that overrun peaked for 100% native WPI solutions does not contradict the results of Zhu and Damodaran (1994). They also found that foam stability peaked at a ratio of 40:60 native to pWPI (Zhu and Damodaran 1994). In our current work, foam stability was maximal for the 100% pWPI solutions. However, it is again noted that the 2 foaming systems are actually examining fundamentally different properties. The Zhu and Damodaran system measures bubble collapse, which results in a pressure increase within the closed column, whereas in our current study, the liquid, as it drains through the foam lamellae, is measured as it collects at the bottom of a whipping bowl and drips through a hole. We suspect the strong correlation observed in our stability measurements with η is a function of this liquid flow, and this was not detected in the Zhu and Damodaran (1994) apparatus. However, it is important to emphasize that measuring the drainage rates of foams does not directly relate to rates of disproportionation or coalescence. Therefore, the decrease in τ stability with increasing pWPI content (Figure 1) must be at least a partial function of either disproportionation and/or coalescence.

Zhu and Damodaran (1994) hypothesized that the native species would adsorb to the interface more rapidly, hence contributing to foamability; whereas pWPI would adsorb after the monomeric species, contributing to the stability of the interfacial protein film. Dynamic surface tension measurements confirm that the native type of WPI adsorbs more rapidly than the polymerized WPI (Figure 3). This is more evident at the lowest protein concentration tested (0.1%), where it was typically observed that γ only declined by about 1 to 3 mN/m for 100% pWPI solutions over 5 min, whereas γ declined by about 12 to 14 mN/m for the native WPI. Clearly, the larger size/smaller diffusion coefficients of pWPI contribute to its decreased adsorbance. However, changes in γ were much more similar at 10% protein, as the initial and final values of γ only varied by about 5 mN/m for all samples tested. This suggests the relative distance for diffusion to the interface is decreased sufficiently at the foaming concentration of 10% protein, such that the adsorbances of native and pWPI are similar within the timeframe tested (about 1 s for 1st measurement).

Interfacial dilatational rheological properties of the various

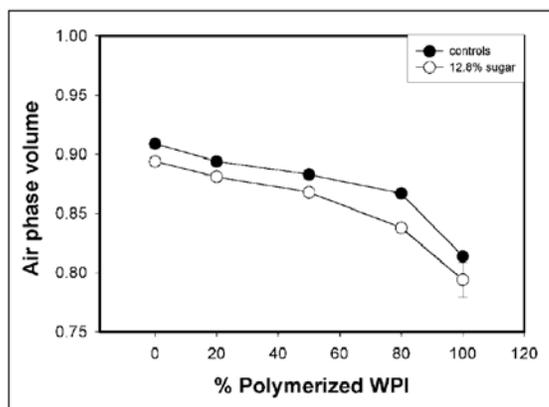


Figure 2—Air phase volume as a function of polymerized whey protein isolate

Table 2—Surface properties of 10% protein solutions^a

Treatment	Π (mN/m)	Π^b (mN/m)	E' (mN/m)	E'^b (mN/m)	E'' (mN/m)	E''^b (mN/m)
Native WPI	23.6a,b	24.3a	49.5d	45.1c	20.7b	20.4b
20% polymer	22.6b	—	64.8f	—	22.5b	—
50% polymer	22.1b	21.4b,c	66.5f	50.6d	22.3b	22.7b
80% polymer	21.2b,c	—	56.0e	—	19.8b	—
Polymerized WPI	20.3c	20.3c	26.6b	8.4a	14.4a,b	10.0a

^aEach value is the average of at least 3 replications. Same letter per measurement type (not column) indicates significant difference ($P \leq 0.05$) within that measurement type. WPI = whey protein isolate.

^b12.8% w/w powdered sugar in solution.

foaming treatments were tested over a range of bulk protein concentrations (10% to 0.1%) and are summarized in Figure 4 and Table 2. It was noted that the elastic component (E') of the interface was greater than the viscous response (E'') for all treatments tested (Table 2). E' is plotted with surface pressure ($\Pi = \gamma_0 - \gamma$, where γ_0 is the surface tension of the solvent, and γ is the surface tension of the solvent on surfactant adsorption) to allow the simultaneous visualization of these parameters. At 1% and 0.1% protein, the data were well described by a straight line ($r^2 \geq 0.98$). The likely explanation of these lines is that within the timeframe tested (about 9 min from drop formation to the last rheological measurement), native WPI completely dominates interfacial adsorption at the 2 lower concentrations tested, with increased adsorption (reflected in increasing Π) of this type of product in turn increasing E' . However, at 10% protein, where Π for all treatments was higher and much more similar, a different trend was noticed for E' versus Π , which followed a parabolic response with increasing pWPI content and peaked at 50%. These data, taken with the dynamic surface tension measurements, suggest that pWPI coadsorbs with native WPI at the foaming concentration, bolstering E' of the interfacial films. These measurements help to explain the maximum in foam stability ob-

served by Zhu and Damodaran (1994) at 60% pWPI, as increased surface dilatational modules (E' and E'') are thought to reduce foam destabilization mechanisms, such as drainage, disproportionation, and coalescence (Prins and others 1998).

Sugar was found to have a minimal effect on Π and E'' of the various treatments (10% protein) at 5 min (Table 2). However, E' measurements were significantly decreased by the presence of 12.8% w/w sugar (Table 2). Compounds such as sucrose are established to stabilize protein unfolding via a mechanism termed preferential exclusion (Lee and Timasheff 1981). Protein interfacial denaturation would be expected to be limited in sugar solutions, possibly explaining the decrease in E' , as interfacial protein unfolding, which exposes reactive functional groups, is expected to contribute to the buildup of this parameter (Murray 1998). Others have indeed noted the presence of sucrose decreased protein denaturation during foaming (Clarkson and others 2000). However, protein interfacial denaturation is hypothesized to decrease with increasing surface pressures (Wustneck and others 1996), meaning denaturation should be minimal at the foaming concentration, where relatively high surface pressures were realized very rapidly, suggesting another mechanism for the decrease in E' upon sugar addition.

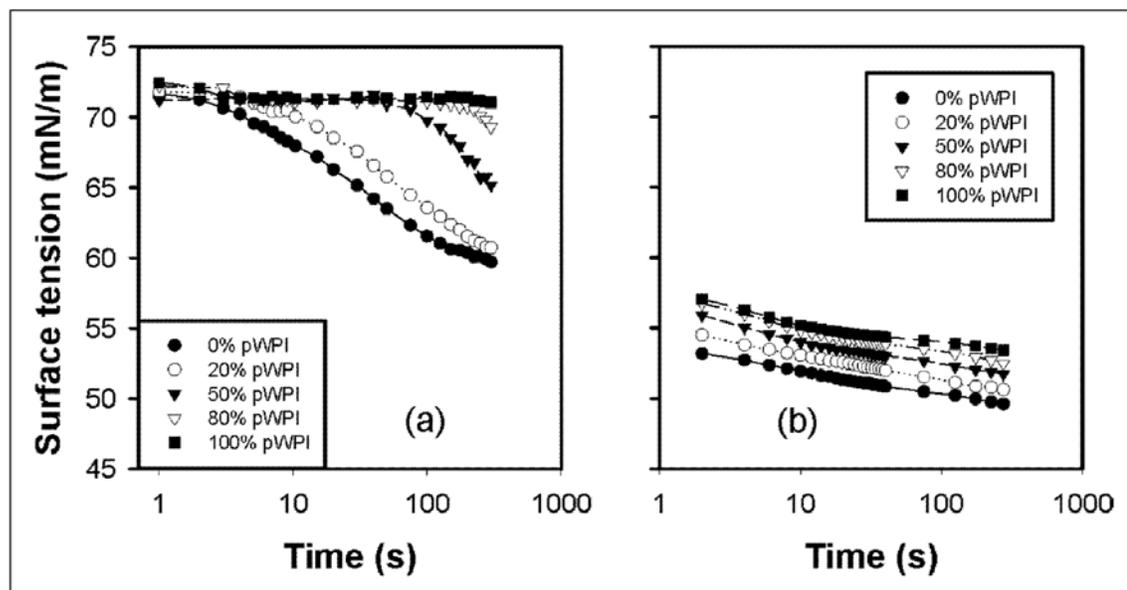


Figure 3—Typical dynamic surface tension measurements: (a) 0.1% protein; (b) 10% protein

The following equation for describing foam τ was developed from theoretical analyses of foam rheology (Princen and Kiss 1989):

$$\tau = \frac{\gamma}{R_{32}} \phi^{1/3} Y(\phi) \quad (4)$$

where R_{32} equals the Saunter mean bubble radius, γ is the equilibrium surface tension, and $Y(\phi)$ is an empirically derived function of the system. This equation has been verified for simplified foaming systems, namely, concentrated emulsions (more monodisperse and more resistant to density driven settling); however, its shortcomings in describing complex foaming systems (such as the current foams) have been previously discussed (Pernell and others 2002; Davis and others 2004). Essentially, this model assumes all foam interfaces are at a constant, equilibrium interfacial tension, when actual foam interfaces are undergoing rapid fluctuations in interfacial tension (Edwards and Wasan 1996). However, ϕ (which directly relates to overrun according to Eq. 3) steadily decreased with increasing pWPI content, whereas γ steadily increased with increasing pWPI content, perhaps accounting for the parabolic response in τ with increasing pWPI content according to Eq. 4. Yet, without an accurate measure of the bubble size distribution, this cannot be stated confidently.

The yield stress of 10% protein foams created from WPI solubilized over a range of electrostatic conditions was found to increase in positive, curvilinear fashion with E' of model WPI interfaces (Davis and others 2004). These interfaces consisted of WPI adsorbed from a bulk protein concentration of 1.84 mg/mL, a concentration that is equal to 10^{-4} M β -lactoglobulin (the primary whey protein). This concentration was chosen because the interfacial concentration of β -lactoglobulin is shown to rapidly approach saturation at concentrations greater than 1 mg/mL (Gauthier and others 2001). The current E' compared with τ values are plotted with the old values of Davis and others (2004) in Figure 5, with the new points generally falling on the curve, despite being measured at a bulk protein concentration that is about 50 times greater. However, it is noted that current Π values were comparable to the Π values of the old points because many of the old points were at electrostat-

ic conditions that favored rapid adsorption, such as pH values near the isoelectric point of the proteins, or in the presence of high salt concentrations. E' values measured at a protein concentration comparable to the old study were dominated by the rapid adsorption of the monomer, giving unrealistic values. This was not a problem in the former study because the diffusion coefficients of native WPI at the various electrostatic conditions did not vary as significantly as those of native WPI and pWPI. These data further suggest a general link between E' and τ , which may simply be a function of the increased foam stability at increased E' values, as discussed earlier. However, the limited theoretical work addressing foam rheology, which takes into account interfacial rheology, does suggest a significant impact from E' on bulk foam rheology, especially for "dry" foams (foams with high ϕ 's, such as the current foams) (Edwards and Wasan 1996).

Conclusions

Yield stress of WPI foams was found to be dependent on the ratio of polymerized to native WPI. A parabolic response was found with a peak occurring at 50% pWPI. Surface measurements suggest that native WPI adsorbed to model interfaces much more rapidly than pWPI. At concentrations typically used for model interfacial measurements, namely $\leq 1\%$ protein, the interfacial elasticity (the dominant contributor to the total resistance of the interface to deformation for all treatments) seemed to be dominated by the adsorption of native WPI. However, at the actual concentration used for the foaming experiments (10% protein), pWPI coadsorbed with native WPI, resulting in a parabolic trend in E' with increasing pWPI content, peaking at 50%. These data corroborate earlier data that suggested the τ of whipped WPI foams is significantly influenced by the rheology of its interfaces, namely, their elastic components.

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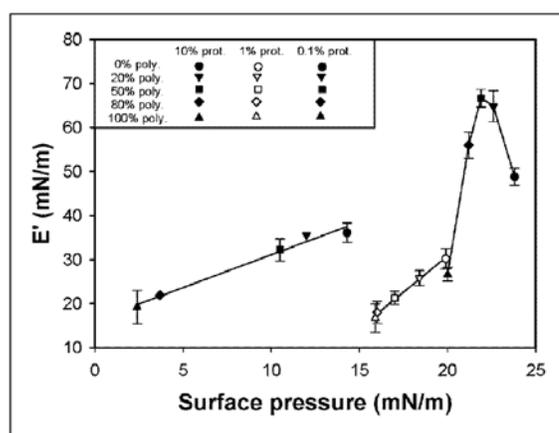


Figure 4—Interfacial dilatational elasticity plotted against surface pressure (5 min) for WPI/pWPI mixtures at 3 different protein concentrations. Straight lines are best linear fits. Spline line added to aid data interpretation.

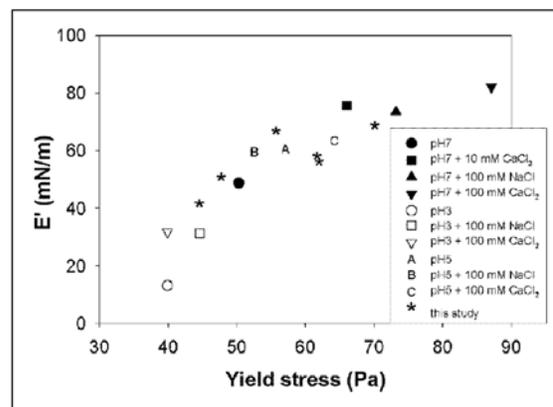


Figure 5—Surface dilatational elasticity (measured at 0.04 Hz after 5 min of aging) plotted against yield stress

ing with data collection. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named nor criticism of similar ones not mentioned.

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CHAPTER 3.

Foaming and Interfacial Properties of Hydrolyzed β -lactoglobulin

**The content of this appendix has been accepted for
publication in *The Journal of Colloid and Interface Science***

Abstract

β -lactoglobulin (β -lg) was hydrolyzed with three different proteases and subsequently evaluated for its foaming potential. Foam yield stress (τ_0) was the primary variable of interest. Two heat treatments designed to inactivate the enzymes, 75°C/30 min and 90°C/15 min, were also investigated for their effects on foam τ_0 . Adsorption rates and dilatational rheological tests at a model air/water interface aided data interpretation. All unheated hydrolysates improved foam τ_0 as compared to unhydrolyzed β -lg, with those of pepsin and Alcalase 2.4L® being superior to trypsin. Heat inactivation negatively impacted foam τ_0 , although heating at 75°C/30 min better preserved this parameter than heating at 90°C/15 min. All hydrolysates adsorbed more rapidly at the air/water interface than unhydrolyzed β -lg as evidenced by their capacity to lower the interfacial tension. A previously observed relationship between interfacial dilatational elasticity (E') and τ_0 was generally confirmed for these hydrolysates. Additionally, the three hydrolysates imparting the highest τ_0 not only had high values of E' (approximately twice that of unhydrolyzed β -lg), they also had very low phase angles (essentially zero). This highly elastic interfacial state is presumed to improve foam τ_0 indirectly by improving foam stability and directly by imparting resistance to interfacial deformation.

Keywords: dilatational modulus; dilatational elasticity; adsorption; interfacial rheology; β -lactoglobulin; peptide; hydrolysate; foam; yield stress; whey protein

Introduction

Proteins function as natural surfactants in many applications that involve foam production. Egg white proteins have traditionally served this role in the food industry, although substitution with other proteins, including those derived from bovine milk, is becoming more prevalent [1]. Common means of evaluating foam surfactants include their capacities to efficiently form foams (foamability), stabilize foams, and impart specific foam rheological properties [2, 3]. Foam rheological studies have received far less attention than those pertaining to foamability and/or foam stability, although understanding the mechanisms responsible for protein-based foam yield stress (τ_0) has been a recent focus for our research group [4-8].

Enzymatic hydrolysis is a common means for improving the foaming potential of protein ingredients [9]. A common approach for evaluating the foaming performance of protein hydrolysates is the whipping of dilute hydrolysate solutions ($\leq 0.05\%$ w/v) in graduated cylinders, after which the initial foam height and its decrease with time are taken as measurements of foamability and foam stability respectively. Accordingly, foamability and foam stability were markedly improved for a variety of hydrolysates as compared to their unhydrolyzed counterparts [10-14]. Chromatographic characterizations of these materials suggest this improvement is partially attributable to the reduced size of peptides as compared to proteins, which promotes a more rapid adsorption at the air/water interface [10, 11, 14, 15]. However, too much hydrolysis may be detrimental for functionality purposes. For example, hydrolysates containing high percentages of large MW fragments (> 7 kDa) were most correlated to improved foam stability in a

comparative study of 44 different hydrolysates [14]. There is also substantial evidence from the hydrolysate-emulsion literature suggesting that extensive hydrolysis can be detrimental to functionality [9]. The relative hydrophobicity of hydrolysates has also been correlated with improved foamability and foam stability [10, 11, 14]. As discussed shortly, very little has been reported on protein hydrolysis as it affects foam rheology.

Direct characterization of hydrolyzed proteins at model air/water or oil/water interfaces has received far less attention than foaming or emulsifying tests of these materials.

Tryptic peptides derived from β -lactoglobulin (β -lg) most effective at decreasing interfacial tension were those containing distinct zones of hydrophobic and hydrophilic regions within a minimum molecular weight allowing this distribution [15]. Increasing levels of hydrolysis (up to 86%) for β -lg variant A with a protease specific for glutamic and aspartic acid residues decreased the interfacial shear elasticity and viscosity of these materials, but resulted in improved foam overrun and stability as determined via a small scale foaming test [16]. This was surprising as improved foaming performance is typically associated with increases in interfacial rheological moduli. Dilatational rheological tests of an amphipathic peptide isolated from a tryptic hydrolysis of β -casein showed surface behavior similar to the intact protein [17]. There has been very little reported on the interfacial dilatational rheological behavior of unfractionated mixtures of protein hydrolysates, which is the typical form of these ingredients.

The capacity to predict and control foam τ_0 has considerable practical significance to the food industry, as this parameter relates well to the empirical concept of foam robustness,

and more robust foams are generally desirable from a processing perspective. Several commercial whey protein hydrolysates were found to improve foam τ_0 as compared to whey protein isolate (WPI) on an equal protein basis, although the mechanism for this improvement was unclear [6]. A potential correlation between interfacial dilatational elasticity (E') and τ_0 has been noted for whey proteins solubilized across a range of electrostatic conditions [7] and varying degrees of polymerization [8]; however, this relationship was untested for hydrolyzed whey proteins. Accordingly, hydrolysates of β -lactoglobulin, the primary whey protein, were prepared from three common food enzymes to further test the validity of this E' vs. τ_0 relationship, and to investigate any other potential mechanisms responsible for foam τ_0 . Furthermore, the effects of two heat treatments designed to terminate enzymatic activity were also investigated, as post-hydrolysis heating of hydrolysates is common practice on an industrial scale, yet its effects on foam functionality were unclear.

Materials

Bovine β -lg (97% protein, dry basis) was obtained from Davisco Foods International Inc. (Le Sueur, MN). β -lg and α -lactalbumin made up 93% and 5% of total proteins respectively as determined by the manufacturer. Trypsin (porcine pancreas, Type II-S, EC 3.4.21.4, No. T-7409), pepsin (from porcine stomach mucosa, EC 3.4.23.1, No. P-7000), Tween 20 (SigmaUltra, P-7949), Hide Powder Azure (No. H-6268) and Fibrin-Blue (No. F-5255) were all purchased from Sigma Chemicals CO (St. Louis, MO). The Trypsin contained 1800 $N\alpha$ -Benzoyl-L-arginine ethyl ester (BAEE) units/mg of trypsin activity and 2 N -Benzoyl-L-tyrosine ethyl ester (BTEE) units/mg of chymotrypsin activity as indicated by the manufacturer. Note that BAEE and BTEE are standard

substrates for determining trypsin and chymotrypsin activity respectively. Alcalase 2.4L[®] (a liquid preparation from subtilisin Carlsberg) was obtained from Novozymes (Franklinton, NC). All other chemicals were of reagent grade quality. Deionized water was obtained using a Dracor Water Systems (Durham, NC) purification system. The resistivity was a minimum of 18.2 M Ω cm.

Methods

Hydrolysis conditions and Degree of Hydrolysis (DH)

β -lg was rehydrated to a protein concentration of 5% w/v for all three hydrolysates.

Trypsin (10% w/v) was rehydrated in 1mN HCl prior to addition with β -lg. Hydrolysis conditions were as follows: 40°C, pH 8.0, Enzyme to substrate ratio (E:S) 1:300 (w/w) based on the dry protein. The pH of the enzymatic reaction was kept constant at 8.0 using the pH-Stat technique [18]. The final degree of hydrolysis (DH) was 5.6% as determined by pH-Stat. These conditions have been previously used to produce tryptic hydrolysates [19, 20]. Alcalase hydrolysis conditions were as follows: 45°C, pH 8.0, E:S 1:20 (v/w). The reaction was allowed to proceed for 3 hours and the pH was not controlled. The final pH of the hydrolysate was 6.2. Prior to pepsin hydrolysis, the β -lg solution was heated at pH 3.0 at 85°C for 15 min to improve digestibility [21]. The solution was allowed to equilibrate to room temperature before enzyme addition. Pepsin hydrolysis conditions were as follows: 40°C, pH 3.0, E:S 1:15 (w/w) based on the dry protein. The reaction was allowed to proceed for 3 hours and the pH was not controlled. The final pH of the hydrolysate was 3.4. The *o*-phthaldialdehyde (OPA) method was used to determine α -amino groups and to calculate the DH for all hydrolysates [22]. In this

method, α -amino groups released by hydrolysis react with the OPA reagent and β -mercaptoethanol to form an adduct that absorbs strongly at 340 nm, quantification of which is indicative of DH.

Termination conditions

Enzymatic reactions were terminated by heating at two different time/temperature combinations: 90°C/15 min [14] and 75°C/30 min. Preliminary experiments using Fibrin Blue and Hide Powder Azure confirmed the efficacy of these treatments [23, 24].

Enzymatic reactions were also placed on ice after hydrolysis to slow enzymatic activity. Samples were frozen before all analyses except foam measurements, which were done immediately after hydrolysate formation and equilibration to room temperature (heated samples).

Turbidity experiments

Samples were diluted to a protein concentration of 1% w/v with different buffers. McIlvain buffers of pH 3.0, 4.0, and 5.0 were prepared using 0.1 M citric acid and 0.2 M dibasic sodium phosphate. Phosphate buffers of pH 6.0, 7.0, and 8.0 were prepared from 0.1 M monobasic sodium phosphate and 0.1 M dibasic sodium phosphate. Carbonate buffer of pH 9.0 was prepared with 0.1 M sodium carbonate and 0.1 M sodium bicarbonate. Samples were analyzed at room temperature and in the presence of SDS (5% w/v) and urea (6 M). Solutions were equilibrated for one hour before turbidity measurements were made in triplicate at 500 nm on a Spectronic 20 Genesys spectrophotometer (Thermo Electron Corporation, Waltham, MA).

Molecular Mass Distribution Profiles

Molecular mass distribution profiles were determined by high performance size exclusion chromatography (HPSEC). Analyses were performed on a Waters HPLC system (Millipore, Milford, MA) consisting of an injector (Rheodyne Model 7725i, Cotati, CA), two pumps (Model 515), and a photodiode array detector (Model 2996) adjusted at 220 nm. Data acquisition and analysis were done using the Empower chromatography software. The analysis was performed with a TSK-Gel G2000 SW_{XL} column (0.78 i.d. x 30 cm) from TosoHaas (Montgomeryville, PA) connected to a guard column (0.6 i.d. x 4 cm) filled with the same matrix. Samples were diluted to a concentration of 1% protein w/v with the mobile phase composed of 0.1% trifluoroacetic acid in 70% aqueous acetonitrile and then filtered on a 0.2- μ m membrane (PVDF). Elution was performed isocratically in the same mobile phase at 28°C with a flow rate of 0.6 mL min⁻¹ over 30 min.

Foam generation

A Kitchen Aid Ultra Power Mixer (Kitchen Aid, St. Joseph's, MI) with a 4.5 qt (4.3 L) stationary bowl and rotating beaters was used for foam formation. 5% w/v protein solutions (225 mL) were whipped at speed setting 8 (planetary rpm of 225 and beater rpm of 737) for 20 min.

Yield stress measurements

Foam yield stress was determined by vane rheometry as recently described [7, 8]. A Brookfield 25xLVTDV-ICP (Brookfield Engineering Laboratories, Inc. Middleboro, MA) viscometer was used at a speed of 0.3 rpm. The vane had a 10 mm diameter and 40 mm length. Maximum torque response (M_0) was documented for each of 3 measurements taken per foam and used to calculate yield stress according to published information [25]:

$$\tau_0 = \frac{M_0}{\left[\frac{h}{d} + \frac{1}{6} \right] \left(\frac{\pi d^3}{2} \right)} \quad [1]$$

where τ_0 is the yield stress, and h and d are the height and diameter of the vane. Three consecutive measurements (4 min max.) were taken per treatment, and each treatment was replicated a minimum of 3 times. This measurement was stable over the entire measurement time. We felt it important to produce foams at protein concentrations actually used in the food industry, i.e. $\geq 5\%$ w/v, as foam rheological behavior is expected to be quite different from the dilute hydrolysate foams described in the introduction.

Overrun

Overrun measurements were begun immediately after the final τ_0 measurement. Foam was carefully scooped from the bowl in a circular pattern with a rubber spatula, filling a standard weigh boat (100 mL) 3 times. The mean value was used to calculate overrun and air phase fraction according to [26]:

$$\% \text{ Overrun} = \frac{(\text{wt } 100 \text{ mL solution}) - (\text{wt } 100 \text{ mL foam})}{\text{wt } 100 \text{ mL foam}} \times 100 \quad [2]$$

$$\text{Air phase fraction} = \phi = \frac{\% \text{ overrun}}{(\% \text{ overrun} + 100)} \quad [3]$$

Overrun measurements were stable over the measurement time (3 min max.). Each treatment was replicated a minimum of 3 times to determine the average overrun.

Interfacial measurements

The foaming solutions or their dilutions were used for interfacial measurements. The following method of interfacial data collection has been recently described [7, 8]. An automated contact angle goniometer (Rame-Hart Inc., Mountain Lakes, NJ) was used for data collection and calculations in combination with the DROPimage computer program [27]. The dynamic surface tension of a 16- μL capillary drop was initially monitored for 5 min with a 1-s resolution. The capillary drop was formed within an environmental chamber at room temperature, in which standing water increased the relative humidity to minimize evaporation effects. Sinusoidal oscillations of the drop's area were then input by a volume amplitude of 0.5 μL , and the resulting change in interfacial tension was used to determine the dilatational modulus. Preliminary work confirmed this strain amplitude was within the linear viscoelastic regime and corresponded to a relative interfacial area change of $\sim 2.3\%$. From the modulus and from the phase angle between the surface area change and surface tension response, the DROPimage software calculates E' and E'' , which are equivalent to and proportional to the elastic and viscous components of the interface, respectively. The details for these calculations have been described elsewhere [27].

Results

Figure 1 shows the molecular mass distribution profiles for Alcalase (A), trypsin (B), and pepsin (C) hydrolysates obtained by HPSEC prior to heat treatment. Alcalase hydrolysates had the broadest distribution and highest proportion of small peptides. Trypsin and pepsin hydrolysates both had narrower molecular mass distribution profiles. Heating at 90°C/15 min or 75°C/30 min induced little to no detectable changes in the hydrolysate profiles for Alcalase and trypsin (data not shown). However, heating at 75°C/30 min induced considerable changes in the pepsin hydrolysate profile (Figure 1D) as seen by the appearance of smaller MW peptides. The profile of the pepsin hydrolysate heated at 90°C/15 min also changed as compared to the nonheated sample, although not as drastically (data not shown). Native β -lg is presented in figure 1E, none of which was detected in any hydrolysate profile.

The degree of hydrolysis for the different hydrolysates is presented in Table 1. Alcalase hydrolysates had the most extensive hydrolysis as compared to trypsin and pepsin hydrolysates, which showed similar results. Residual activity was observed for the nonheated hydrolysates as evidenced by their higher degrees of hydrolysis. Surprisingly, heating the pepsin hydrolysate at 75°C/30 min induced more hydrolysis than heating at 90°C/15 min.

Figure 2 summarizes τ_0 and overrun of foams prepared from the three hydrolysates and the unhydrolyzed substrate, β -lg (pH 7.0). Note these solutions were all at a protein concentration of 5% w/v. Data for whey protein isolate solutions at protein

concentrations of 5% and 10% w/v protein were included for comparison with previous work [4-8]. Heating at 90°C/15 min reduced τ_0 for all hydrolysates, but this effect was minimal for the trypsin hydrolysate. The foaming behavior of the trypsin hydrolysates was more similar to β -lg than either pepsin or Alcalase. Foam τ_0 of hydrolysate solutions heated at 75°C/30 min was more similar to unheated hydrolysates than hydrolysates heated at 90°C/15 min. Both heat treatments minimally affected overrun, with a slight negative effect detectable, especially for the pepsin hydrolysates. The decrease in overrun upon increasing the WPI protein concentration from 5% to 10% w/v is likely an artifact of the measurement resulting from increased drainage in the less concentrated solution [2, 8]. It is notable that in a screening study of various hydrolysates prepared from WPI, of which β -lg typically makes up $\geq 70\%$ of the protein, Alcalase hydrolysates had better overrun and stability (5% w/v whipped protein foams) as compared to those of trypsin and pepsin [28].

Heat treatments induced noticeable turbidity for both the Alcalase and pepsin hydrolysates at their respective pH's of 6.2 and 3.4. The aggregates causing this turbidity development were insoluble, as they would eventually sediment if given enough time. Turbidity was quantified by optical density measurements at 500 nm as a function of pH, both in the presence and absence of the chaotropic agents, SDS and Urea (Figure 3). All heated and unheated trypsin hydrolysates displayed a sharp peak in turbidity at pH 4.0, in good agreement with similarly prepared hydrolysates, as many of the generated peptides have pI's near 4.0 [20]. Both SDS and urea fully solubilized these aggregates. SDS fully solubilized all samples regardless of conditions, except for the Alcalase hydrolysates

heated at 90°C/15 min. This reflects the charged surfactant's capacity to interact with hydrophobic protein residues while simultaneously imparting a repulsive charge [29]. Both Alcalase and pepsin hydrolysates were more soluble than β -lg in the absence of heat (data not shown), which behaved very similarly to trypsin hydrolysates, in agreement with previous findings that have shown hydrolysis to improve solubility [9]. 6 M urea reduced turbidity for heated Alcalase and pepsin samples, suggesting H-bonding is partially responsible for the observed aggregation, although the reduction of hydrophobic interactions can't be excluded [30].

The interfacial adsorption rate of all hydrolysates (5% w/v) was faster than unhydrolyzed β -lg as determined by dynamic surface tension measurements (Figure 4a). Note that adsorption rates were qualitatively assessed by visualizing the rate of surface tension change, and quantitatively assessed by the surface tension observed at 5 min. Heating at 90°C/15min did not change adsorption of the Alcalase hydrolysates, decreased adsorption of the trypsin hydrolysates, and increased the adsorption rate of the pepsin hydrolysates (Figures 4a and 4b). Dynamic surface tension profiles were generally similar for hydrolysates heated at 90°C/15 min and 75°C/30 min (data not shown). Note the β -lg was at neutral pH and the reduced adsorption observed upon heating was a function of the molecule forming soluble, disulfide linked aggregates, which diffuse more slowly due to their larger size [8].

Figure 5 shows E' of the various hydrolysates and unhydrolyzed β -lg, all at a concentration of 5% w/v. All samples were aged 5 min and the frequency of oscillation

was 0.04 Hz. In the absence of heat, E' was notably improved for all hydrolysates as compared to β -lg. Note again the β -lg was at neutral pH and the heat treatments, both of which induced the formation of soluble, disulfide linked aggregates, slightly increased E' . The effects of this phenomenon on WPI interfacial rheological behavior have been recently documented [8]. E' of the unheated hydrolysates was similar for pepsin and Alcalase and both were notably higher than that of trypsin. Both heat treatments reduced E' for all hydrolysates, and this reduction was greater for those of Alcalase and trypsin as compared to pepsin hydrolysates.

Figure 6 shows the phase angle of the various hydrolysates and their dilutions as a function of surface pressure (Π). Π is defined as the net change in surface tension for the solvent (water) upon surfactant adsorption at a given time (5 min in this case).

Variations in Π were a function of the hydrolysate concentrations as listed in the figure legend. Note the phase angle corresponds to the viscous and elastic response ratio within the dilatational modulus, with higher phase angles indicative of a greater viscous response. Data for unhydrolyzed β -lg at pH 7.0 is included with the pepsin hydrolysates for comparison with all hydrolysates. The concentration of β -lg was 10%, 5%, 1% and 0.1% w/v from highest to lowest Π .

Discussion

Hydrolysates were characterized by HPSEC, in which components are separated by selective exclusion from a porous matrix based on differences in their hydrodynamic volumes, with smaller molecules eluting last. Alcalase is a serine alkaline protease

produced by a selected strain of *Bacillus licheniformis*. Its main enzyme component, subtilisin Carlsberg, has broad specificity, hydrolyzing most peptide bonds, preferentially those containing aromatic amino acids residues [31]. Within whey proteins, Alcalase was observed to have a high specificity for not only aromatic amino acid residues (Phe, Trp, and Tyr) but also for acidic (Glu), sulfur-containing (Met), aliphatic (Leu and Ala), hydroxyl (Ser), and basic (Lys) residues [32]. The heterogeneity of peptides generated by Alcalase likely explains the broad HPSEC distribution and high proportion of small peptides (Figure 1a). Its high DH value supports this hypothesis (Table 1). Trypsin specifically cleaves at lysine and arginine residues for which there are 18 total sites in the β -lg primary sequence [33], explaining its relatively narrow HPSEC peptide profile (Figure 1B).

Pepsin has a fairly broad specificity with a preference for cleaving after hydrophobic residues [34]. The HPSEC profile of unheated pepsin hydrolysate (Figure 1C) and its DH (Table 1) suggests this hydrolysis was less extensive than either Alcalase or trypsin. As previously mentioned, β -lg is inherently resistant to pepsin digestion unless preheated [21], as were the current pepsin hydrolysates. Pepsin inactivation at 75°C/30 min increased the DH (Table 1), in agreement with its HPSEC profile, which was broader than its unheated counterpart (Figures 1C and 1D). It seems that after the initial 3 hr hydrolysis, heating at 75°C/30 min allowed more of the partially digested β -lg substrate to be accessed by pepsin prior to its inactivation. We suspect that heating at 90°C/15 min inactivates pepsin more rapidly, minimizing any further hydrolysis, as supported by the lower DH of this hydrolysate (Table 1).

Solution pH of the hydrolysates was not adjusted prior to heat treatments to simulate immediate heat inactivation of such enzyme based ingredients. Accordingly, the decision was made to not to adjust the pH of the hydrolysates prior to the interfacial measurements in order to be consistent with foaming measurements. The interfacial properties of proteins are well documented to be affected by the solution pH [7, 35, 36]. A prominent example is the typical increase in adsorption rates and interfacial rheological moduli as the pH approaches a protein's isoelectric point, as the net neutrally charged molecules adsorb more efficiently and form intermolecular interactions between adsorbed species more effectively, due to decreased electrostatic repulsion [36]. Accordingly, the pH of the current hydrolysates was also expected to significantly influence the surface properties (and foaming behavior) of these materials. Work is currently ongoing to investigate pH effects on the foaming and interfacial behaviors of these hydrolysates.

Increased hydrolysate DH seemed to promote a more rapid adsorption at the air/water interface as indicated by dynamic surface tension measurements (Figure 4A and Table1). Increasing the DH should 1) yield a higher percentage of smaller peptides and 2) increase the potential for exposing previously buried hydrophobic residues, both of which should promote a more rapid adsorption [3]. However, due to the different specificities of each enzyme and hence different distributions of peptides within each hydrolysate, a definitive statement relating DH and adsorption rates is not possible.

Heating at both time/temperature combinations induced aggregation for the Alcalase and pepsin hydrolysates (Figure 3). Enhanced aggregation in the bulk phase should limit

adsorption at the air/water interface as the two processes are essentially competing for the peptides' hydrophobic contacts [37, 38]. Adsorption of the trypsin hydrolysates was reduced upon heating (Figure 4), even though it showed minimal heat induced aggregation near its pH of 8.0 (Figure 3). We therefore suspect this reduced adsorption was due to the formation of soluble, disulfide linked aggregates in the trypsin hydrolysate, as discussed previously for β -lg. This phenomenon readily occurred in similarly prepared trypsin hydrolysates at ambient temperatures [19], and heating should promote further this reaction. Adsorption of alcalase hydrolysates did not change upon heating at 90°C/15min, despite extensive aggregation (Figure 3 and Figure 4). This was surprising as these aggregates were insoluble. Perhaps the remaining soluble peptides are more surface active, or the insoluble aggregates are somehow contributing to the decrease in interfacial tension. More work is necessary to better understand this phenomenon. Heating at 90°C/15min actually increased the adsorption rate of the pepsin hydrolysates (Figure 4B). However, analysis of the pepsin HPSEC profile suggested a higher content of smaller peptides upon heating at 90°C/15 min, which seemingly override any negative aggregation effects by promoting a more rapid adsorption. Indeed, adsorption of the pepsin hydrolysate heated at 75°C/30 min was even more rapid than that heated at 90°C/15 min (data not shown), consistent with the increased DH observed for this hydrolysate as discussed previously.

E' primarily reflects the inherent rigidity of the adsorbed surfactant and the magnitude of the lateral interactions between adsorbed surfactants [39]. Comparative studies have shown that rigid proteins better transmit forces across the interface upon deformation as

compared to flexible proteins such as β -casein [40, 41]. One could imagine a very slight hydrolysis to potentially increase the flexibility of β -lg; however, no intact β -lg was detected in any of the current hydrolysates. Hence, the generated peptides are of a simpler structure and able to sample fewer conformations than unhydrolyzed β -lg. Once adsorbed at the air/water interface, these peptides might better transmit interfacial stresses, as there would be less energy dissipated in structural rearrangements. This may partially explain the high values of E' observed for many hydrolysates as compared to β -lg; however, there is a broad range of peptides produced for each hydrolysate and their exact interfacial compositions are unclear. Furthermore, interfacial peptide-peptide attractions have not been considered, but will be discussed next.

E' of the unheated Alcalase and pepsin hydrolysates (~ 140 mN/m) were much greater than β -lg at pH 7.0 (Figure 5). We can speculate on the interfacial peptide-peptide interactions for the Alcalase hydrolysates by comparison with a similarly prepared material [29, 32]. This earlier Alcalase hydrolysate was derived from WPI and gelled at higher concentrations (20% w/v) due to hydrophobic interactions between minimally charged peptides at the hydrolysate's natural pH of 6.0 [29, 32]. Comparison of the two Alcalase hydrolysates revealed very similar interfacial behaviors (data not shown). Therefore, we hypothesize that these same hydrophobic associations between minimally charged peptides are also present at the interface, and are primarily responsible for this hydrolysate's high value of E' . The unheated pepsin hydrolysates were considerably less hydrolyzed than that of Alcalase (Table 1), which might promote lower values of E' as discussed earlier; however, again this is ignoring the contribution from intermolecular

peptide-peptide interfacial attractions. We can't speculate too confidently on these interactions for pepsin hydrolysates; however, they are clearly strong. It is noteworthy that despite having drastically different HPSEC profiles and pH levels, the unheated pepsin and Alcalase hydrolysates behaved very similarly from a dilatational interfacial rheological perspective.

Heat-induced aggregation in the Alcalase and pepsin hydrolysates seemed to hinder peptide-peptide interactions at the interface, resulting in the reduced values of E' (Figure 5). As discussed previously, aggregation hinders accessibility of functional groups, i.e. hydrophobic clusters of amino acids that contribute to both the initial peptide adsorption and peptide-peptide interfacial attractions. The aggregates formed for β -lg upon heating at pH 7.0 were disulfide linked and soluble and actually increased E' . This was surprising as E' of WPI, which is typically $\geq 70\%$ β -lg, decreased upon formation of similar aggregates [8]. Similarly, E' decreased for the heated trypsin hydrolysates which showed very little aggregation near their pH value of 8.0 (Figure 3) and were expected to have formed disulfide linkages [19]. More work is ongoing to better understand the effects of these different aggregate types on dilatational rheological behavior.

In addition to their high magnitudes of E' , striking were the very low phase angles observed at a protein concentration of 5% w/v for the unheated Alcalase hydrolysate, unheated pepsin hydrolysate and pepsin hydrolysate heated at 75°C/30 min (Figure 6). Note the phase angle of unhydrolyzed β -lg steadily increased upon increasing protein concentration/ Π , as has been previously observed for several proteins [42]. This

increased viscous response is due to increasing interfacial relaxations, including diffusional exchange with the bulk solution. Indeed, the dilatational viscosity (oil/water) of an isolated peptide derived from β -casein increased with increasing peptide concentration, and was speculated to be caused by a diffusional relaxation mechanism [17]. However, it has been concluded that in time scales ranging from 1 to 1000s, relaxation methods within the interface must also be present [42]. These include structural rearrangements of the surfactant, which would be less likely at higher concentrations/ Π 's due to surfactant crowding at the interface [43]. This might explain the drastic drop in phase angle observed for these three hydrolysates at higher concentrations, coupled with their loss of structure upon hydrolysis (as discussed previously). However, due to the wide range of peptides produced by different enzymes and experimental conditions, a variety of interfacial behaviors are not surprising. Accordingly, the phase angle of all hydrolysates did not show this dramatic decrease at higher Π 's (Figure 6).

Frequency sweeps were conducted to better understand hydrolysate interfacial behaviors, while simultaneously comparing them to β -lg and Tween20®, a typical nonionic small molecular weight surfactant (SMWS). The rapid adsorption and low values of surface tension observed for many of the hydrolysates (Figure 4) suggested they might behave more like a typical SMWS, although the initial E' values suggested more of a protein-like behavior (Figure 5). Indeed, work with synthetic peptides revealed that minor amino acid substitutions can drastically alter a peptide's capacity in transmitting uniaxial stretching forces at the interface, from much less to much greater than β -lg [44]. Data for the

unheated Alcalase hydrolysate is presented in Figure 7, along with β -lg and Tween20. Measurements were made sequentially from lowest to highest frequency prior to an initial 5 min aging. The low values of E' and E'' observed for the lowest concentrations of β -lg and Alcalase hydrolysate were a function of not enough protein/peptide having adsorbed. Work with spread monolayers of β -lg suggests it undergoes non-diffusional relaxations occurring on the order of 20 s [45]. This timeframe is in the same magnitude with many of the frequencies tested in Figure 7, explaining the frequency dependence of E' observed at higher β -lg concentrations, in addition to the increased likelihood of interfacial desorption upon increasing concentration [46]. In contrast, the frequency independence and much lower values of E' observed for Tween20 solutions are typical of a SMWS, as these interfacial layers completely adjust to perturbations within these measurement timeframes.

Increasing the Alcalase hydrolysate concentration from 1% to 5% w/v saw E' become more frequency independent and E'' drop to essentially zero (Figure 7). This interface effectively behaved as a perfectly elastic 2-D solid. It seems that at 5% w/v, due to the decreased diffusion distances, these small, hydrophobic hydrolysate molecules adsorb quite rapidly, forming very strong and elastic interfaces. Interfacial relaxations, which are manifest in E'' , seem to be reduced due to the simplified internal structure of the peptides, and/or they may be occurring more rapidly than the measurement timeframe. Unheated and 75°C/30 min pepsin hydrolysates displayed similar responses to interfacial dilatational frequency sweeps as a function of concentration (data not shown), despite their different compositions and pH levels. Frequency sweeps of the trypsin hydrolysates

revealed interfacial rheological behavior more similar to β -lg. It seems all hydrolysate fractions do behave much more like proteins than typical SMWS's.

Theoretical analyses of foam rheology predict a strong dependence on interfacial rheology [47]. Recent experimental observations for highly concentrated, protein-stabilized emulsions, which share many similarities with foams, found their dimensionless bulk elasticity, $G'/(r\tau)$ to be positively correlated with E' [48].

Accordingly, previous work with WPI foams (10% w/v protein) revealed a positive, curvilinear relationship in the plot of E' vs. τ_0 [7, 8]. This trend was not as obvious for the current hydrolysates (Figure 8a). However, there was still a general positive correlation between the two measurements. When these measurements are combined with the data collected for WPI [7, 8], the current values all fell on or above the previous values (Figure 7b). Foams of purified β -lg typically have higher τ_0 's than those of WPI, and E' of adsorbed β -lg is generally higher than that of WPI [7]. Therefore, the shift to higher regimes seen in Figure 7b is partially explained by the substrate being β -lg and not WPI. The data for pepsin hydrolysates heated at 90°C/15 min and Alcalase hydrolysates heated at 75°C/30 min, strongly suggest that foam τ_0 depends on other factor(s) besides E' .

It is noteworthy that the three foams with the highest τ_0 were formed from hydrolysates that had *both* high values of E' and very low phase angles (essentially zero). This highly elastic interfacial state may promote increased foam stabilization, which would indirectly promote higher foam τ_0 by selecting for smaller bubbles [49, 50]. This is assuming high

values of E' and/or low phase angles do indeed retard foam destabilization mechanisms. However, a more direct influence on foam τ_0 may be coming from the interfacial tension gradients, which are manifest in the value of E' . The influence of these gradients on foam rheology becomes most important in “dry” foams, i.e. foams with air phase volumes (ϕ) above approximately 0.74 [47]. Note the ϕ 's of all current foams are greater than 0.9. These interfacial tension gradients impart resistance to interfacial deformation and were shown to be a primary contributor to a foam's dilatational viscosity in theory [47]. Accordingly, it seems logical these interfacial tension gradient effects are also present in these experimental systems and are directly influencing foam τ_0 .

Conclusions

Hydrolysis of β -lg with Alcalase and pepsin produced fractions that formed foams with significantly increased τ_0 as compared to unhydrolyzed β -lg. Trypsin hydrolysis only slightly improved foam τ_0 . Interfacial characterization of the hydrolysates revealed that samples that induced high foam τ_0 generally produced interfaces with high E' ; however, this was not a prerequisite. Furthermore, the three hydrolysate fractions imparting the highest values of τ_0 were all characterized by high values of E' and phase angles close to zero. Interfacial rheological frequency sweeps confirmed the formation of these extremely elastic interfaces with essentially no viscous response. Heating the hydrolysates at 75°C/30 min and 90°C/15 min both successfully terminated enzymatic activity for the three hydrolysates. However, heating at 75°C/30 min better preserved foam τ_0 and this generally reflected in higher E' and/or a low interfacial phase angle.

More work is ongoing to better understand some of the unique interfacial behaviors observed in these hydrolysates.

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Figure 1.

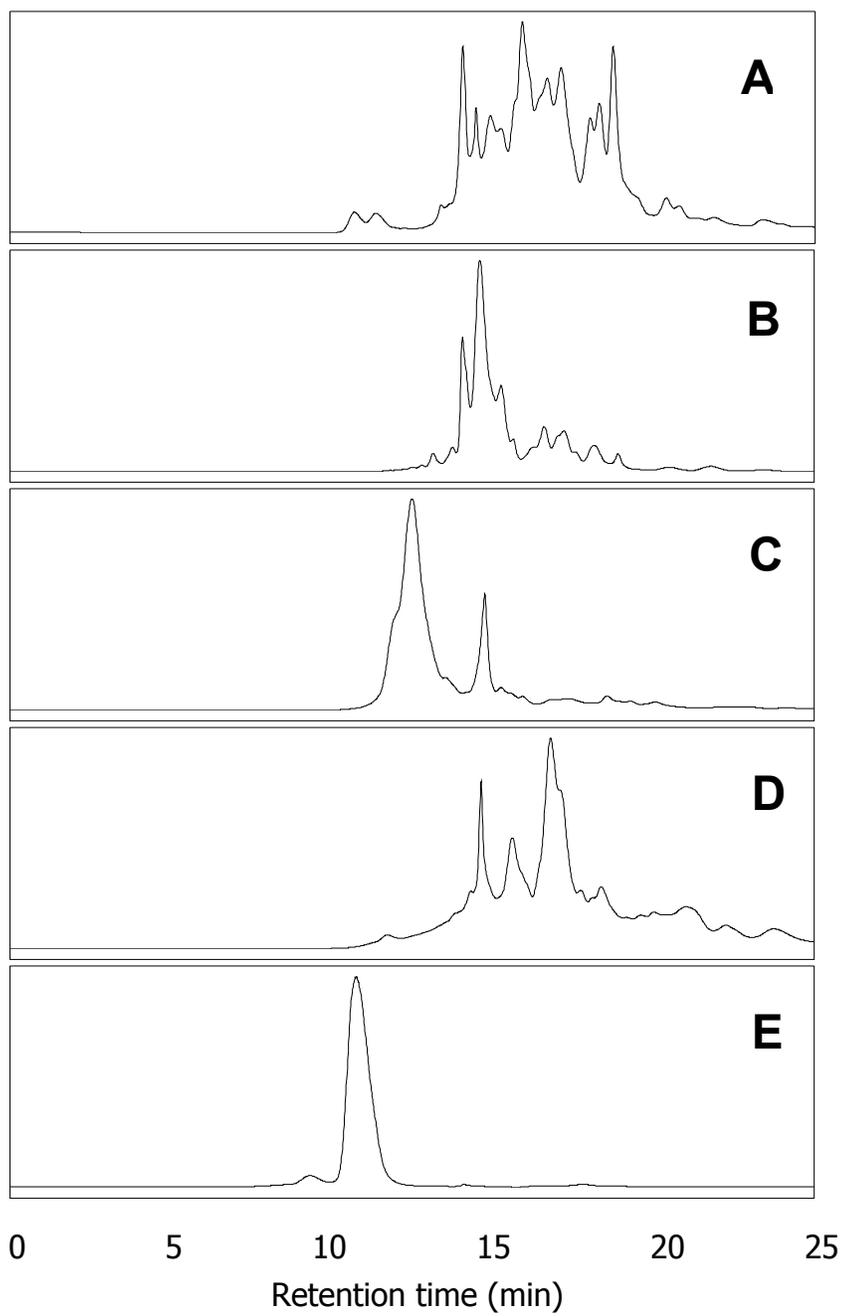


Figure 2.

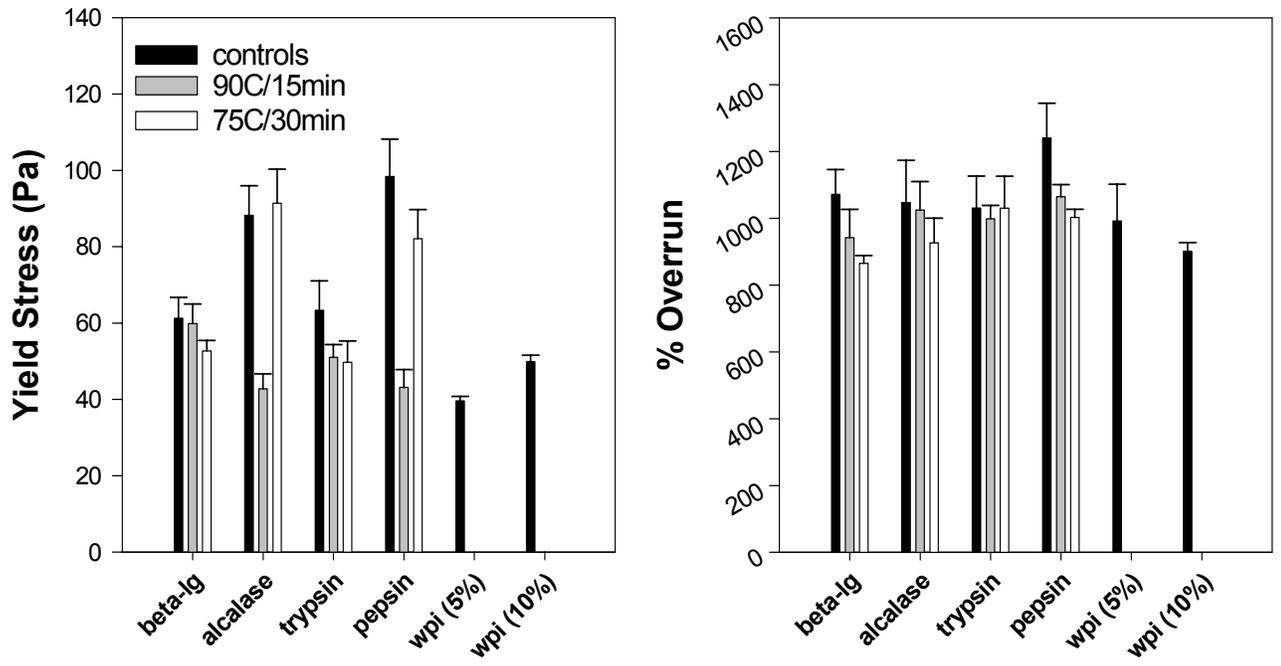


Figure 3.

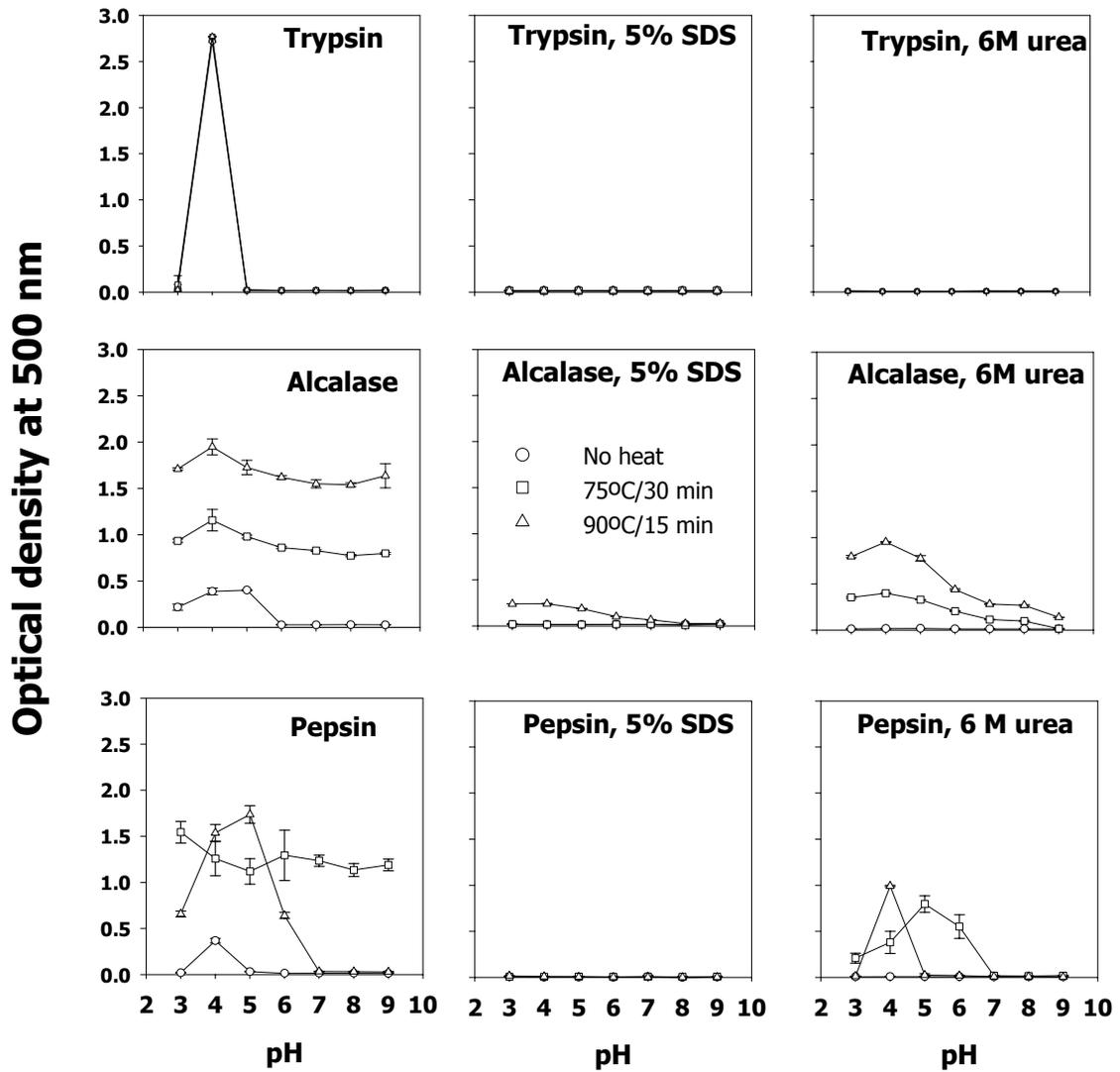


Figure 4.

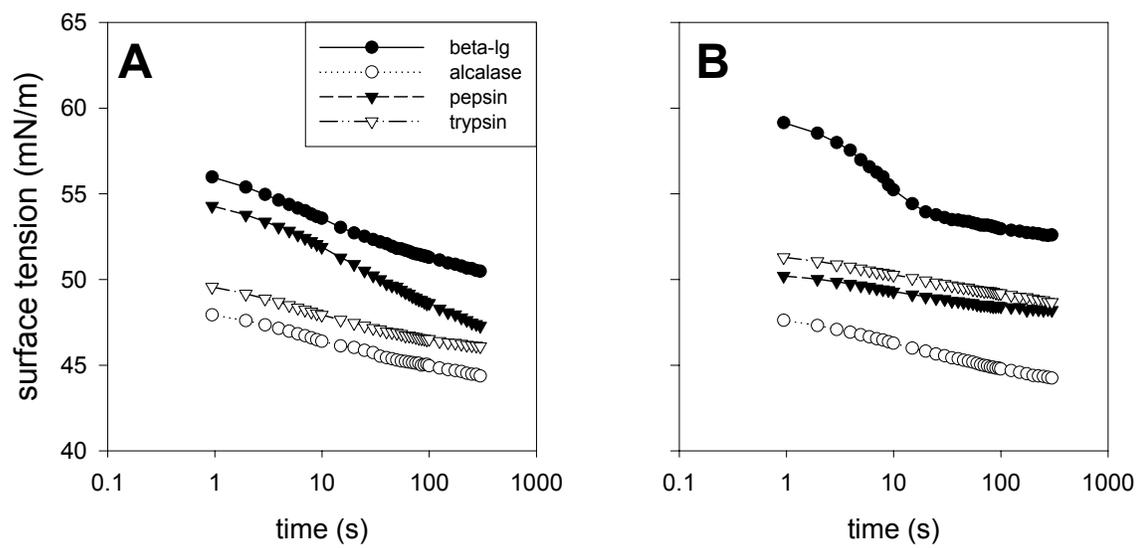


Figure 5.

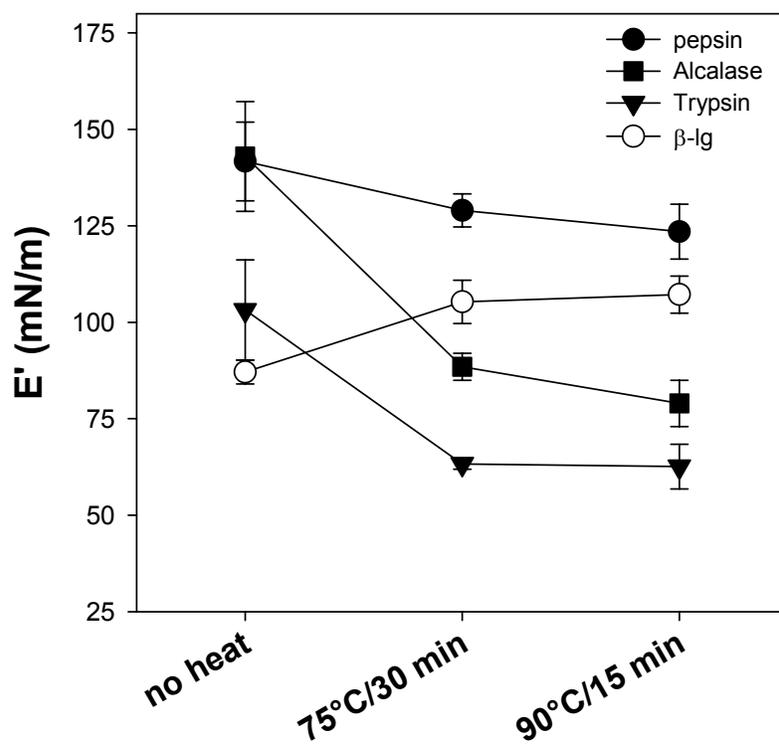


Figure 6.

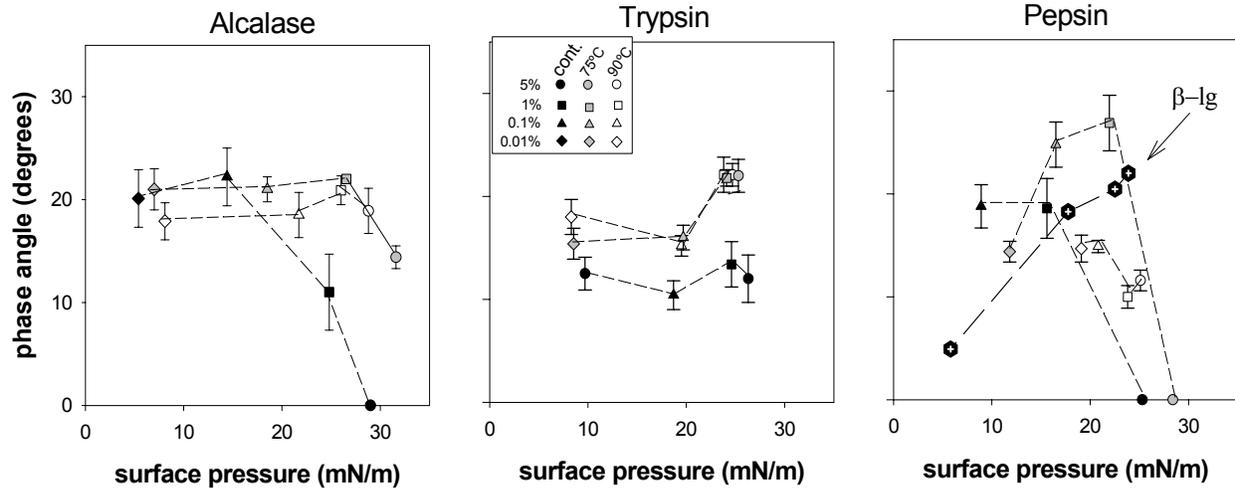


Figure 7.

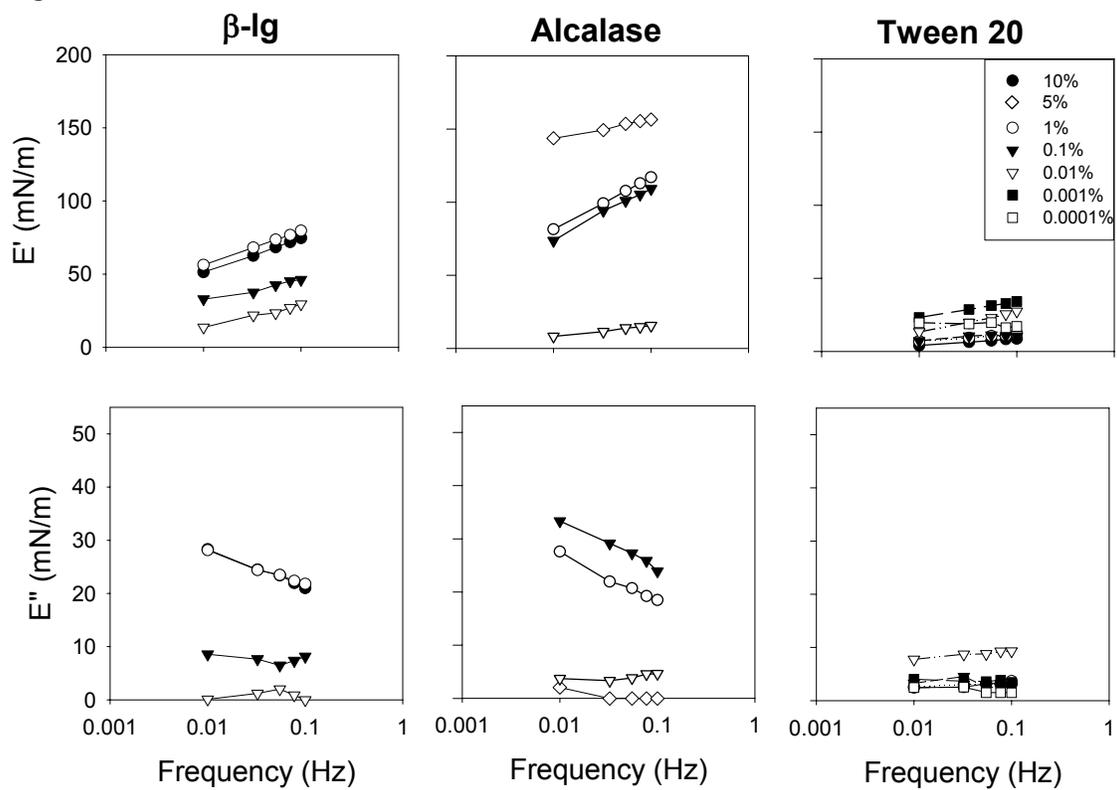


Figure 8.

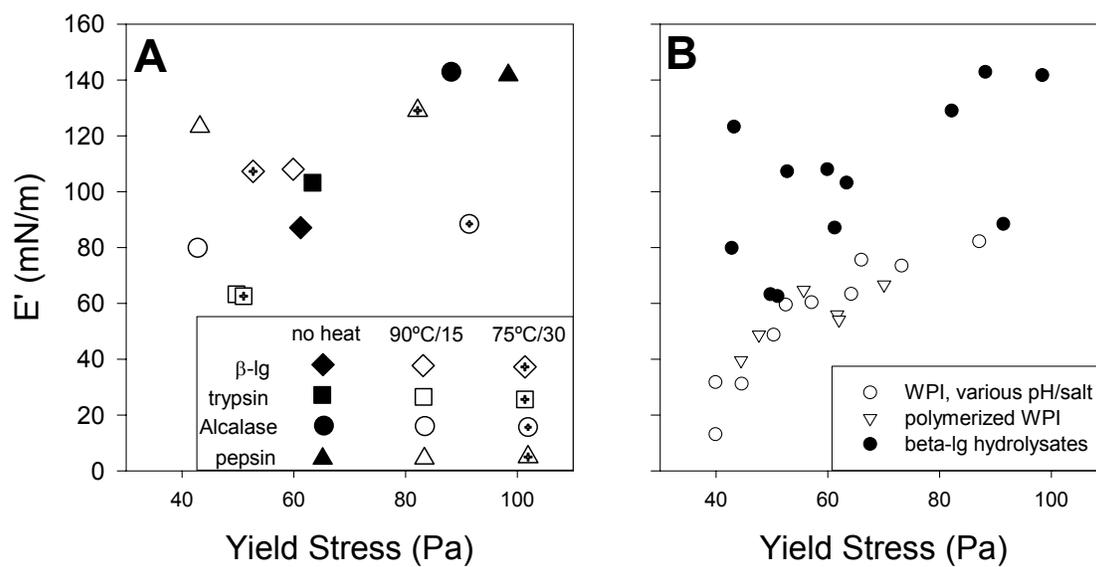


Table1. Degree of Hydrolysis (%) as determined by OPA method¹.

Hydrolysate	Heat treatment		
	<i>no heat</i>	<i>75°C/30 min</i>	<i>90°C/15 min</i>
<i>Alcalase</i>	15.6	14.2	13.7
<i>Trypsin</i>	6.7	4.6	4.8
<i>Pepsin</i>	5.2	8.4	4.7

1. Values are the average of three replications.

CHAPTER 4.

Comparisons of the Foaming and Interfacial Properties of Whey Protein Isolate and Egg White Protein

The contents of this chapter will be submitted for publication:

Davis, J.P. and Foegeding, E.A.

Abstract

Whipped foams (10% w/v protein, pH 7.0) were prepared from three commercially available ingredients, including whey protein isolate (WPI) and two egg white protein (EWP) based ingredients, and subsequently compared based on foam yield stress (τ_0), overrun and drainage stability. A range of tests were conducted at a model air/water interface via pendant drop tensiometry to better understand foaming differences among the three ingredients. The highest τ_0 and resistance to drainage were observed for standard EWP, followed by EWP with added 0.1% w/w sodium lauryl sulfate, and then WPI. Previously observed relationships between τ_0 and interfacial rheological measurements did not hold across the protein types; however these interfacial measurements did effectively differentiate foaming behaviors within EWP-based ingredients and within WPI. Addition of 25% w/w sucrose to the solutions increased τ_0 and drainage resistance of the EWP-based ingredients, but it decreased τ_0 of WPI foams and minimally affected their drainage rates. These differing sugar effects were reflected in the interfacial measurements, as sucrose addition increased the dilatational elasticity and decreased the interfacial phase angle for both EWP-based ingredients, while sucrose addition imparted the exact opposite effects on WPI. Several other interfacial behaviors are identified and discussed that further explain differences in foaming properties between the three ingredients. Many of these observations should be of practical use for those designing aerated food products containing sugars and/or proteins.

Introduction

Foam is a dispersion of gas bubbles within a liquid or solid continuous phase. These materials are important to the structure and texture of many food products, including various cakes, confections, meringues, etc [1]. Two common and important ingredients in many of these products are proteins and sugars. With regards to the foam properties, proteins function as surfactants by adsorbing at the freshly created aqueous/water interface during bubble formation [2]. This adsorption lowers the interfacial tension, which promotes bubble formation. Immediately after and during the initial adsorption, protein-protein attractions at the interface can result in network formation, which promotes bubble stability [3]. Besides their obvious contribution to product flavor, sugars can also contribute to the functional properties of foam. Sugars are known to improve the stability of foams to gravity induced drainage, primarily by their capacity to increase solution viscosity [4, 5]. However, studies at model air/water interfaces, also suggest sugars affect the interfacial behavior of proteins by exerting an influence on the protein structure [6-9].

There are various means of assessing the foaming performance of proteins, including their capacity to form (foamability), stabilize, and impart specific foam rheological properties. Controlling and predicting foam rheology is especially important when considering the final structural stability and texture of foamed food products. The most important physical factor governing foam rheology is the volume of gas (ϕ) incorporated into the continuous phase. Foams transition from viscous fluids to semi-solid like structures as ϕ increases from zero above the random close pack volume, $\phi_{rcp}=0.64$ [10].

Above ϕ_{rcp} , the formerly spherical bubbles begin contacting one another, forming so called “polyhedral” or “dry” foams. There is an ever developing quantitative framework to describe the unique rheological behaviors of polyhedral foams and concentrated emulsions, as the two systems share many similarities [3, 10].

A notable solid like behavior of polyhedral foams is their exhibition of a yield stress (τ_0), and a simple and effective method has been developed to measure τ_0 of whipped protein foams (considered polyhedral based on measurements of ϕ) via vane rheometry [11]. Initial work with this method established that it takes less protein and less whipping time for egg white protein (EWP) to produce foams with significantly improved τ_0 as compared to those prepared from whey protein isolate (WPI) [12]. Despite some limited correlations, it was generally found that differences in ϕ or equilibrium surface tensions (γ) for the two protein types were inadequate to explain the differences observed in τ_0 [12, 13]. This, despite the importance of these variables (γ and ϕ) within theoretical equations applied to such systems [10, 14, 15]. Indeed, others have experimentally verified that the shear elastic modulus (G') depends on ϕ for both concentrated emulsions [10, 16] and whipped foams prepared from EWP solubilized in high contents of invert sugar [17]. As discussed by Dimitrova and others, most models pertaining to polyhedral foam or concentrated emulsion rheology implicitly assume constant interfacial tension during perturbation [18]. However, there is a limited amount of theoretical work that suggests the interfacial rheological properties of the surfactant can significantly influence bulk foam or emulsion rheology [19, 20]. Experimental evidence for such phenomena is beginning to emerge. For example, data for protein-stabilized, concentrated emulsions

revealed a positive correlation between the dimensionless bulk elasticity, $G'/(γ/r)$ of the emulsions and the interfacial dilatational elasticity (E') of the stabilizing proteins, where r is equal to the radius of the dispersed phase [18]. In our own lab, recent work with whey proteins suggest a link between the dilatational rheological properties of the air/water interface and foam $τ_0$. Specifically, proteins and/or peptides which induce high values of E' and/or a low viscous modulus at a model air/water interface seem to promote high values of $τ_0$ when used to produce foams [21-23]. However, comparison of these interfacial and foaming measurements have not been extended to whipped foams prepared from other proteins, specifically EWP, which is the traditional foaming agent of choice in the food industry.

The overall goal of the current work is to determine the interfacial basis, if any, behind the different foaming properties of EWP and WPI, with an emphasis on the proteins' interfacial dilatational rheological behaviors. A secondary goal of this work is to assess the effects of relatively high concentrations of sucrose on the foaming and interfacial behavior of EWP and WPI. Work with model systems generally suggest that adsorption rates of globular proteins are suppressed at interfacial boundaries in the presence of sugars [7, 8, 24], although there is also evidence that sucrose addition may increase globular protein adsorption [6]. Interfacial rheological data of proteins in the presence of sugars is limited, with the available data suggesting high sugar contents (1 M) decrease the interfacial dilatational viscoelasticity of proteins, specifically bovine serum albumen [25]. A better understanding of sugar/protein interactions both at the interface and in

foaming systems is of significant practical interest to those preparing aerated food products containing protein and sweeteners.

Materials

Commercial samples of WPI (BiPro, 93.9% protein, dry basis) and β -lactoglobulin (Biopure, 97.0% protein, dry basis) were supplied by Davisco Foods International, Inc. (Le Sueur, MN). Within the β -lactoglobulin (β -lg) sample, β -lg made up approximately 93% of the total protein as determined by the manufacturer. Two types of spray dried egg white protein (82.4% protein, dry basis) were obtained from Primera Foods (Cameron, WI): 1) standard egg white protein (std-EWP) and 2) high whip egg white protein (hw-EWP). These products are essentially identical except the hw-EWP had not more than 0.1% sodium lauryl sulfate added as a whipping agent. Sucrose (SigmaUltra, $\geq 99.9\%$ purity) was purchased from Sigma Chemicals CO (St. Louis, MO). All other chemicals were of reagent grade quality. Deionized water was obtained using a Dracor Water Systems (Durham, NC) purification system. The resistivity was a minimum of 18.2 M Ω cm.

Methods

Hydration

Samples were initially hydrated to 10% w/v protein. Prior to the final volume adjustment, the pH of all solutions was adjusted to 7.0. When required, sucrose was added to the protein solutions on a % w/w basis.

Foam generation

A Kitchen Aid Ultra Power Mixer (Kitchen Aid, St. Joseph's, MI) with a 4.5 qt (4.3 L) stationary bowl and rotating beaters was used for foam formation. 10% w/v protein solutions (225 mL) were whipped at speed setting 8 (planetary rpm of 225 and beater rpm of 737) for 20 min for WPI solutions and 15 min for EWP solutions.

Yield stress measurements

Foam yield stress was determined by vane rheometry [11]. A Brookfield 25xLVTDV-ICP (Brookfield Engineering Laboratories, Inc. Middleboro, MA) viscometer was used at a speed of 0.3 rpm. The vane had a 10 mm diameter and 40 mm length. Maximum torque response (M_0) was documented for each of 3 measurements taken per foam and

$$\tau_0 = \frac{M_0}{\left[\frac{h}{d} + \frac{1}{6} \right] \left(\frac{\pi d^3}{2} \right)} \quad (1)$$

used to calculate yield stress according to published information [26, 27]:

where τ_0 is the yield stress, and h and d are the height and diameter of the vane. Three consecutive measurements (4 min max.) were taken per foam, and each solution type was replicated a minimum of 3 times.

3.4 Overrun

Overrun measurements were begun immediately after the final τ_0 measurement. Foam was carefully scooped from the bowl in a circular pattern with a rubber spatula, filling a

standard weigh boat (100 mL) 3 times. The mean value was used to calculate overrun and air phase fraction according to [1]:

$$\% \text{ Overrun} = \frac{(\text{wt } 100 \text{ mL solution}) - (\text{wt } 100 \text{ mL foam})}{\text{wt } 100 \text{ mL foam}} \times 100 \quad (2)$$

$$\text{Air phase fraction} = \phi = \frac{\% \text{ overrun}}{(\% \text{ overrun} + 100)} \quad (3)$$

Overrun measurements were stable over the measurement time (3 min max.). Each treatment was replicated a minimum of 3 times to determine the average overrun.

Stability measurements

Foam drainage was measured based on the method of [28]. Drainage measurements were begun immediately after the final overrun measurement. The time for ½ of the pre-foam mass to drain through a hole in a whipping bowl was taken as a measurement of foam stability. Note that the mass of foam removed during the overrun measurements was subtracted when calculating ½ of the pre-foam mass. The starting time for these measurements was taken as immediately after foam formation.

Interfacial measurements

The foaming solutions or their dilutions were used for interfacial measurements. Pendant drop tensiometry is an established method for measuring surfactant behavior at liquid phase boundaries [29, 30]. An automated contact angle goniometer (Rame-Hart Inc., Mountain Lakes, NJ) was used for data collection and calculations in combination with

the DROPimage computer program [31]. Most measurements were made from vertical drops (16 μl) dangling from a capillary into an environmental chamber with standing water at its bottom to minimize evaporation. Other measurements were made from an inverted needle immersed in the solution of interest, in which a bubble of air (15 μl) was introduced at the tip of the needle. All measurements were made at room temperature. When required, changes in the surface tensions of the drops or bubbles were monitored with a 1-s resolution. Sinusoidal oscillations of the drops' or bubbles' areas were input by a volume amplitude of 0.5 μL , and the resulting change in interfacial tension was used to determine the dilatational modulus. From the modulus and from the phase angle between the surface area change and surface tension response, the DROPimage software calculates E' and E'' , which are equivalent to and proportional to the elastic and viscous components of the interface, respectively. The details for these calculations have been described elsewhere [31]. Frequencies applied in this work ranged from 0.01 Hz to 0.1Hz. Preliminary work confirmed this strain amplitude was within the linear viscoelastic regime for all samples at all frequencies and corresponded to a relative interfacial area change of $\sim 2.3\%$ for both the bubbles and drops.

Density determination

Densities of the component phases are required inputs for the determination of interfacial tension from the shape analysis of drops and bubbles [32]. Accordingly, a Mettler-Toledo DE40 density meter (Mettler-Toledo, Columbus, OH) equipped with a viscosity correction card was used to determine the density of each solution at 23 $^{\circ}\text{C}$. The

accuracy of the instrument was $1 \times 10^{-4} \text{ g/cm}^3$ and every solution was measured in duplicate and averaged prior to interfacial measurements.

Results & Discussion

The foaming properties of the different protein solutions (10% w/v, pH 7.0), both in the presence and absence of 25% w/w sucrose, are summarized in figure 1. Foam yield stress (τ_0) was significantly greater for both the standard egg white protein (std-EWP) and the high whip egg white protein (hw-EWP) as compared to WPI (figure 1A). This is in agreement with previously reported data for WPI and std-EWP [11, 12]. Overrun was slightly higher for WPI than either std-EWP or hw-EWP in the absence of 25% w/w sucrose (Figure 1B). Sugar addition significantly decreased overrun for all three foaming ingredients (Figure 1B). The time required for $\frac{1}{2}$ of the pre-foam mass to drain through a hole near the base of the whipping bowls was taken as a measurement of foam stability [21, 28]. As seen in Figure 1C, half life was significantly greater for the std-EWP and hw-EWP foams as compared to WPI, both in the presence and absence of 25% w/w sucrose. Sucrose addition significantly increased foam half life for the std-EWP and hw-EWP foams, whereas sucrose addition minimally affected the drainage rates of the WPI foams (Figure 1C). Previous work found the addition of 10% sucrose decreased foam overrun and increased the stability against drainage of whipped WPI solutions and improved foam stability against drainage [33].

Foam yield stress (τ_0), like all foam rheological measurements, strongly depends on the amount of air incorporated into the continuous phase or its air phase volume (ϕ).

Application of equation 3 to the overrun measurements presented in figure 1B reveal all of the current foams have ϕ 's ≥ 0.88 , well above ϕ_{rcp} , meaning these foams can be considered polyhedral. Most equations describing polyhedral foam rheology predict τ_0 to increase with increasing ϕ and/or decreasing bubble size [14, 15]. A simple comparison of Figures 1A and 1B reveals that τ_0 and overrun, which is directly proportional to ϕ as seen in equation 3, do not positively correlate in the current foams. However, such conclusions are limited without an accurate description of the bubble size distribution, a parameter that also significantly influences foam rheology [14]. Confocal microscopy is one technique applied to characterizing bubble sizes in foams [12, 17]. Surprisingly, direct comparison of 10% w/v protein foams of WPI and std-EWP, each solubilized in the presence of approximately 16.2% w/v powdered sugar, revealed no difference in bubble size distribution [12]; however, this may reflect a limitation of the method and not an actual physical phenomenon. Lau et al. observed qualitative differences in bubble size over whipping time with EWP solubilized in a high content of invert sugar; however the phase volumes of such materials were significantly lower ($\phi \leq \sim 0.54$) [17].

Comparison of τ_0 of the various foams to their respective stabilities against drainage (Figures 1A and 1C) reveals a positive correlation between the two measurements. This seems logical as increased foam stability to drainage should correlate with both higher ϕ 's and smaller bubbles, both of which should increase τ_0 [14]. Interestingly, addition of 25% w/w sucrose significantly improved the two EWP based ingredients resistance to drainage, whereas for WPI, sucrose addition minimally affected drainage rates (Figure 1C). If the increased resistance to drainage was solely attributable to an increase in

continuous phase viscosity, one might expect a uniform improvement in drainage for all three foaming ingredients. Since this was not observed, it seems likely that the sucrose addition was affecting the functional properties of the various proteins differently, as discussed later.

Adsorption rates at the air/water interface of the three foaming solutions were measured by qualitatively assessing the rate of surface tension (γ) decline of freshly formed pendant drops [34-36]. Data for 10% w/v protein solutions are presented in Figure 2. Adsorption rates were most rapid for the hw-EWP solution, followed by std-EWP and then WPI, both in the presence and absence of 25% w/w sucrose. As mentioned previously, the hw-EWP ingredient contained approximately 0.1% sodium lauryl sulfate, in addition to the albumen protein also found in the std-EWP. Sodium lauryl sulfate is a typical small molecular weight surfactant (SMWS), which are characteristically more effective than proteins at rapidly decreasing γ [37]. Furthermore, work with protein/SMWS mixtures have shown that small amounts of SMWS relative to protein, can profoundly increase the rate of γ decline relative to that observed for proteins in the absence of SMWS [37]. Therefore, the presence of sodium lauryl sulfate in the hw-EWP ingredient likely explains its more rapid decrease in γ as compared to WPI and std-EWP, which contained only protein as surfactants.

Conflicting reports exist in the literature as to the effects of added sucrose on protein adsorption. Bovine Serum Albumin (BSA) was found to adsorb more rapidly at the air/water interface in the presence of 1M sucrose during the 1st stage of adsorption, in

which diffusion dominates this process [6]. A potential explanation was that the protein molecule would be more compact in sugar solutions, due to the well established phenomenon of preferential hydration [38], and hence adsorb more rapidly. It was also noted that the increased solution viscosity imparted by the sugar solutions should limit diffusion to the interface, meaning protein adsorption in sugar solutions should be a balance of these two phenomena. In a separate study, increasing concentrations of sucrose, up to 40% w/w (~1.4 M), were found to decrease the adsorption rate of BSA [7]. Potential explanations included the increased solution viscosity, the potential for direct sucrose-protein interactions with which would decrease the molecule's hydrophobicity, and preferential hydration of the proteins. Ovalbumin, the primary egg white protein, was also found to adsorb less rapidly at the air water interface in the presence of sucrose [24]. Mixing calorimetry data suggested this protein participated in hydrogen bonding with the sucrose molecule, potentially decreasing its hydrophobicity and hence its surface activity.

It is noted that in the above studies, the protein concentrations were all several orders of magnitude more dilute than concentrations (5-10% w/v) typically found in industrial food foams. In the current work, adsorptions of 10% w/v protein solutions were measured in the presence or absence of 25% w/w sucrose (Figure 2B). Sucrose addition did slow the rate of surface tension decline for both WPI and std-WPI, but less so for hw-EWP.

Potential reasons for such decreases have already been discussed. If sucrose addition was restricting surfactant adsorption primarily via an increase in solution viscosity, it could be hypothesized all surfactants would show proportional decreases in surface tension

decline, which does not seem to be the case. However, if sucrose is affecting the structure of the surfactant molecules, these effects should be minimal for the sodium lauryl sulfate present in the hw-EWP, due to its simpler structure as compared to proteins, potentially explaining the lessened effect sucrose addition had on hw-EWP adsorption.

As seen in Figure 1, the addition of 25% w/w sucrose to each protein solution decreased foam overrun. It is established that overrun measurements can be influenced by drainage rates, that is, decreased liquid drainage increases foam density and hence decreases overrun, while increased liquid drainage decreases foam density, making overrun measurements higher [5]. We have suspected this phenomena in earlier work [21] and it can not be ruled out with the current foaming solutions. An alternative explanation for the drop in overrun upon adding 25% w/w sucrose is the reduced adsorbance observed in these foaming solutions upon equivalent sucrose addition (Figure 2). This is because the capacity of a surfactant to rapidly decrease γ is thought to promote bubble formation and hence increase ϕ [39].

Previous work with whey protein: 1) solubilized across a range of electrostatic conditions [23], 2) in the presence of various amounts of polymerized whey protein [21], and 3) after hydrolysis with various enzymes [22], revealed potential relationships between interfacial rheology and foam rheology. Specifically, proteins and/or peptides which induce high values of E' and/or a low viscous modulus at a model air/water interface seem to promote high values of τ_0 when used to produce foams. E' is the amount of recoverable energy upon dilatational interfacial deformations and can be thought of as the

stiffness of a surfactant covered interface to dilatational motions [40]. The phase angle is proportional to ratio of the viscous modulus (energy lost upon dilatational interfacial deformations) and elastic modulus, with higher phase angles indicative of an increased viscous modulus [40]. For the above solutions, interfacial dilatational rheological properties of the various solutions were analyzed via an oscillating pendant drop [30]. Conditions for these interfacial measurements were specific, and included a 16 μl capillary drop which had been aged 5 min, prior to oscillation at 0.04 Hz with either a 1 μl or 0.5 μl amplitude, corresponding to approximately 5% and 2.3% area changes respectively. Note the amplitude was reduced for several of the highly elastic β -lactoglobulin hydrolysates to ensure a linear viscoelastic response. The same interfacial test (0.5 μl amplitude) was applied to the current solutions, both in the presence and absence of 25% w/w sucrose, and the resulting E' values are plotted with τ_0 in Figure 3. The protein concentration for the interfacial measurements was 10% w/v, identical to that actually used in the foaming measurements. It is clearly seen in Figure 3, that EWP-based foams have significantly higher values of τ_0 , despite lower and/or equivalent values of E' at a model air/water interface. Striking also were the differing effects addition of 25% w/w sucrose had on the foaming ingredients, as sucrose addition increased E' for EWP based ingredients, whereas sucrose addition decreased this parameter for WPI (Figure 3). In the absence of sugar, the phase angles for WPI and std-EWP were equivalent (approximately 20°) whereas the phase angle for hw-EWP was greater at approximately 25° (data not shown). Phase angle measurements will be discussed in more detail later.

To further explore the interfacial rheological behaviors of these various foaming ingredients, it was decided to increase the frequency of oscillation to 0.1 Hz for several reasons, which included: 1) The perturbations actual foams experience during their formation and subsequent processing are likely much more rapid than even 0.1 Hz, which is approaching the upper frequency limit of the instrument, 2) The limiting interfacial dilatational elasticity (E_0) of proteins should be approached under a given set of conditions as the frequency of oscillation is increased [41]. 3) Increases in frequency allows for more information to be collected within a given measurement time.

Data for WPI, std-EWP and hw-EWP, all in the absence of sucrose, are presented in figure 4, where the first measurable data point of E' is plotted as a function of surface pressure (Π). Note that $\Pi = \gamma_0 - \gamma$, where γ_0 is the surface tension of the solvent (water in this case), and γ is the surface tension of the solution at a given time. The frequency of oscillation was 0.1 Hz and was begun immediately after drop formation. Both the foaming samples and their dilutions were analyzed, with the goal of dilution being to better understand the effect of bulk protein concentration of interfacial rheology. These measurements were made between 50 and 100 s for all solutions except for those of maximal dilution (0.013% w/v), for which there was typically a time lag prior to real data being collected. This is because a minimal adsorbed amount (Γ) is necessary to induce an interfacial rheological response, and for the maximally diluted samples, this minimal adsorption took longer to reach due to diffusion considerations [41].

It is noted that Π decreased for all solutions upon increased dilution of the foaming agents (Figure 4). This was expected, as the capacity of a surfactant to decrease γ , and hence increase Π , is closely related to its bulk concentration, primarily due to diffusion considerations [41]. It is seen in Figure 4, that E' of WPI displayed a sigmoidal response with increasing concentration/ Π , with E' ultimately peaking at 10% w/v protein. In contrast, E' of the std-EWP and hw-EWP solutions peaked at lower concentrations/ Π 's than WPI, with peak values occurring near 0.625% w/v protein for std-EWP, and between 0.063% and 1.25% w/v protein for the hw-EWP.

As seen in figure 4, the bulk concentration at which interfacial rheological measurements are made strongly influences the measurements of E' . The concentration at which E' peaks for WPI is at the highest concentration tested (10% w/v protein), whereas for std-EWP and hw-EWP, this peak occurs at lower concentrations. As discussed by Lucassen-Reynders, the capacity of a surfactant to stabilize interfaces does depend on E' ; however, it is not a simple proportionality [42]. Instead, foaming agents are most effective at concentrations such that E' increases as the bulk concentration of surfactant decreases. This is because during the dynamics of foam formation and breakdown, surfactant is constantly being depleted, either by expansion of the interfaces, or through losses to drainage [42]. As seen in Figure 4, this condition is fulfilled with the two EWP based ingredients, but not for WPI. The peak in E' followed by a gradual decline with increasing concentration is expected for all SMWS's, as this effect is not necessarily a function of any interfacial intermolecular interactions, which are minimal for this class of surfactants. Instead, it results from increasing bulk surfactant concentrations leveling off

the gradients in interfacial tension which are manifest in E' [42]. This likely explains the response of hw-EWP as a function of concentration, since it contained approximately 0.1% sodium lauryl sulfate as an additional whipping agent. As discussed next, protein-protein interactions significantly impact measurements of E' as their bulk concentration is increased. Regardless, std-EWP does pass through a maximum in E' at concentrations lower than that utilized for foaming in the current work, providing a helpful insight into its improved foaming performance as compared to WPI.

Interfacial measurements were extended to longer times to observe aging effects on the interfacial dilatational rheology of the various foaming solutions and their dilutions. Data for WPI, std-EWP and hw-EWP, all in the absence of sucrose, are presented in Figure 5, where both E' and the phase angle of the various solutions are plotted against Π . The non-diluted solutions (10% w/v protein) and maximally diluted samples (0.013% w/v protein) were each analyzed for ~ 1 hr, whereas the other samples were typically analyzed for approximately 20 min. The frequency of oscillation was 0.1 Hz and was begun immediately after drop formation.

It was observed that the slope of E' vs Π for WPI increased as the bulk protein concentration was increased (Figure 5), which is in general agreement with similar data for WPI [43] and theoretical equations for protein adsorption/interfacial rheology [2]. A maximum in E' was observed for WPI at the highest protein concentration tested (10% w/v), prior to a slight decrease in this parameter upon increased aging. Analysis of the phase angles for WPI as a function of Π suggested a transitional behavior between

approximately 0.63 and 1.25% w/v protein (Figure 5). At concentrations $\geq 1.25\%$, the phase angle decreased sharply with increasing Π , meaning the interfacial layer was becoming more elastic and less viscous with time. At concentrations up to approximately 0.63%, the phase angle was essentially increasing, as a minimal interfacial concentration was building, prior to the point where the interface starts becoming more elastic.

The interfacial rheological behavior of the std-EWP and hw-EWP ingredients displayed several notable differences as compared to WPI (Figure 5). The slope of E' vs Π did increase with increasing concentration/ Π for both EWP based ingredients, but not as drastically as observed for WPI (Figures 5). Also, E' of undiluted std-EWP and hw-EWP solutions showed no decline over the 1 hr test period, whereas WPI did display a maximum in E' followed by a slight decrease (Figure 5). Transitions in which the phase angle began to decrease with age were also observed for both the std-EWP and hw-EWP; however these transitions occurred at lower concentrations, somewhere between 0.13% and 0.31% for both EWP based ingredients (Figure 5).

Surface equation of states developed for SMWS's are inadequate to describe the complex adsorption behaviors of proteins [2]. All protein adsorption studies are characterized by extreme non-ideal behavior such that Π is not proportional to the surface concentration (Γ) even at very low surface pressures [2]. This non-ideal thermodynamic behavior ultimately results from both reorientations of proteins and protein-protein interactions at the interface [41]. In plots of E' vs. Π this non-ideal behavior is manifest in the steep slopes often observed for various types of proteins [2, 41]. Analysis for the current

protein ingredients reveals that WPI shows extreme non-ideal behavior at much lower concentrations than either EWP based ingredient. That is, a rapid increase in the slope of E' vs. Π is observed at a surface pressures above approximately 15 mN/m, with values typically ranging between $\Delta E'/\Delta \Pi = 15-16$. Patino et al applied such an approach to WPI adsorbed at the oil/water interface using pendant drop tensiometry (0.1Hz, 15% area amplitude, pH 7.0, I=0.05 M) and this data shares many similarities with the current data [43]. These authors reported a rapid increase in the slope of E' vs Π , at surface pressures above approximately 12.5 mN/m, which generally agrees with the current data for WPI at the air/water interface. The slope of E' vs Π was considerably less steep (approximately 4) for WPI adsorbed at the oil/water interface which may reflect difference in the two types of interfaces.

In contrast to WPI, the slopes of E' vs Π for the std-EWP did not intensify as rapidly upon increasing protein concentration (Figure 5). This suggests the adsorbed form of the std-EWP proteins is more consistent across the concentration regimes of adsorption. Similarly, the hw-EWP ingredient also displayed less non-ideality in its plot of E' vs Π , as only at the highest concentration tested, did the slope of E' vs Π become noticeably more steep.

To better understand the interfacial rheological behavior of WPI, identical measurements were conducted with β -lg as it is the primary whey protein and thought to dominate the functionality of WPI. This data is summarized in Figure 6. This protein behaved

similarly to WPI although it was generally more uniform. It is noted that maximums in E' (initial values) occur at concentrations below 10% for purified β -lg, unlike WPI.

It is clear from Figures 5 & 6, that the concentration of protein (or SMWS) strongly influences the interfacial rheological response of such materials. With the primary goal of interfacial measurements being the replication of conditions actually found in protein-based foams, it seems logical to utilize concentrations for interfacial measurements at the same concentration utilized in the foaming application. Accordingly, it was decided to investigate the effects of added sucrose on foaming ingredient interfacial rheology, at a protein concentration of 10% w/v. This data is summarized in figure 7. The frequency of oscillation was 0.1 Hz and was begun immediately after drop formation, and all samples were tested for 20 min. Note that in calculating Π for the sucrose containing solutions, values of ~ 74.4 and 77.0 mN/m were used for γ_0 , as these were experimentally determined for 25 and 50% w/w sucrose solutions in water respectively, and in general agreement with previously reported data concerning such solutions [44]. As noted earlier, sucrose addition affected the different foaming ingredients quite differently. Increasing concentrations of sucrose decreased E' for WPI in addition to lowering the phase angle of this material, whereas exactly opposite effects were observed within the EWP-based ingredients (Figure 7). Earlier work with BSA did find sucrose addition (1 M) to decrease the interfacial viscoelasticity of this molecule [25]. The reasons for these contrasting effects for WPI and EWP are not clear; however this does seem to reflect in the foam properties: including foam τ_0 and drainage rates. The slope of E' vs. Π was

decreased for all samples upon sucrose addition; however, this was more apparent for the two EWP based ingredients (Figure 7).

Conclusions

Interfacial tests at a model air/water interface were utilized to investigate differences in foaming behaviors between WPI and std-EWP and hw-EWP. Adsorption rates at the air/water interface were most rapid for hw-EWP, followed by std-EWP and then WPI. The rapid adsorption of hw-EWP was attributable to the additional 0.1% w/w sodium lauryl sulfate added to this ingredient. Addition of 25% w/w sucrose slowed the rate of surface tension decline for WPI and std-EWP in agreement with previous researchers examining protein adsorption in the presence of sugars, but sucrose addition minimally affected adsorption of the hw-EWP. Addition of 25% w/w sucrose significantly improved the τ_0 and resistance to drainage of the two EWP-based foams; however, equivalent additions of sucrose to WPI resulted in reduced τ_0 and similar drainage rates. Interfacial rheological tests revealed sucrose to be affecting the foaming ingredients differently, with it increasing E' and lowering the interfacial phase angle of std-EWP and hw-EWP, while imparting opposite affects to WPI. Previous work has established that increases in E' and/or decreases in interfacial phase angle correlate with improved τ_0 , and this was also found to be true with the current solutions.

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Figure 1.

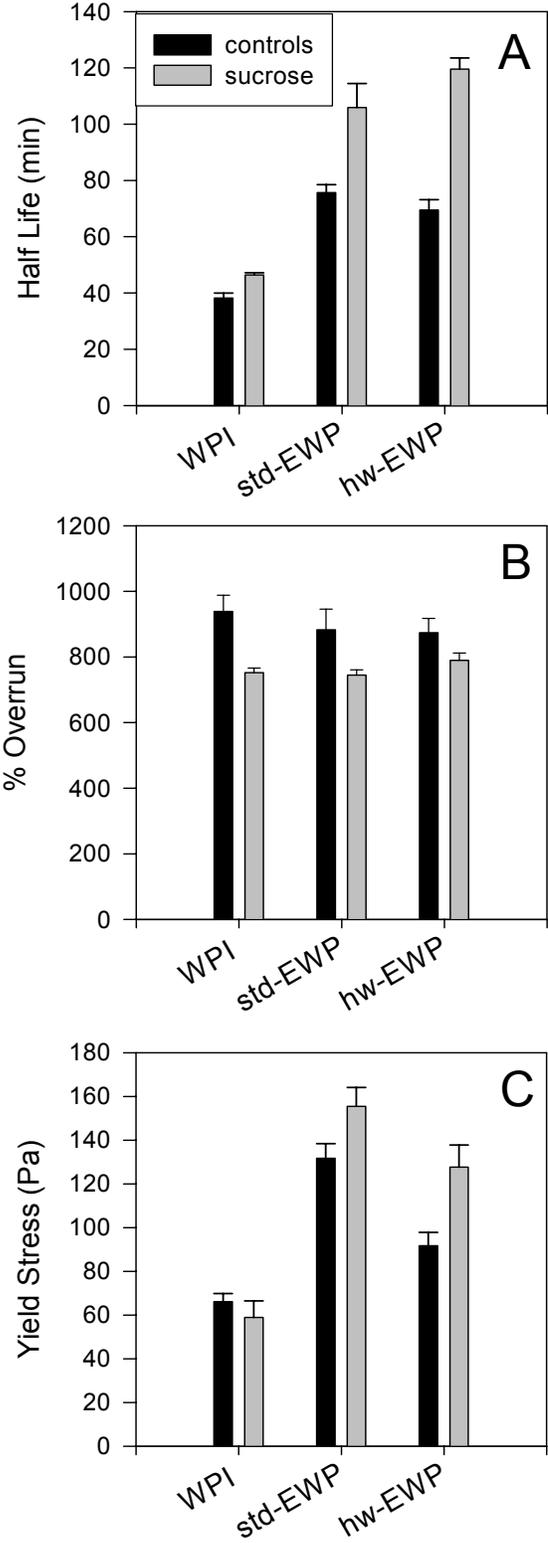


Figure 2.

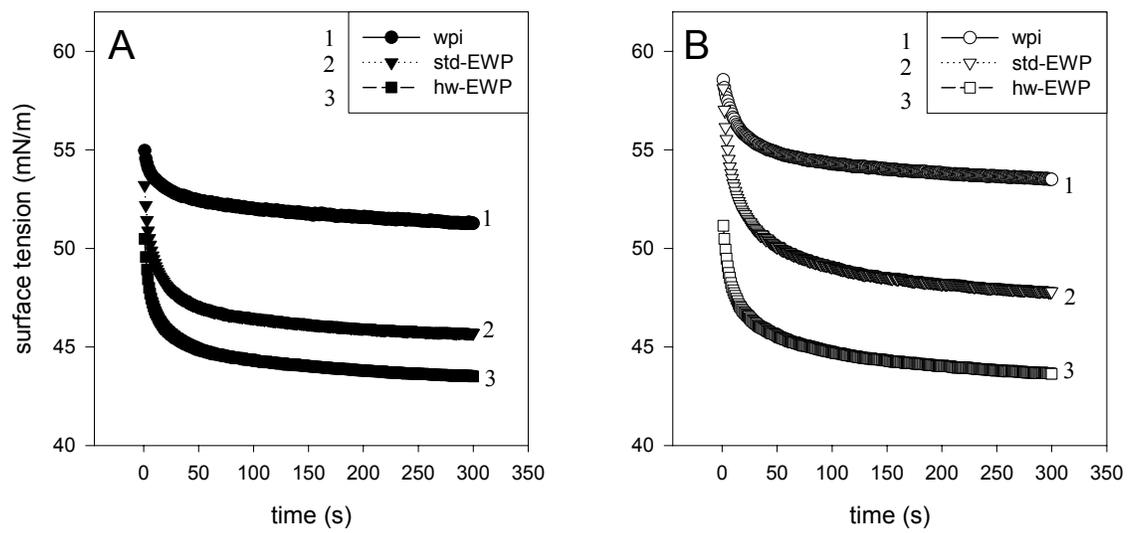


Figure 3.

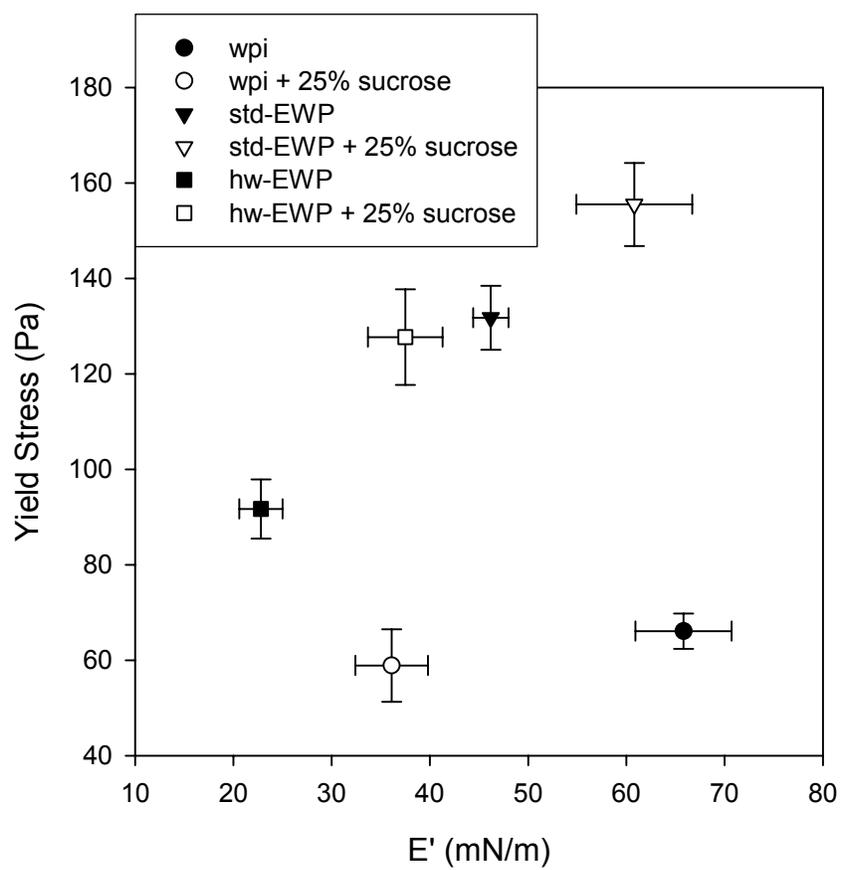


Figure 4.

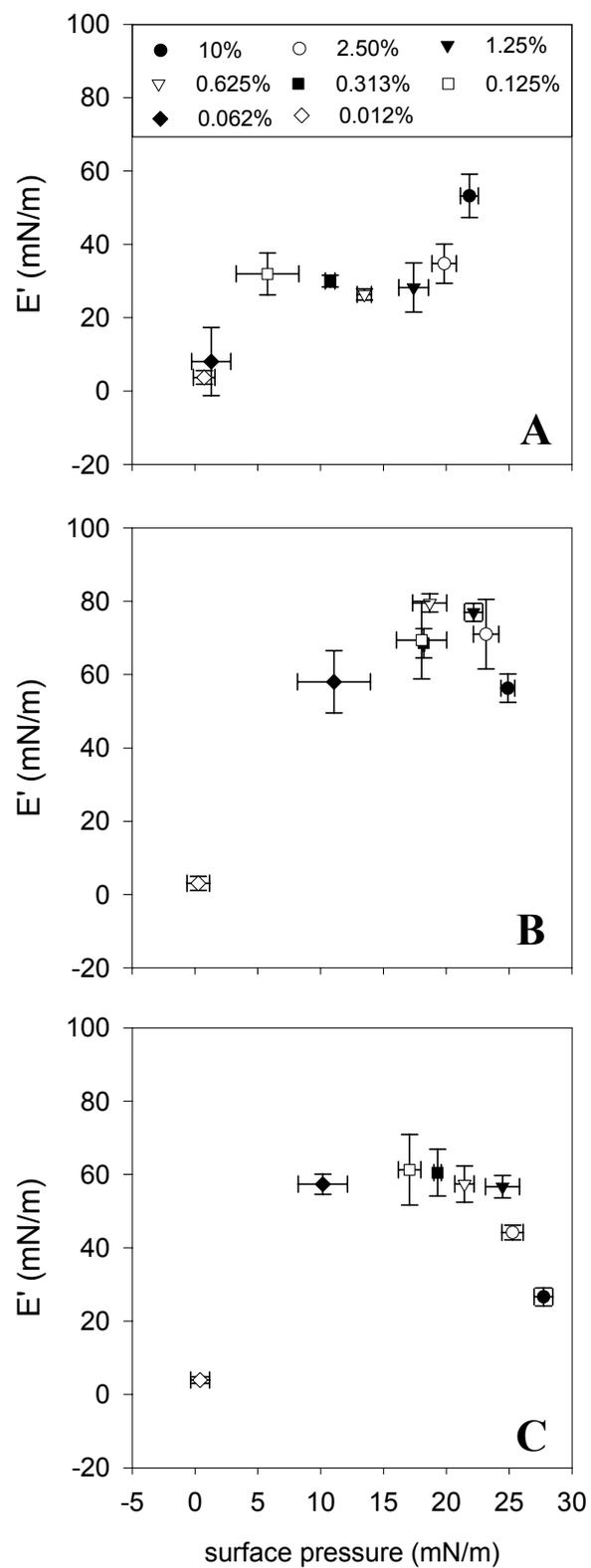


Figure 5.

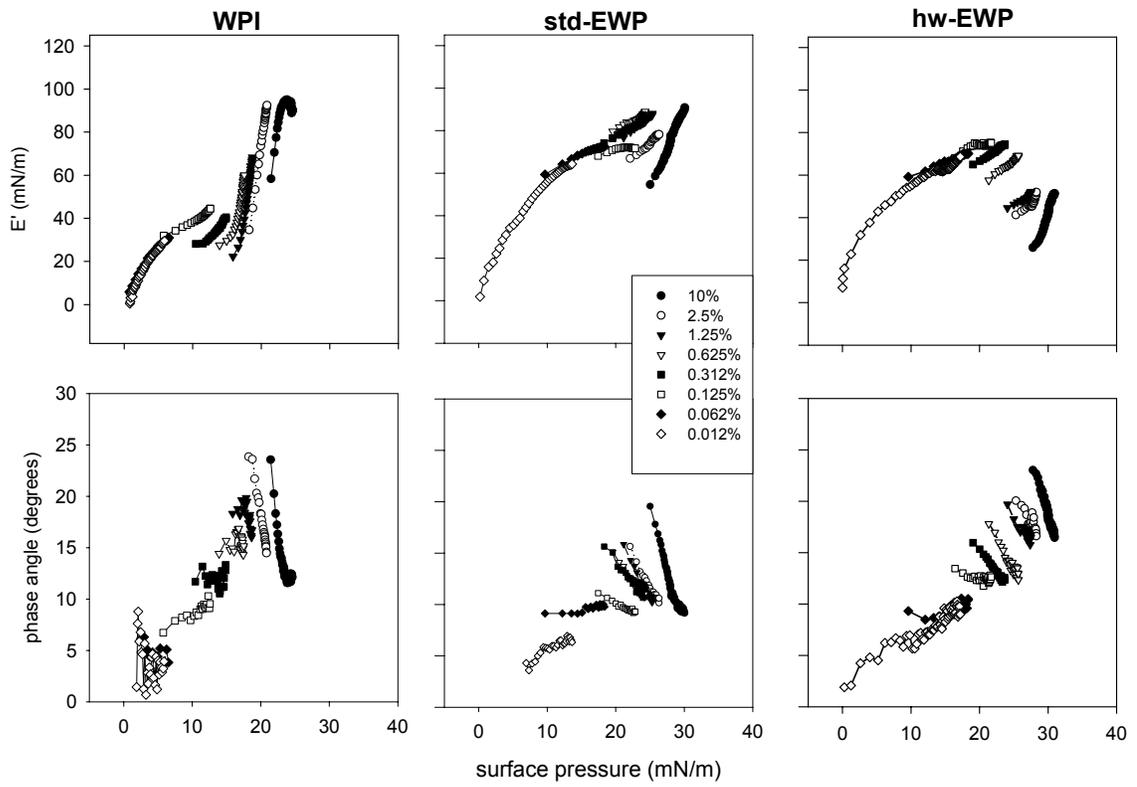


Figure 6.

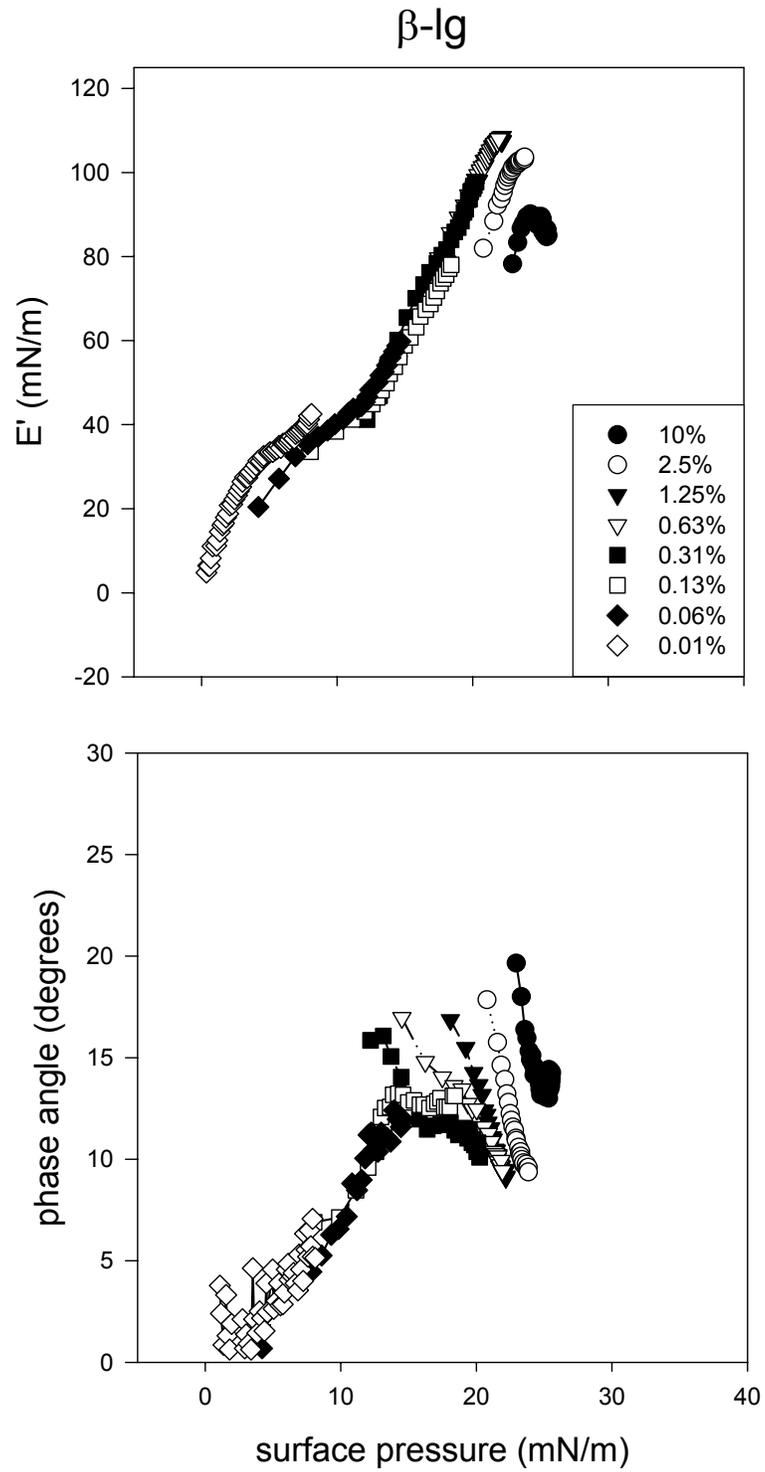
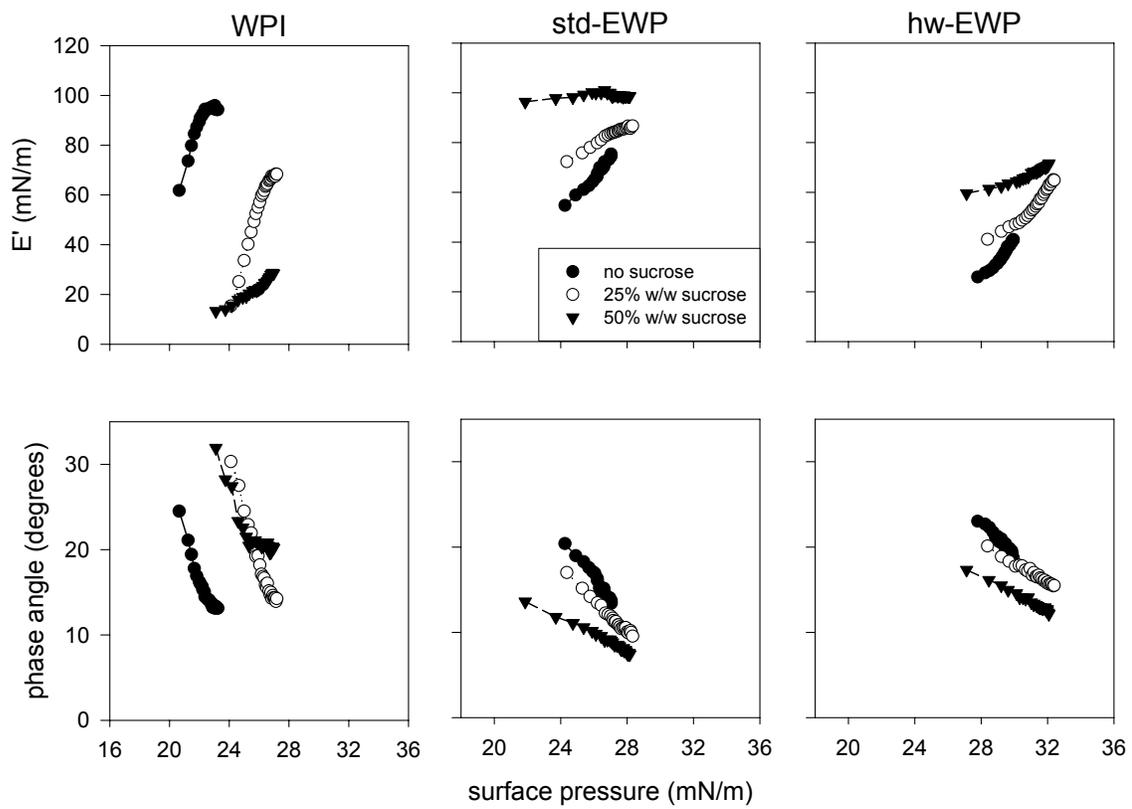


Figure 7.



Dissertation Summary

The yield stress of protein-based foams was systematically investigated for several different protein types, most of which were commercially available ingredients, with an emphasis on whey protein isolate (WPI). Foams were whipped via a bench top mixer after solubilizing the proteins ($\geq 5\%$ w/v) under a variety of conditions. After whipping the foams, typical measurements included yield stress (τ_0), overrun (used to calculate foam air phase volume, ϕ) and drainage stability. A variety of interfacial measurements were also conducted to aid interpretation of the foam data. This emphasis on interfacial phenomena was justified, as proteins function in foams as surfactants, that is they rapidly adsorb at the air/water interface of freshly created bubbles. This adsorption lowers the interfacial tension of this interface, which in turn promotes bubble formation and stability. Once adsorbed at the air/water interface, lateral interactions between neighboring proteins can result in the formation of a protein network with its own rheological properties. These two phenomena, protein adsorption and interfacial network formation, were previously established to strongly influence bulk foam properties such as overrun and drainage stability; however, it was not clear what interfacial behaviors (if any) influenced bulk foam rheology, i.e. yield stress. Accordingly, measurements of protein adsorption and interfacial dilatational rheology were conducted at a model air/water interface, to better understand potential relationships between protein interfacial behaviors and foam τ_0 .

In the first study, the yield stress of WPI foams as affected by electrostatic forces was investigated by whipping 10% w/v protein solutions prepared over a range of pH levels

and salt concentrations. Foam yield stress, foam overrun, protein adsorption rates and interfacial rheological measurements were all found to be highly dependent on the solution pH and salt content. The following equation, derived from a theoretical analysis of spatially periodic foam cells was used as a starting point to analyze the data:

$$\tau_0 = \frac{\gamma}{R_{32}} \phi^{1/3} Y(\phi) \quad (1)$$

where γ is an equilibrium surface tension of the bubbles (assumed uniform throughout the foam), $\phi^{1/3}$ is the cube root of the air phase volume, R_{32} is the Sauter mean bubble radius and $Y(\phi)$ is an experimentally derived function of the system. While knowingly oversimplified for real foams, this equation had been partially verified experimentally in model systems, i.e. concentrated emulsions, as the dispersed phase of these systems is much more stable to disproportionation, and their size distribution is much more controllable, i.e. a more monodisperse distribution (model assumption) can be more closely approached. Note γ and ϕ were experimentally determined, whereas R_{32} was assumed constant based on previous work. Analysis of the data revealed none of the expected correlations suggested by equation 1. In regards to the surface measurements it was noted that several samples could have very similar equilibrium surface tensions, while having drastically different dilatational rheological moduli, i.e. E' (storage modulus) and E'' (loss modulus). Analysis of the data did reveal a positive, curvilinear correlation between E' and foam τ_0 for 10 different solution conditions, ranging from pH 3.0 to pH 7.0, both in the presence and absence of NaCl and CaCl₂ (0 to 100 mM). This was the first time a potential link was noticed between interfacial rheological measurements and foam yield stress.

In the second study, the potential of polymerized (p-WPI) as a functional foam ingredient was evaluated. Note that p-WPI are soluble, disulfide linked aggregates of native WPI created by a controlled heating process. Various ratios of native WPI and p-WPI were created and foam τ_0 , ϕ and drainage stability were all evaluated. Again, interfacial measurements aided data interpretation. Interfacial measurements were known to be strongly dependent on the concentration used for such measurements. In the previous work, a concentration of $\sim 0.2\%$ w/v protein was selected, as the literature suggested β -lactoglobulin (β -lg), the primary whey protein, rapidly approaches interfacial saturation levels at this concentration. For the p-WPI study, 3 concentrations were selected for interfacial measurements: 10%, 1%, and 0.1% w/v to better understand the effect of this variable. Interestingly, the same correlation between E' and τ_0 was noted for these solutions, but only when the interfacial measurements were conducted at a concentration of 10% w/v. This was because at 10% w/v the relative diffusion distances at the model interface were significantly reduced, and the larger p-WPI adsorbed with native WPI on a similar time scale (5 min measurement). This underscored the importance of selecting the proper concentration for interfacial measurements to best reflect the foam data. This study further strengthened the hypothesis that there was a link between the interfacial rheology of a protein and its potential at providing a specific foam τ_0 .

In the third study, β -lactoglobulin (β -lg) was hydrolyzed with three different proteases and subsequently evaluated for its foaming potential. Two heat treatments designed to inactivate the enzymes, $75^\circ\text{C}/30$ min and $90^\circ\text{C}/15$ min, were also investigated for their effects on foam functionality. All unheated hydrolysates increased τ_0 as compared to

unhydrolyzed β -lg, whereas heating the hydrolysates decreased τ_0 ; however, heating at 90°C/15 min decreased τ_0 much more than heating at 75°C/30 min. These trends were generally reflected in the interfacial measurements (5% w/v, same as foaming data) with increasing values of E' corresponding to increase in τ_0 . However, it was also noted that the three hydrolysates with the highest values of τ_0 not only had very high values of E' , they had viscous moduli of essentially zero. This highly elastic interfacial state was presumed to improve foam τ_0 indirectly by improving foam stability and directly by imparting resistance to interfacial deformation.

For the final study, it was decided to directly compare the foaming and interfacial properties of WPI and egg white protein (EWP), which is the traditional foaming agent of choice in the food industry. Previous work suggested discrepancies in the foaming behaviors between these two ingredients; however it was unclear if there was indeed an interfacial basis for these differences. Furthermore, the effects of 25% w/w sucrose were also evaluated for its effect on foaming and interfacial behavior. Note that foams often appear in foods containing high contents of sugars, i.e. confections. Previously observed relationships between τ_0 and interfacial rheological measurements did not hold across the protein types; however these interfacial measurements did effectively differentiate foaming behaviors within EWP-based ingredients and within WPI. Addition of 25% w/w sucrose to the solutions increased τ_0 and drainage resistance of the EWP-based ingredients, but it decreased τ_0 of WPI foams and minimally affected their drainage rates. These differing sugar effects were reflected in the interfacial measurements, as sucrose addition increased the dilatational elasticity and decreased the interfacial phase angle

(ratio of E' to E'' , with lower phase angles indicative of an increased elastic response) for both EWP-based ingredients, while sucrose addition imparted the exact opposite effects on WPI.

This work has clearly demonstrated a link between the capacity of a protein to increase foam yield stress and the interfacial rheological behaviors it imparts. Specifically, proteins which impart high values of E' and/or low phase angles seem predisposed to form foams with high values of E' . This is the first time such observations have been noted. Hence, this research has revealed a rapid means of screening new proteins as whipping agents and/or predicting the potential impact of a processing change on foam behavior.

Appendix 1.

Advances in Understanding and Predicting Whey Protein Functionality

The content of this appendix has been published:

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Trends in Food Science & Technology, 2002, 13: 151-159

Advances in modifying and understanding whey protein functionality

E. Allen Foegeding*,
Jack P. Davis, Dany Doucet
and Matthew K. McGuffey

Southeast Dairy Foods Research Center, North
Carolina State University, Department of Food
Science, Box 7624, 236 Schaub Hall, Raleigh, NC
27695-7624, USA (tel.: +1-919-513-2244; fax: +1-
919-515-7124.; e-mail: allen_foegeding@ncsu.edu)

Whey protein ingredients are used for a variety of functional applications in the food industry. Each application requires one or several functional properties such as gelation, thermal stability, foam formation or emulsification. Whey protein ingredients can be designed for enhanced functional properties by altering the protein and non-protein composition, and/or modifying the proteins. Modifications of whey proteins based on enzymatic hydrolysis or heat-induced polymerization have a broad potential for designing functionality for specific applications. The effects of these modifications are demonstrated by discussing how they alter gelation and interfacial properties.

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Whey protein ingredients and functionality

When casein is formed into a cheese network or removed from milk to produce caseinate or casein

* Corresponding author.

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ingredients, the remaining whey fluid contains approximately 0.6% protein and 93% water (Foegeding & Luck, 2002). Whey can be processed into a food ingredient by simple drying, or the protein content can be further increased by removing lipid, minerals and lactose. Producing whey protein concentrates (25–80% protein) and isolates ($\geq 90\%$ protein) requires that large amounts of non-protein compounds be removed, therefore separation technologies were the focus during the early development of whey protein concentrates and isolates (Goldsmith, Amundson, Horton, & Tannenbaum, 1970; Timmer & van der Horst, 1997). While separation technologies continue to be developed, direct modification of the proteins has been an active area in recent years. Proteins can be modified by: (1) covalently attaching other compounds such as carbohydrates (Chevalier, Chobert, Popineau, Nicolas, & Haertle, 2001; Losso & Nakai, 2002); (2) causing non-covalent and/or covalent interactions among proteins to produce aggregates or polymers or (3) hydrolyzing proteins to various degrees (van der Ven, Gruppen, de Bont, & Voragen, 2001).

Proteins provide various functions in food quality and stability. They form interfacial films that stabilize emulsions and foams; can interact to make networks associated with gels (e.g., cheese and meat) and edible films; and produce stable sols for nutritional drinks (often referred to as *thermal stability*). The ability of proteins to provide these properties is called *protein functionality*, and is dependent not only on proteins but also on other ingredients and processing operations. Nevertheless, one often speaks in general terms of increasing or decreasing protein functionality.

This review of protein modifications and functional consequences will focus on the areas of gelation, foam and emulsion formation. Emphasis will be given to theoretical models and the modifications of polymerization and hydrolysis, with hydrolysis discussed as a separate section. These modifications represent the extremes of what happens when you effectively increase or decrease molecular size.

Focusing on a limited number of functional properties and modifications was done to allow for more in-depth coverage.

Gelation of whey proteins

Gels are formed when proteins interact and produce a clastic network. Whey protein gels have been classified as

fine stranded, mixed or particulate based on rheological and microstructural properties (Clark, Judge, Richards, Stubbs, & Suggett, 1981; Foegeding, Gwartney, & Errington, 1998; Stading, Langton, & Hermansson, 1993). Recently, Gosal and Ross-Murphy (2000) reviewed the physics of globular protein gelation with an emphasis on models describing their fundamental structure and rheology.

What determines whey protein gel rheology and microstructure?

A series of studies have established that the incorporation of protein into the gel network at and after the gel point determines whey protein (WP) gel microstructure and rheology (Verheul & Roefs, 1998a,b; Verheul, Roefs, Mellema, & de Kruijff, 1998). The lower kinetic limit for β -lactoglobulin denaturation (68.5°C) (Iametti, De Gregori, Vecchio, & Bonomi, 1996) at long times (~24 h) was used to slow the process so the development of rheological and microstructural properties could be observed. The amount of aggregated protein forming the incipient gel network seems to determine gel network type (microstructure) and permeability (a function of microstructure and other properties). Gel permeability decreased only slightly upon further heating after the gel point (Fig. 1). In contrast, the elasticity (G') is zero at the gel point and increased with the total amount of protein incorporated into the established gel network (Verheul & Roefs, 1998a,b). The gel permeability decreased as the concentration of aggregated protein at the gel point increased. Permeability and G' scale as a power law with concentration of whey protein isolate (WPI) with exponents of

-2.35 ± 0.15 and 4.5 ± 0.5 , respectively (Verheul & Roefs, 1998a).

Gelation models

One approach to characterize disordered structure is through fractal analysis (Pedreschi, Aguilera, & Brown, 2000). The fractal dimension (d_f) is a non-integer value that expresses the self-similarity of gel structure and can be probed through either rheological or microstructural methods. β -Lactoglobulin (β -Lg) forms fractal aggregates (Aymard, Durand, Nicolai, & Gimel, 1997; Aymard, Gimel, Nicolai, & Durand, 1996; Le Bon, Nicolai, & Durand, 1999) that further associate into a three-dimensional network. The fractal nature of these networks is a current subject of debate (Kavanagh, Clark, & Ross-Murphy, 2000).

The d_f relates to the two limiting cases of aggregation kinetics: reaction-limited cluster aggregation (RLCA; d_f of 2.2–2.1) and diffusion-limited cluster aggregation (DLCA; d_f of 1.7–1.8) (Bijsterbosch, Bos, Dickinson, van Opheusden & Walstra, 1995). In RLCA, there is a repulsive energy barrier to aggregation so many collisions must occur before two particles collide with the correct orientation and “stick”. With DLCA, the energy barrier has been overcome so all collisions result in the proteins “sticking”. The aggregation kinetics of β -Lg are highly sensitive changes in electrostatic properties and will switch between RLCA and DLCA with changes in pH and salt concentration (Aymard *et al.*, 1997; Vreeker, Hoekstra, Denboer, & Agterof, 1992).

A compelling aspect of the fractal approach is that scaling behavior or elastic properties can be used to describe structural elements within the microstructure.

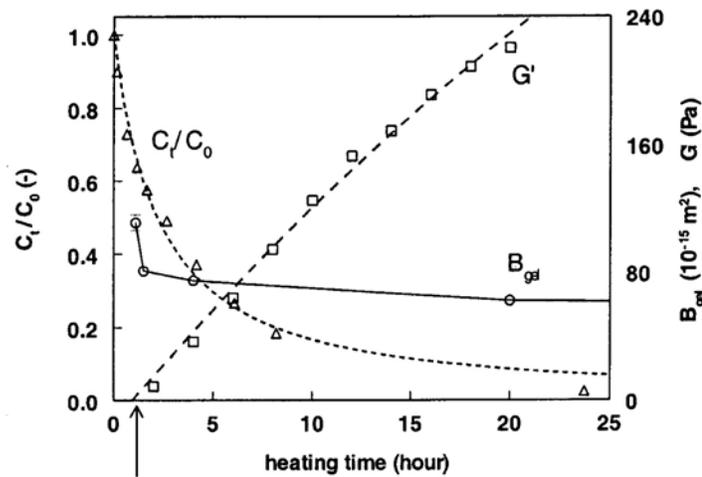


Fig. 1. Fractional native WPI concentration (C_1/C_0) (Δ), B_{gel} (O) and G' (\square) as a function of heating time at 68.5°C for an initial WPI concentration of 44.5 g/l and 0.4 mol/dm³ NaCl; the arrow indicates the gel point. (Reprinted from (Verheul & Roefs, 1998b) with permission from Elsevier Science).

When the interactions within fractal flocs are weaker than the interactions between flocs, the network is considered strong-linked and breaking will occur within the fractal flocs (Shih, Shih, Kim, Liu, & Aksay, 1990). The opposite case is called “weak-linked.” The critical strain (strain limit of the linear viscoelastic region) increases with increasing network concentration in weak-linked networks, and decreases with increasing network concentration in strong-linked networks (Shih *et al.*, 1990). Therefore, simple experiments where protein concentration is varied and rheological properties are measured may reveal if the network is strong- or weak-linked.

Table 1 summarizes fractal dimension values for WPI and β -Lg gels cited in the literature, which were obtained by either rheological or optical methods. Rheological methods are the most common for determining d_f (for a theoretical description see Bremer, Bijsterbosch, Walstra, & van Vilet, 1993; Shih *et al.*, 1990), and this value often correlates with the d_f calculated from microstructural analysis (Hagiwara, Kumagai, & Matsunaga, 1997; Marangoni, Barbut, McGauley, Marcone, & Narine, 2000). Gels of WPI formed at pH 7 with 25 mM NaCl demonstrate RLCA ($d_f \approx 2.2$), where low ionic strength leads to a large electrostatic energy barrier to aggregation (Ikeda, Foegeding, & Hagiwara, 1999). Gels of WPI formed at pH 7 with a NaCl concentration of 50, 80, 500 and 1000 mM NaCl demonstrate DLCA, indicating this electrostatic barrier has been overcome (Ikeda *et al.*, 1999).

The fractal approach can yield inconsistent values (see Table 1) and must be applied with caution. Verheul and Roefs (1998a) noted that fractal law assumes cluster-cluster aggregation starting at $t = 0$ and ending at the gel point. In reality, only a fraction of WP is aggregated at the gel point and aggregates are incorporated into the network after the gel point, which makes interpretation difficult (Verheul & Roefs, 1998a). Kavanagh *et al.* (2000) have acknowledged that WPI gels are self-similar

over some length scales, but have rebuked this approach because the concentration dependence of the elastic modulus does not follow a simple power law, measurements are made too far from the critical gelation threshold, and G' is not extrapolated to infinite time. Their branching model successfully correlates G' to the molecular weight (MW) and crosslink density of network chains for β -Lg gels (Gosal & Ross-Murphy, 2000; Kavanagh *et al.*, 2000). The limitations of fractal models were also discussed in proposing an isotropic force percolation model for protein gels (van der Linden & Sagis, 2001).

Cosolute effects on thermal gels

The effects of ionic strength and various salts on whey protein gelation have been reviewed (Foegeding *et al.*, 1998); therefore, this will be an update on the progress made in understanding electrostatic effects of whey protein gelation.

Electrostatic effects on the aggregation kinetics, rheology and microstructure of particulate gels have been studied (McGuffey & Foegeding, 2001; Verheul and Roefs, 1998a,b). As pH is increased from 6.0 to 7.5 in the presence of 0.5 M NaCl, the rate of protein aggregation and G' increase whereas the permeability decreased (Verheul & Roefs, 1998b). Essentially, as pH is increased away from the pI, the increased electrostatic repulsion and reactivity of the sulfhydryl group causes the protein to unfold more extensively and aggregate more rapidly; this produced a more elastic, less permeable, gel network. As NaCl concentration increased (at neutral pH), WPI gels have a more open, coarse structure with increasing permeability and decreasing G' that may be related to a decrease in the solvent quality or an alteration in the aggregation kinetics (McGuffey & Foegeding, 2001; Verheul & Roefs, 1998a). Particulate WPI gels with equivalent fracture stress and strain values can be formed by either varying the pH from 5.2 to 5.8 with

Table 1. Reported values for fractal dimension of WPI or β -Lg gels from various studies

Protein source	Solvent conditions	Thermal treatment ^a	Method of analysis	Fractal dimension	Reference
β -Lg	pH 7; 50 mM HEPES	95°C for 10 min	Rheology	2.17	(Hagiwara <i>et al.</i> , 1997)
	pH 7; 30 mM CaCl ₂ & 50 mM HEPES		Rheology	2.69	
			Microstructure	2.70	
β -Lg	pH 7.5; no salt	90°C for 1 h	Rheology	2.91	(Stading <i>et al.</i> , 1993)
	pH 5.3; no salt		Rheology	2.46	
WPI	pH 7; no salt	80°C for 20 min	Rheology	2.7	(Fernandes, 1994)
WPI	pH 6.8; 0.2–3.0 M NaCl	68.5°C for 20 h	Microstructure	2.40	(Verheul <i>et al.</i> , 1998)
WPI	pH 7; 25 mM NaCl	Held at 90°C for 1 h (testing done at 90°C)	Rheology	~2.2	(Ikeda <i>et al.</i> , 1999)
WPI	pH 7; 50,80,500,1000 mM NaCl	80°C for 30 min	Rheology	~1.8	(Marangoni <i>et al.</i> , 2000)
	pH 7; 30 mM CaCl ₂		Rheology	2.57	
			Microstructure	2.45	
	pH 7; 300 mM NaCl		Rheology	2.44	
			Microstructure	2.45	

^a Samples analyzed at ambient temperature unless otherwise noted (see actual papers for heating and cooling rates).

no added salt, or by adding NaCl (0.2–0.6 M) at pH 7 (McGuffey & Foegeding, 2001). While identical in fracture properties, the strain hardening trends in the non-linear region of stress versus strain curves were different. Addition of the disulfide blocker *n*-ethylmaleimide at levels less than the amount of total sulfhydryls changed the non-linear region such that gels had similar values for strain hardening, without altering fracture properties. This showed that electrostatic properties were the major factor determining fracture properties, whereas sulfhydryl/disulfide interactions were important to the non-linear region.

Whey protein polymers and cold set gelation

Many food applications are sensitive to the high heat required to gel proteins, so a great deal of attention has recently been devoted to developing whey proteins as cold gelling ingredients. The mechanisms and processes involved in the cold-gelation of whey protein polymers (WP-polymers, also referred to as heat-denatured, pre-heated or aggregated) have been reviewed (Bryant & McClements, 1998). Essentially, whey proteins are heat-polymerized (i.e. aggregated) at conditions that do not yield a gel (low ionic strength and high pH), then the solvent quality is altered to form a gel under "cold" conditions (typically 20–37°C). Since the excellent review of Bryant and McClements (1998), new investigations have focused on understanding how preheating and cold gelling conditions affect the final gel properties.

Increasing the concentration of protein in solutions used to make WP-polymers (heated at 80°C for various times) results in increased whey protein polymer size (Ju & Kilara, 1998) and intrinsic viscosity (Vardhanabhuti & Foegeding, 1999). The WP-polymers are formed primarily through disulfide bonding with some non-covalent interactions, and have flow properties similar to common food hydrocolloids (Mleko & Foegeding, 1999; Vardhanabhuti & Foegeding, 1999). In general, when making WP-polymers, the higher the protein concentration, heating time and temperature, the more viscous the dispersion and rigid the gel (Bryant & McClements, 2000b; Ju & Kilara, 1998; Vardhanabhuti & Foegeding, 1999).

Solvent quality is altered to facilitate interactions among WP-polymers. Adding NaCl (100–400 mM) to WP-polymer solutions results in gels with an increase in turbidity and G^* , and a decrease in phase angle (indicating an increase in elasticity) (Bryant & McClements, 2000c). Adding CaCl_2 increases turbidity, fracture stress, and G^* and decreases fracture strain (Barbut & Foegeding, 1993; Bryant & McClements, 2000b). When WP-polymer gels of equal ionic strength were made with CaCl_2 or NaCl, gels containing CaCl_2 were more rigid due to calcium being more effective at screening charges and its ability to form ion-bridges between negative charges on proteins (Bryant & McClements,

2000b; Vardhanabhuti, Foegeding, McGuffey, Daubert, & Swaisgood, 2001).

Acidifying WP-polymer dispersions with glucono- δ -lactone induces weak network formation (Ju & Kilara, 1998). Even at pH < 5, disulfide bonds are formed between aggregates to strengthen the network, though gel microstructure is determined by the initial non-covalent interactions (Alting, Hamer, de Kruif, & Vischers, 2000). The presence of up to 8% w/w sucrose when forming WP-polymers (Kulmyrzaev, Bryant, & McClements, 2000) decreases turbidity, increases gelation time and decreases G^* . The decrease in gelation rate at low sucrose concentration is due to an increase in continuous phase viscosity, which decreases protein collision frequency. At high sucrose concentrations (> 10%), the protein-protein interactions are thermodynamically preferred over protein-solvent interactions leading to stronger gels formed more quickly than if < 10% sucrose is present.

Mixtures of WP-polymers and hydrocolloids can undergo phase separation. Bryant and McClements (2000a) found that adding xanthan gum to WP-polymer dispersions increases turbidity, gelation rate and final gel rigidity. Without salt, dispersions of these two biopolymers form a two layer separation, but with 200 mM NaCl, xanthan-rich regions are dispersed within a WP gel network (Bryant & McClements, 2000a).

Interfacial properties

Food foams and emulsions such as ice cream, soufflés, frothed drinks, dressings, etc. are extremely popular among consumers (Bryant & McClements, 1998; Campbell & Mougeot, 1999). The formation and stability of these foods primarily depends on the behavior of surface-active agents, and whey protein ingredients are often chosen for this functional role (Dickinson, 1999). Simple system investigations into the properties (formation, composition, rheology, etc.) of adsorbed protein layers, are providing fundamental insights into the relationships between protein interfacial behavior and the bulk properties of real food foams and emulsions (Dickinson, 2001; Wilde, 2000). Recent reviews (Dickinson, 1999, 2001) have summarized much of the current research pertinent to the properties of adsorbed whey proteins (most notably β -Lg).

Lateral interactions among adsorbed proteins at the interface (air/water or oil/water) can lead to an interfacial layer with measurable rheological properties. The rheology of interfaces has long been thought to be important to the stabilization of foams and to a lesser extent emulsions, so not surprisingly, the literature in this area is quite extensive (Wilde, 2000). The following articles are excellent reviews on the rheology of adsorbed milk protein layers (Dickinson, 1999) and protein interfacial layers in general (Prins, Bos, Boerboom, & van Kalsbeek, 1998). The rheology of model systems,

consisting of both milk proteins (often β -Lg) and small MW surfactants (monoglycerides, Tween 20, sodium dodecyl sulfate, etc.), has also received a great deal of attention due to its relevance in actual food systems. The following articles provide thorough reviews on recent advances pertaining to the behavior of combined protein/surfactants layers (Bos & van Vilet, 2001; Miller *et al.*, 2000; Prins *et al.*, 1998).

The relationships between measurements taken from isolated interfacial protein layers and actual bulk foam or emulsion properties are not well understood. This was recently illustrated in the work of Ipsen *et al.* (2001), who examined the effects of limited enzymatic hydrolysis on the interfacial shear rheology and foam overrun of purified β -Lg A. Increasing the degree of hydrolysis steadily decreased both interfacial shear elasticity and viscosity values as compared to the unhydrolyzed control. However, the highest foam overrun was found for the sample with maximum hydrolysis (86%).

A foaming property of interest for liquid foams is yield stress, τ , which relates to the empirical concept of foam "stiffness" (Pernell, Foegeding, & Daubert, 2000). The τ of egg white protein foams is higher (~ 150 Pa) than whey protein isolate foams (~ 50 Pa) (Pernell *et al.*, 2000). The theoretical model of Princen and Kiss (1989) predicts that τ depends on surface tension, air phase volume fraction, and bubble size. However, none of these factors explained the difference between egg white and whey protein foams (Pernell, Foegeding, Luck, & Davis, 2002). The potential importance of protein interactions (and possibly networks) in the lamellae was suggested. Whey protein ingredients can be modified to increase foam yield stress by increasing the ratio of β -Lg to α -lactalbumin, or hydrolysis (Luck, Bray, & Foegeding, 2002).

The adsorption of proteins at the interface is extremely sensitive to protein structure, as was recently demonstrated by Mackie, Husband, Holt, and Wilde (1999) who compared the adsorption of the A and B genetic variants of β -Lg at the air/water interface. These variants differ by only two amino acids, but the B variant was shown to adsorb more rapidly via surface tension measurements. The valine (A) to alanine (B) substitution at residue 118 was hypothesized to effect adsorption minimally (at least initially), since neither amino acid is charged, both are hydrophobic, and their location is within the interior of the molecule. However, the substitution at position 64, aspartic acid (A) to glycine (B) is on the surface of the molecule. Variant B therefore has a lower charge and higher hydrophobicity both of which were hypothesized to contribute toward its increased adsorbance.

Enzymatic hydrolysis of whey protein

Whey protein hydrolysates (WPH) have been on the market for many years because they possess excellent

nutritional value and functional properties. Enzymatic hydrolysis is generally used to produce WPH because it occurs under mild conditions and thus retains nutritional quality. The applications for these products include but are not limited to: improved heat stability; reduced allergenicity; production of bioactive peptides, tailoring amounts and size of peptides for special diets; and altering the functional properties of gelation, foaming and emulsification.

Native whey proteins are not always hydrolyzed easily so the susceptibility to hydrolysis can be increased by different means such as heat treatment, sulfitolysis, high pressure, medium polarity changes and esterification (Chobert *et al.*, 1997; Kananen *et al.*, 2000; Maynard, Weingand, Hau, & Jost, 1998). Whey protein hydrolysates designed for nutritional applications (sports nutrition, enteral formulas, hypoallergenic infant formulae, etc.) have a high degree of hydrolysis and therefore a high content of short peptides. Short chain peptides are less antigenic due to elimination of sequential epitopes (Siemensma, Weijer, & Bak, 1993). The diminished or lack of secondary structure in peptides explains heat stability, in that there are minimal changes in structure upon heating. Furthermore, a product with a low content of free amino acids is absorbed more efficiently because of intestinal absorption differences between peptides (primarily di- and tripeptides) and free amino acids (Boza *et al.*, 2000; Clemente, 2000). To obtain that kind of profile, an extensive enzymatic hydrolysis is required, preferentially with an endoprotease, in order to avoid the presence of free amino acids in the final product.

Gelation

Hydrolysis can be used to change conditions required for gelation (e.g., temperature) along with the physical properties of gels. When β -Lg was subjected to limited proteolysis with immobilized trypsin, it had a lower gel point and gelled more rapidly than native β -Lg at 80°C (Chen, Swaisgood, & Foegeding, 1994). Incubation of a heat-polymerized WPI solution with a variety of proteinases (trypsin, papain, pronase and protease) caused gelation (Sato, Nakamura, Nishiya, Kawanari, & Nakajima, 1995). Gelation properties of WPI were significantly improved at neutral pH by limited hydrolysis with a protease from *Bacillus licheniformis* (BLP) (Ju, Otte, & Madsen, 1995). Gels formed from BLP-hydrolyzed WPI consisted of small aggregates that were speculated to be formed during hydrolysis, prior to heat-induced gelation (Otte, Ju, Skriver, & Qvist, 1996). It was confirmed that limited hydrolysis of WPI by BLP leads to formation of a soft gel, with a microstructure similar to a heat-induced gel (Otte, Ju, Faegermand, Lomholt, & Qvist, 1996). The formation of aggregates and a soft gel prior to heating is thus one way that heat-induced gelation properties of whey proteins are altered

by hydrolysis. Otte *et al.* (1997) showed that hydrolysis of β -Lg with BLP produced soft gels, indicating that other proteins found in WPI were not a requirement for soft gel formation. The aggregates derived from β -Lg hydrolysis consisted of a range of peptides of intermediate size (2–6 kDa) held together by non-covalent interactions (Otte *et al.*, 1997). The aggregates consisted of 6–7 major peptides of which the fragment f135–158 seemed to be the initiator of aggregation. This fragment contains several basic and acid amino acids alternating with hydrophobic amino acids, which is in accordance with formation of non-covalently linked aggregates (Otte, Lomholt, Halkier, & Qvist, 2000).

In producing whey protein hydrolysates, it is desirable that the protein concentration be as high as possible to minimize the cost of drying. A problem encountered when hydrolyzing at high protein concentrations is gelation at normal processing temperatures (40–50°C). Unlike above, where hydrolysis is used to improve gelation properties, in this case, the prevention of gelation is desired. Gelation occurs during extensive hydrolysis (degree of hydrolysis > 18%) of whey protein isolate with Alcalase 2.4L* (Doucet, Gauthier, & Foegeding, 2001). Figure 2 shows the mechanical spectrum and their respective slopes (n) for enzyme- and heat-induced gels. A flat slope is indicative of a strong, elastic gel. Both gels have similar characteristics and are predominantly elastic in nature. The types and amounts of aggregating peptides produced are important and research is needed in this area to better understand the gelation mechanism.

Interfacial properties

Whey protein hydrolysates designed for foaming and emulsification applications are generally hydrolyzed to a lower extent. However, there are some contradictory findings on how the degree of hydrolysis affects the functionality.

The fact that peptides are smaller than proteins generally allows for a more rapid adsorption, which in turn allows for better foam or emulsion formation (Wilde & Clark, 1996). Additionally, hydrolysates are generally more soluble over a wider range of environmental conditions, such as pH and ionic strength, which also explains some of the improved surface-activity of hydrolysates (Turgeon, Gauthier, & Paquin, 1991). Studies have shown that the relative distribution of hydrophobic and hydrophilic amino acids found in a protein or peptide is important for its adsorption (Singh & Dalgleish, 1998).

It has been speculated that smaller peptides do not form the same inter-molecular interactions as larger proteins, making the interfacial network less stable (Singh & Dalgleish, 1998; Wilde & Clarke, 1996). However, foam yield stress and overrun is improved by hydrolysates ranging in degree of hydrolysis from 5.4 to 15.9% (Luck *et al.*, 2002). A probable explanation is that since degree of hydrolysis varies strongly with enzyme and substrate (Singh & Dalgleish, 1998; Turgeon, Gauthier, Molle, & Leonil, 1992), the link with functional properties will vary. Distinct regions of hydrophobic and hydrophilic amino acids with a minimum in size seem to be the most desirable for interfacial

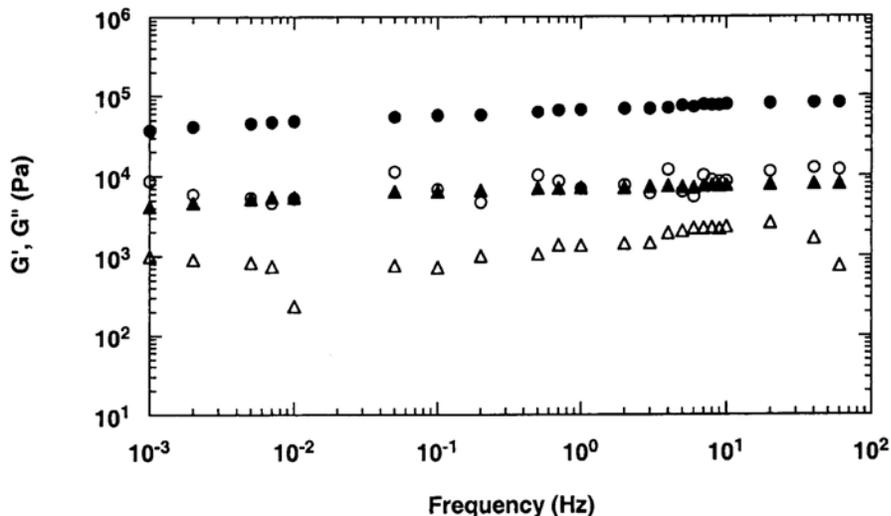


Fig. 2. Influence of frequency on storage (circle) and loss (triangle) moduli for an enzyme-induced WPI gel with Alcalase 2.4L* (20% solution at 45°C, E:S 1:10) and for a heat-induced WPI gel. Enzyme ($n=0.052$) and heat-induced ($n=0.067$) gels are represented by open and closed symbols respectively.

properties (Turgeon *et al.*, 1992). Therefore, while the degree of hydrolysis and molecular weight distribution are good general indicators of hydrolysate properties, the functional properties are determined by the specific peptides produced. Recent work has shown that emulsion-forming behavior of WPH is generally independent of the molecular weight distribution and degree of hydrolysis (van der Ven *et al.*, 2001). Another study has demonstrated that adsorption of β -Lg peptides of known sequences at the oil/water interface is influenced more by the amphiphilic character than the peptide length (Rahali, Chobert, Haertle, & Gueguen, 2000). Caessens, Daamen, Visser, Gruppen, & Voragen (1999a) subjected purified β -Lg to limited hydrolysis (1, 2 and 4%) by three different enzymes (plasmin, trypsin, and an endoproteinase from *Staphylococcus aureus* V8) and screened the resulting hydrolysates for their foaming and emulsifying properties. Overall, the plasmin hydrolysates displayed superior interfacial functionality. This was attributed to the high concentration of both large molecular weight and hydrophobic peptide fragments retained in this hydrolysate. However, limited hydrolysis by the other enzymes also improved the functionality of β -Lg. Most of the peptides were disulfide linked β -Lg fragments suggesting that considerable SH/SS exchange takes place during plasmin hydrolysis. None of the peptides identified consisted of more than two intermolecular disulfide linked β -Lg fragments (Caessens, Daamen, Gruppen, Visser, & Voragen, 1999b).

A way to improve the functionality of whey protein hydrolysate is by membrane separation (e.g. ultrafiltration and nanofiltration) to obtain fractions with varying degrees of functionality (Huang, Catignani, & Swaisgood, 1996; Turgeon *et al.*, 1991, 1992). Nanofiltration can be used to lower salt content and modify peptide content of whey protein hydrolysates (Pouliot, Gauthier, & L'Heureux, 2000; Pouliot, Wijers, Gauthier, & Nadeau, 1999; Wijers, Pouliot, Gauthier, Pouliot, & Nadeau, 1998). Enzymatic hydrolysis of whey proteins can produce bitter tasting peptides, and may limit their applications in foods. Control of the degree of hydrolysis, combined with ultrafiltration, can be used to reduce the bitterness of whey protein hydrolysates (Ziajka & Dzwolak, 1999).

Conclusions

Our understanding of whey protein functionality has been advanced by studies concerning molecular mechanisms of functional properties, along with investigations on the functional consequences of protein modifications. Modifying whey proteins by forming larger (WP-polymers) or smaller (hydrolysates) molecules or aggregates enhances traditional functional properties and creates new ones (e.g., cold gelation). The opportunities for improved and uniquely functional

whey protein ingredients will continue to foster research in this area.

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APPENDIX 2.

Factors determining the physical properties of protein foams

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E. Allen Foegeding, P.J. Luck and J.P. Davis

Abstract

Protein foams are an integral component of many foods such as meringue, nougat and angel food cake. With all these applications, the protein foam must first obtain the desired level of air phase volume (foamability), and then maintain stability when subjected to a variety of processes including mixing, cutting and heating. Therefore, factors determining foamability and stability to mechanical and thermal processing are important to proper food applications of protein foams. We have investigated the effects of protein type, protein modification and co-solutes on overrun, stability and yield stress. The level of overrun generated by different proteins was: whey protein isolate hydrolysates > whey protein isolate = β -lactoglobulin = egg white > α -lactalbumin. The level of yield stress generated by different proteins was: egg white > whey protein isolate hydrolysates \geq β -lactoglobulin > whey protein isolate > α -lactalbumin. Factors that decreased surface charge (pH \sim pI or high ionic strength) caused a more rapid adsorption of protein at the air-water interface, generally increased dilatational viscoelasticity and increased foam yield stress. The elastic component of the dilatational modulus of the air-water interface was correlated with foam yield stress. The properties of foams did not predict performance in making angel food cakes. A model for foam performance in angel food cakes is proposed.

Keywords: foams, proteins, air-water interface, surface tension

Introduction

Processed foods come in a variety of colors, flavors and textures intended to delight consumers. Foam formation can provide a range of unique textures that are associated with many foods including cake, bread, ice cream and confectionary products [1].

Understanding the physical and chemical mechanisms responsible for the foaming characteristics of these systems is of interest for several reasons. First and most obvious, consistently producing high-quality products is the goal of every manufacturer. Second, processors would like flexibility in their choice of ingredients, so substitution of milk, soy or other proteins for egg white is of interest. The latter goal prompted early investigations into the foaming and interfacial properties of proteins [2].

When proteins are used as foaming agents in foods they contribute to the formation and stability of the foam structure. Destabilization of quiescent foams is determined by factors such as film drainage, film rupture and disproportionation [3, 4]. Conditions become more complex with foods such as meringue, angel food cake or nougat because stability must be maintained during additional processing. With meringue, the foam must show little expansion or collapse when heat-setting causes drying, and converts the foam from a liquid to a solid. In angel food cake, the protein foam is mixed with wheat flour (starch and protein) and sugar, then the mixture is baked to produce a cake. During baking, the foam-flour-sugar mixture must expand to a desired volume before heat-setting causes drying and converts the foam from a liquid to a solid. Nougat presents the complication of maintaining a foam structure in a high sugar/low moisture environment [5].

From the above discussion, it is apparent that manufacturing aerated foods involves a series of reactions involving proteins. The transport of individual proteins from the bulk aqueous phase to the air/water interface, and formation of the interfacial film, occurs in all protein-stabilized foams [3, 4]. Subsequent processing involving mixing, heating and drying affect proteins at the interface and those within the continuous phase. The dynamics involved in processing, such as simultaneously expanding and drying, makes them very difficult to investigate. Once a foam is formed, it can be characterized by air phase volume (overrun), rheological properties and stability [1]. Precise measurement of these properties is problematic for many protein foams due to destabilization processes occurring simultaneously with foam formation and immediately after formation.

In this manuscript we will focus on the foaming properties of whey and egg white proteins (using egg white as a point of comparison). We will first briefly review age-tested culinary principles to provide some background for factors determining overall product quality. Subsequently covered will be physical and chemical properties of air/water interfaces and foams. Emphasis will be given to rheological properties of the interface and bulk properties of foams (overrun, stability and yield stress). Finally, angel food cake will be used as an example of how well our knowledge of foaming properties predicts performance in an aerated food.

Culinary aspects of foams

Egg white has long been valued for its varied functionality in the culinary world. One of the most important properties is its capacity to form voluminous and highly stable foams. Many aerated products begin with the formation of a type of egg white foam called a meringue. Initial whipping generates slight foaming while homogenizing the whites. Whipping is continued until “soft peaks” are formed, and then sugar is slowly added. The amount of sugar added depends on the final application of the meringue and the lightness (overrun) and stability of the required foam [6]. Soft meringues have sugar at less than or equal the amount of egg whites [7]. Hard meringues have twice the amount of sugar than egg white and are stiffer than soft meringues [7]. The mention of “soft peak” and “soft” and “hard” meringues indicate that bulk rheological properties of foam are used as quality indicators in culinary applications.

Meringue can be further processed into several different aerated products. It can be applied to the top of a pie and set by baking at 180 °C for 15 min. Meringue can also be piped into ‘kiss shapes’ for cookies, then baked at 120 °C for 5 hrs. If an angel food cake is desired, a mixture of cake flour and sugar are folded into the meringue and baked at 180 °C for a short time. The resulting cake is light in texture with a slight chewiness. Each of these applications is again dependent on the ratio of sugar to egg white, as well as, the amount of dehydration and thermal treatment that occurs during baking.

A different method of making meringue is classically known as an Italian meringue. Sugar and water are heated to the “soft ball” stage (115 °C), and then slowly added to a

previously whipped hard meringue. This type of meringue is the beginning of nougat. Nougat has a higher sugar to egg white ratio, approximately 6 times the amount of sugar than egg white. Nougat's ability to be cut into candy or be taken further by adding inclusions (e.g., nuts) or coating may be attributed to the heat setting of egg white with hot sugar syrup.

Trial and error over the years has yielded tips for improving egg white foam properties. Dating back to the early 19th century, cooks have whipped egg whites in copper bowls to enhance stability. Many recipes recommend that egg whites are at room temperature prior to whipping to increase final foam volume [8]. Salt and acid, usually cream of tartar (tartaric acid), are added while whipping to enhance stability of the end foam [8]. Therefore, some key observations associated with increase stability and/or overrun are: 1) addition of acid and/or sugar, 2) whipping in a copper bowl and 3) mild heat treatment of egg white solution. Mechanisms associated with these factors will be discussed in the following sections.

Proteins at air/water interfaces

The spontaneous adsorption of proteins from solution to the air/aqueous interface is of central importance to their foaming performance. This phenomenon is thermodynamically favorable due to the simultaneous dehydration of the hydrophobic interface and hydrophobic portions of the protein [9]. Hydrophobic patches on a protein's surface initially drive this process, and surface hydrophobicity has been correlated with improved foaming properties [10-12]. Once contacts are made with the

interface, natural flexibility within the molecules can expose previously buried hydrophobic portions to the interface, potentially leading to interfacial denaturation of the molecules [13].

The most obvious outcome of protein adsorption is a reduction in interfacial (surface) tension. Note the surface tension of water is ~ 72 mN/m at room temperature and equilibrium surface tensions of concentrated protein solutions are often around 45 mN/m [14]. More important than equilibrium surface tension values is the capacity of the protein to rapidly decrease surface tension, as this has been correlated with improved foamability [4]. Indeed, the increased adsorption rate of protein fragments generated during hydrolysis, due to their larger diffusion coefficients as compared to intact proteins, seems a primary cause behind the improved foaming properties of various protein hydrolysates [15-17].

Intermolecular interactions between adsorbed proteins can lead to an interfacial film with measurable rheological properties. Interfacial rheology has long been recognized as a primary factor contributing to the bulk behavior of foams and emulsions. Two general types of measurements are commonly reported in the scientific literature, those based on either shear or dilatational deformations. Detailed descriptions of the principles behind these measurements as well as their pros and cons have been well documented [18].

What follows is a brief review of protein interfacial rheology that is by no means comprehensive. The interested reader is directed to several recent and excellent reviews on protein interfacial rheology and other measurements of proteins at model interfaces

[18, 19]. A notable example we do not cover is the large amount of work being applied to understanding mixed protein/small molecular weight surfactant systems [20, 21].

The interfacial dilatational modulus (E) was originally defined by Gibbs, and is the change in interfacial tension (γ) upon a small change in interfacial area (A) at a constant shape [22]:

$$E = d\gamma/d\ln A$$

This modulus can be thought of as a surfactant covered interface's total resistance to dilatational deformations, i.e. stretching and compression. This modulus contains both an elastic (E') and viscous (E'') component corresponding to the energy recovered and lost during an interfacial deformation. Interfacial shear rheology involves measuring the forces encountered upon shearing a surfactant covered interface parallel to the interfacial plane, note the interfacial area doesn't change during such deformations, but the shape does. Both a shear elasticity (G) and shear viscosity (η) can be defined, again corresponding to the energy recovered and lost during such deformations [23]. The primary difference between dilatational and shear interfacial rheology is that the surfactant concentration at the interface remains essentially constant during the latter.

Interfacial viscoelasticity of protein films depends on numerous factors including the type of protein, cosolutes present and thermal history of the solution. Due to the variety of amino acids contained in a typical protein, a range of intermolecular interactions are possible at the interface, including hydrogen bonding, hydrophobic contacts, electrostatics, disulfide bond formation and van der Waals interactions [14]. Numerous

studies have found that globular proteins such as β -lactoglobulin, ovalbumin and lysozyme tend to form more viscoelastic films as compared to less ordered proteins such as β -casein [19]. This is explained by the flexible β -casein not transmitting force across the interface as efficiently as the more rigid, globular proteins [24]. Recently, dilute mixtures of β -casein and β -lactoglobulin adsorbed at the air/water interface were characterized via dilatational and shear interfacial rheology [25]. Simple models adapted from 3-D rheology were found capable of discerning the nature of the adsorbed layer using both techniques. Shear measurements were more sensitive to the interactions between adsorbed proteins, while the dilatational data reflected both interactions between the proteins and composition.

Electrostatic interactions play a significant role in both protein adsorption and interfacial rheology. Foaming properties have been reported as optimal for a range of proteins near their isoelectric points (pI) [26-29]. Accordingly, protein adsorption to the interface is generally most rapid at this pH as electrostatic repulsion is minimized for the net neutrally charged proteins [23]. Furthermore, viscoelasticity of interfacial films generally peaks for a range of proteins near their pI's [23, 24, 26, 28, 30]. NaCl addition to whey protein isolate solutions at pH levels above or below their pI's increased protein adsorption as evidenced by dynamic surface tension measurements [26]. This was explained by the salt counter ions screening the charged protein molecules. Concomitant increases in dilatational elasticity were observed for these adsorbed layers upon NaCl addition, although this increase was minimal for whey protein isolate (WPI) at pH 3.0 as compared to the same solution at pH 7.0. The weakened dilatational elasticity of WPI

layers adsorbed at pH 3.0 potentially explains notable decreases in foam yield stress [26] and foam stability [29] for WPI foams formed at acidic pH. From the above discussion of electrostatic interactions it is clear that the age-tested benefit of adding acid to egg white improves foaming properties by decreasing egg white pH (7 – 8.5 for fresh eggs and up to 9.7 for stored eggs) such that it is closer to the pI's of the egg proteins [31].

Small amounts of positive, multivalent cations can significantly increase the dilatational viscoelasticity of adsorbed whey proteins (net negatively charged) via specific electrostatic bridging interactions with corresponding improvements in various foaming properties [26, 32]. Copper has a unique association with egg white foams. Whipping egg whites in a copper bowl has been recommended for centuries as a way to improve foam stability [33]. This effect was speculated to be due to an increased stability of ovotransferrin to denaturation when copper is bound [33]. A more recent investigation found that surface tensions of the egg whites was essentially unchanged upon copper addition, although the dilatational elasticity was significantly increased, primarily explaining the increase in foam stability [34]. It is possible, though unproven, that there is a connection between the increased stability to denaturation and interfacial dilatational elasticity.

Heating is an important processing step for many products that consist of protein foams. The effect of heat (20 to 80 °C) was examined for adsorbed WPI using pendant drop tensiometry [35]. Competing effects between the increased fluidity of the adsorbed layer

and increased elasticity from interfacial gelation were noted. Rheological changes were heat dependent.

Foamed food products often contain high contents of sugars and proteins. An understanding of the behaviors of these compounds both within solution and at the air/water interface is desired to better predict or control bulk foaming properties. The short-time adsorption rates of BSA were found to increase in the presence of increasing concentrations (up to 1 M) of sucrose [36]. A potential explanation was that the protein molecule would be more compact in sugar solutions, due to preferential hydration, and hence adsorb more rapidly. It was also noted that the increased viscosity of concentrated sugar solutions should limit diffusion to the interface, meaning protein adsorption in sugar solutions would be a balance of these two phenomena. In contrast, the adsorption of ovalbumin was found to decrease in the presence of sucrose, as observed by dynamic surface tension measurements [37]. Light scattering and mixing calorimetry data suggested that ovalbumin participated in hydrogen bonding with the sucrose molecule, increasing its hydrophilicity and hence decreasing its surface activity.

Foams

Producing a foam involves the generation of a protein film surrounding a gas bubble and the packing of gas bubbles into an overall structure. One of the first differences seen between studies of interfacial protein films and foams is the lack of stability of the latter. While a model interface can be observed for hours or days to establish equilibrium, a foam made with whey or egg white proteins starts changing within minutes of formation.

Therefore, properties of protein foams are measured under non-equilibrium conditions, complicating interpretation within and between investigations. However, some general observations can be made.

The foamability of a protein solution will depend on the aeration process (Wilde et al., 1996). In experimental systems the aeration process is usually whipping or sparging. Typical overrun values given for beaten egg white and meringue range from 500 to 800% [1]. Whey and egg white protein foams have been shown to vary experimentally in overrun from 500 – 1700%, depending on factors such as pH, co-solutes and protein concentration [26, 27, 38-41]. While there may be differences among studies due to foaming method and/or overrun measurement, it is clear that whey and egg white proteins have very similar abilities to incorporate air into a foam structure.

Destabilization of protein foams occurs due to creaming, drainage (from lamellae and plateau borders), bubble coalescence and disproportionation (Dickinson, 1992). The high void fraction of air for meringue and whey or egg white foams (> 80%) means that drainage will be a problem, while creaming may occur in nougat with a void fraction of air of 30 to 40% [1]. In protein foams it is most likely that several of the destabilization mechanisms are occurring simultaneously. This makes detection of individual processes difficult so methods sensitive to a variety of processes, such as conductivity or fluid drainage rate, are often used [39]. Whey protein isolate foams were found to be less stable against gravity induced drainage as compared to egg white foams at a concentration of 5% protein [42]. Within whey protein isolate foams, modifications that

increase the foaming solution viscosity increases stability by decreasing drainage rate (Figure 1; [38]).

Foam rheological properties

As previously mentioned, foams are often characterized in culinary arts by their textural (rheological) properties. Whipping to a “soft peak” and forming “soft or hard” meringues are some examples. Therefore, in addition to overrun and stability, empirically assessed rheological properties are used to determine stages of formation and quality of foams. When one considers these textures are usually assessed by a cook probing a foam with a spatula, the foam yield stress would appear as a logical property to measure by a more quantitative means.

In understanding foam rheological properties there are some assumptions that are made concerning foam structure. One starting point for theoretical treatment of foams is where the air phase consists of hexagonally close-packed monodispersed cylindrical drops (Princen, 1983) and assume a two-dimensional foam of hexagonal cell structure at an air volume fraction (ϕ) of 1 [43, 44]. When deformed, the hexagonal cells deform then “hop” to a location adjacent to the previous one (Figure 2.; [43, 44]. That model predicts that yield stress (τ_0) of foams and concentrated emulsions depends on the interfacial surface tension (γ), radius of the undeformed drops (R), the air phase fraction (ϕ), and the dimensionless contribution of each drop to the yield stress [44]. The relationship was extended by Princen & Kiss [45] to three-dimensional systems, producing the relationship seen in the following equation.

$$\tau_0 = \frac{\gamma}{R_{32}} \phi^{1/3} Y(\phi)$$

The radius of the un-deformed drops is replaced with the surface-volume or Sauter mean drop radius (R_{32}), and $Y(\phi)$ is used as an experimentally derived function to estimate the yield stress contribution from each unit cell.

Pernell, Foegeding & Daubert [46] adapted the vane method of Dzuy and Boger [47, 48] for measuring yield stress of foams. The advantage of this method is that the shear plane occurs along a circumference outlined by the diameter of the vane blades and thus circumvents problems related to slip in traditional rheometer testing cells (e.g, parallel plates). Egg white foams were shown to have yield stress values ranging from 100 to 150 Pa while whey protein isolate foams, of equal or higher protein concentration, had values ranging from 55 to 80 Pa. Yield stress in egg white foams was relatively stable while the yield stress of whey protein isolate foams decayed over the first three minutes after formation [41]. The air phase volume, surface tension and bubble size of egg white and whey protein isolate foams were very similar [41]. Based on the model of Princen et al. [43], the only variable remaining is the fitted parameter of $Y(\phi)$. When compared with $Y(\phi)$ values from a concentrated (ϕ ranging from 0.833 to 0.975) paraffin oil emulsions, the $Y(\phi)$ of egg white and whey protein isolate foams were higher at lower air phase fractions (Figure 3). That is to say, the adjustable factor $Y(\phi)$ determined for egg white foams at $\phi = 0.86 - 0.89$ were similar to paraffin oil emulsion values for $\phi = 0.94-0.98$.

There are a number of factors that affect the yield stress of foams made with whey proteins. Addition of salts (NaCl or CaCl₂), glycine and lactose to whey protein isolate, α -lactalbumin or β -lactoglobulin alter yield stress and overrun [40]. As predicted from equation 1, yield stress was found to be proportional to the cube root of the gas phase volume. However, deviations were seen, with CaCl₂ generally showing higher than predicted yield stress values. Since CaCl₂ should affect the interfacial properties of the proteins, the relationship between interfacial rheological properties and yield stress was investigated [26]. Whey protein isolate solutions at varying pH (3, 5 and 7) and concentrations of salts (NaCl or CaCl₂) were whipped into foams and the foam yield stress was compared with the surface tension and interfacial dilatational elasticity. There was no relationship found between yield stress and surface tension; however, one was established between foam yield stress and interfacial dilatational elasticity (Figure 4). This agrees with recent observations for highly concentrated protein emulsions in which the dimensionless bulk elasticity, $G'/(r\gamma)$, was correlated with the dilatational elasticity of the various protein interfaces [49]. Note that γ and r are the interfacial tension and mean drop radius respectively. This suggests that the model proposed by Princen et al. [45] could be modified by replacing surface tension with interfacial dilatational elasticity.

An additional factor to consider is that the relationship between air phase fraction and yield stress for egg white foams suggested that the bubbles were at a higher phase fraction than calculated [41]. This could be due to inaccurate determination of air phase fraction or the possibility of some connectivity between the protein in the lamellae and that on the bubble surface. Determining connectivity between proteins in the lamellae

and on the bubble surface is not simple. It possibly could be done based on changes in rheological properties. The yield stress of concentrated water-in-oil emulsions shows a marked increase when the dispersed phase fraction goes from 65 to 70% [50], suggesting that determining the change in yield stress in foams as it transitions out of the close-packed region may be a way to determine the close-packed air phase fraction. However, the instability of protein-based foams makes this approach problematic. Additional research is needed in this area.

As previously mentioned, the addition of mild heat to protein solutions has been associated with improved foaming properties. Heating of protein solutions under proper ionic and pH conditions causes denaturation and aggregation such that soluble aggregates or whey protein polymers (based on covalent intermolecular linking) are formed [51]. Whey protein polymers have a much higher intrinsic viscosity than the native globular protein [51]. As a way to understand the effect of mild heating, the foaming properties of mixtures of whey protein polymers and native whey protein mixtures were investigated [38]. Increasing the relative percentage of whey protein polymers in the solution decreased air phase volume but made the foams more stable by decreasing drainage rate. The increase in stability was directly associated with an increase in protein solution viscosity, thereby slowing the drainage rate. Increasing the percentage of whey protein polymers up to 50% increased yield stress but stability of the yield stress measurement decreased [38]. This coincides with culinary practices in that a little heating helps but too much decreases foaming properties. Moreover, it was found that changes in foam yield stress coincided with changes in interfacial dilatational elasticity (Figure 4). It appears

that the interconnecting of proteins at the air-water interface forming an elastic network is important to the yield stress of foams.

Comparison among foam properties and food performance: Angel food cake

Angel food cake is traditionally made by combining an egg white meringue with flour and sugar to form a batter that is baked to form a solid foam cake. Egg white is composed of a variety of proteins that range in chemical properties (molecular weight, pI, glycosylation, phosphorylation and sulfhydryl/disulfide content; [31]). Not all individual egg white proteins have the ability to function in making angel food cake. Ovalbumin (54% of total protein) and globulins (8% of total protein), when tested individually, are the only two that produce angel food cakes with volumes equal or greater than egg white [52, 53].

Protein foams have to possess a range of properties to function in angel food cakes. After whipping to the proper volume and rheological properties (stiff peak), it must be stable when the flour is folded into the foam to form the batter. During the cooking process, the batter expands and ultimately sets into a solid foam structure. Batters made with egg white or whey proteins will expand similarly until the internal temperature reaches 80 to 85 °C (Figure 5; [54]). As the temperature increases, the batter will continue to expand at different rates, ultimately decreasing slightly and “setting” into a solid foam, or collapsing then forming a solid foam of much lower volume. Note that the collapse seen in Figure 5 occurs prior to cooling and thus is not associated with decreased gas volume. Therefore, final cake volume is a function of expansion and prevention of collapse [54-

56]. When a protein foam made from a 10% w/v protein egg white solution is used, the cake is able to set without collapse. In contrast, when cakes are made using 10% w/v whey protein foams, or 5% w/v egg white protein foams, they collapsed [54]. The transition where expansion stopped and either a set or collapsed cake was formed coincided with starch gelatinization and a major increase in the elastic modulus of the batter [54]. There was an apparent minimal amount of egg white protein needed to stabilize the cake; and a failure of whey proteins to stabilize the expanded foam and prevent collapse.

The following model for angel food cake is proposed based on the results of several investigations [54, 55]. An initial foam is formed with an overrun ranging from 500 to 800% (corresponding to an air phase fraction of 83 to 88%). Wheat flour and sugar are mixed with the meringue forming a batter. As temperature increases below protein denaturation temperature (~ 70 °C), the bubbles can expand, coalesce or break, with the net result producing an overall volume increase. At temperatures above 70 °C the combined processes of protein denaturation and starch gelatinization determine if the cake volume will set or collapse. Denaturation and aggregation of proteins surrounding the air bubbles and within the continuous phase produce a gel network that is interdispersed with starch granules. The uptake of water into the granules during gelatinization contributes to the overall rigidity of the network, and/or may cause rupture of air bubbles and loss of gas resulting in decreased cake volume (i.e., collapse).

The proposed model can be used to explain treatments that improve or decrease cake volume. Cakes made with foams produced from 2% or 5% w/v egg white solutions showed collapse [54]. This is most likely due to the amount of protein in the continuous phase being either below the critical concentration to form a gel, or form such a weak gel that it could not withstand the stresses associated with starch granule swelling. An alternative hypothesis is a loss of protein from the continuous phase to the expanding bubble surface area. Assuming 100 ml of a 5% w/v protein solution is whipped into 800 ml of foam (700% overrun), there would be 700 ml of air bubbles surrounded by 100 ml of protein solution in the continuous phase of lamellae and Plateau borders. Assuming that all the bubbles are uniform spheres with radii of 20 μm , then each bubble will have a volume of $3.3 \times 10^{-8} \text{ cm}^3$ and the number of bubbles in 700 ml of air would be 2.09×10^{10} . With initial protein coverage of 2 mg/m^2 , then the protein interface would account for 0.105 g of the initial 5 g of protein. That leaves the majority, 4.9 g, of protein in the continuous phase. Cake volume is generally between 2 to 3 fold larger than batter volume [54]. A 3 fold expansion would result in a bubble radius of 29 μm with bubble volume of $1.02 \times 10^{-7} \text{ cm}^3$. Assuming no change in bubble number during heating and a 2 mg/m^2 surface coverage of protein, then the protein at the interface would account for 0.22 g, reducing the continuous phase concentration to 4.78% w/v. The reduction of continuous phase protein due to increasing bubble surface area is small and would only appear to be significant if the concentration was close to the critical concentration for gelation.

Cakes made with whey protein foams collapse, resulting in cake volumes that are 42% the volume of cakes made with egg white foam (foams made from 10% w/v protein solutions;[54]. The collapse is similar to what is observed for cakes made with lower protein concentration, 2 and 5%, egg white foams [54]. However, whey protein isolate forms strong gels at 10% w/v protein [52] and therefore insufficient protein to form a gel network does not seem plausible. Increasing whey protein concentration to 20% w/v produces cakes with volumes similar to those made with 10% w/v egg white protein; however, the texture is highly elastic and should be considered a different material than a cake [54]. Angel food cake volume can be improved when whey protein foams are modified by applying a mild heat treatment to the solution prior to foaming (i.e., formation of soluble whey protein polymers) or addition of xanthan gum to the foaming solution. Both treatments increase the viscosity (7 mPa s for whey protein polymers and 29 mPa s for xanthan gum) of the continuous phase and may, therefore, slow the destabilization process leading to collapse. However, egg white protein solutions have viscosities of 3 mPa s, lower than the prior mentioned solutions, so they prevent collapse by some means other than viscosity increase [54]. It remains unclear why egg proteins prevent collapse, and unmodified whey proteins cannot, in angel food cake.

Conclusions

Much is known about proteins adsorbed at the air-water interface. Precise measurements of interfacial tension, rheological properties, as well as many other physical and chemical characteristics, are possible. The study of interfacial properties is facilitated by the possibility of establishing equilibrium or near-equilibrium conditions. Investigating

protein foams is more complex in the number of variables and the relative instability of these systems. Nonetheless, much information has been gained by investigating factors that determine foamability, rheological properties and stability. What's lacking is understanding how interfacial and foam properties determine final performance in a food foam during processing. Foams may undergo heat and mass transfer, along with volume changes, when formed into the desired food structure. Much research is needed to understand the complex series of reactions that go into making protein foam-based aerated foods.

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Figure 1.

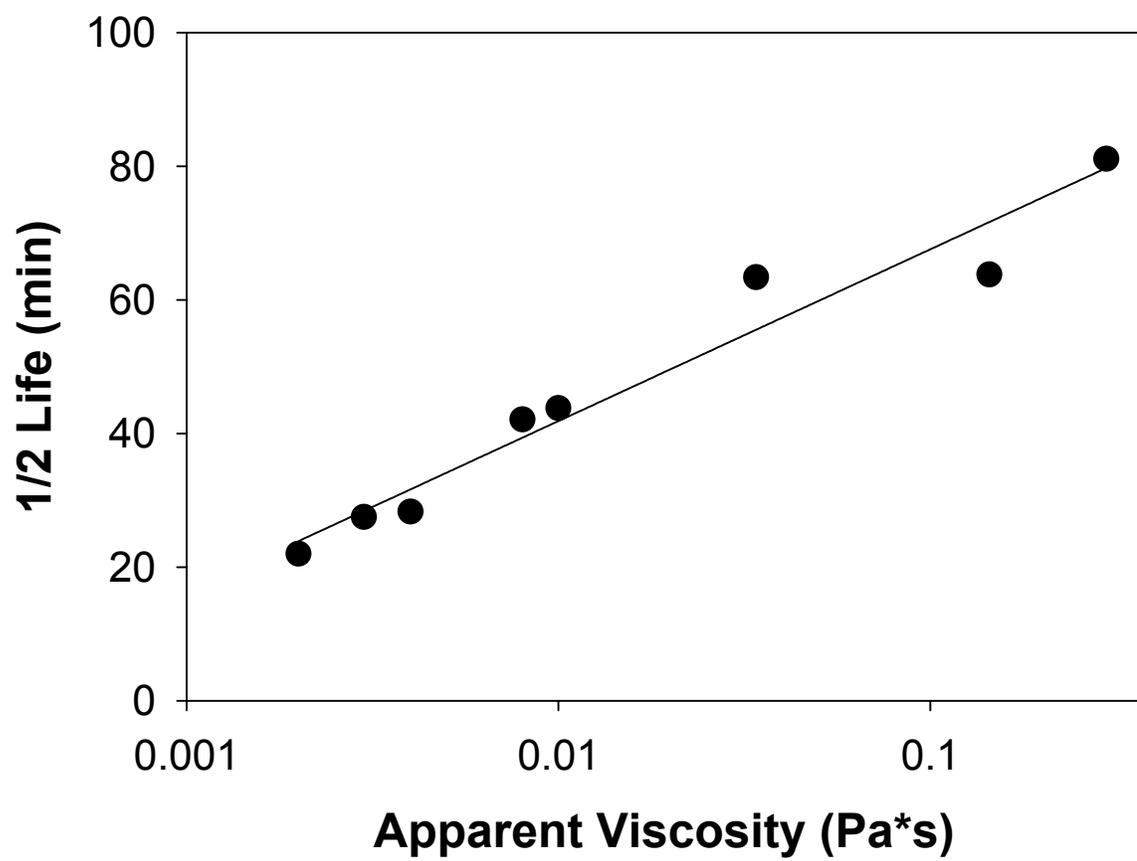


Figure 2.

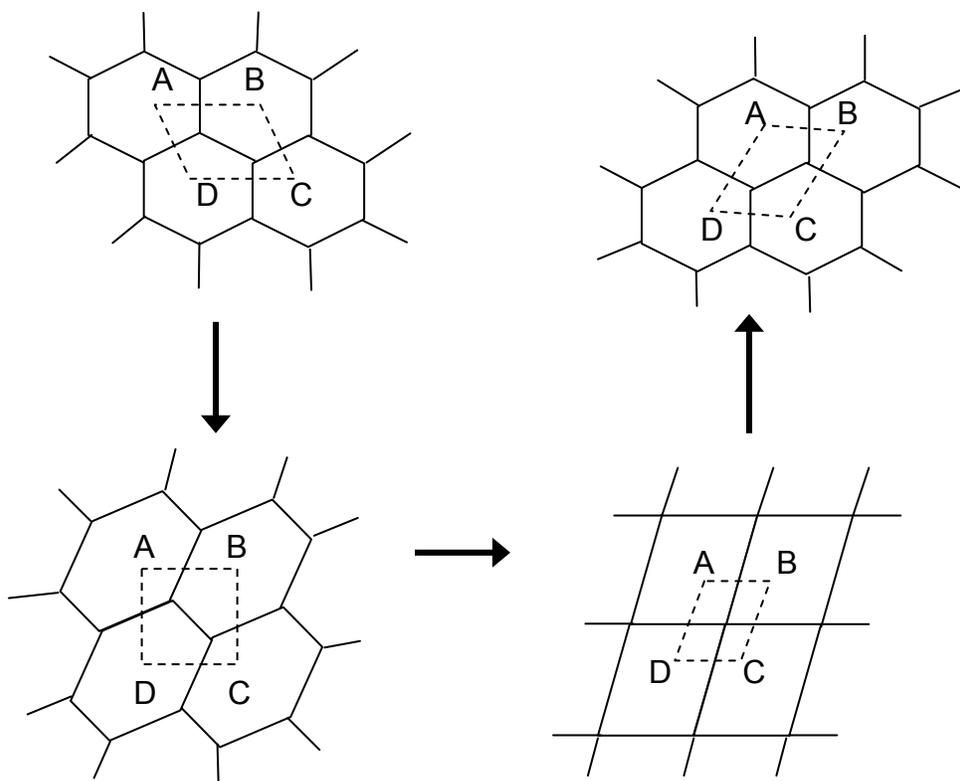


Figure 3.

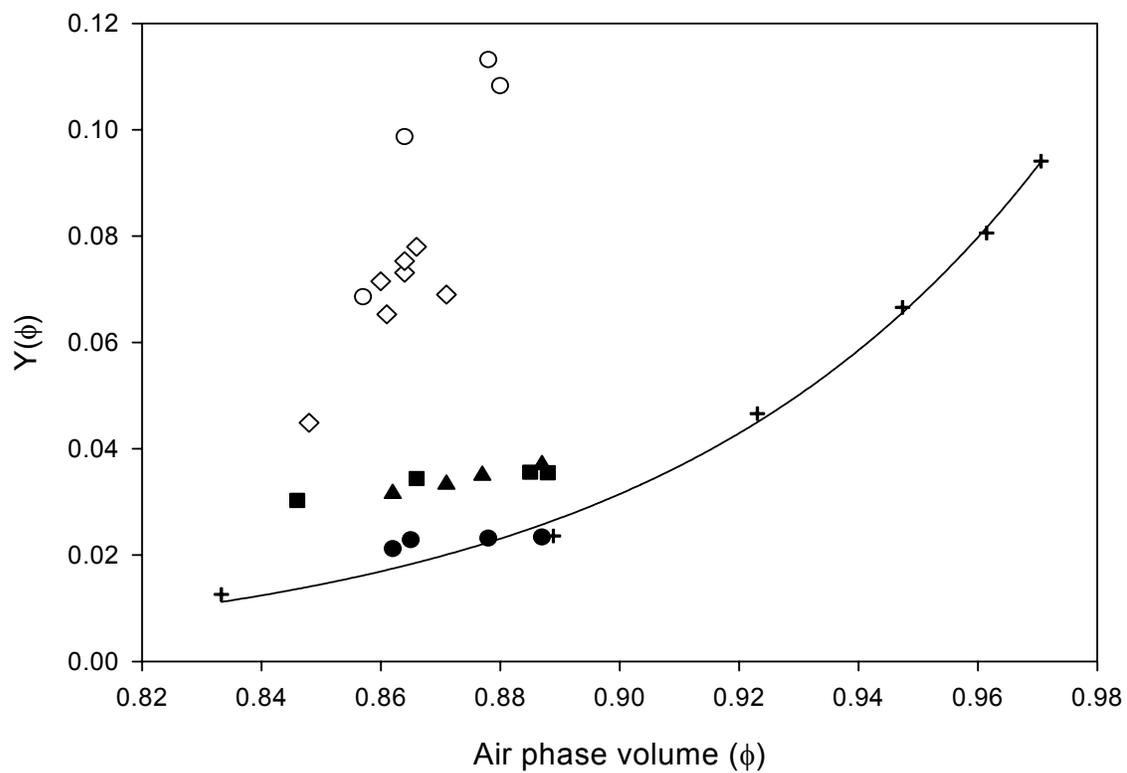


Figure 4.

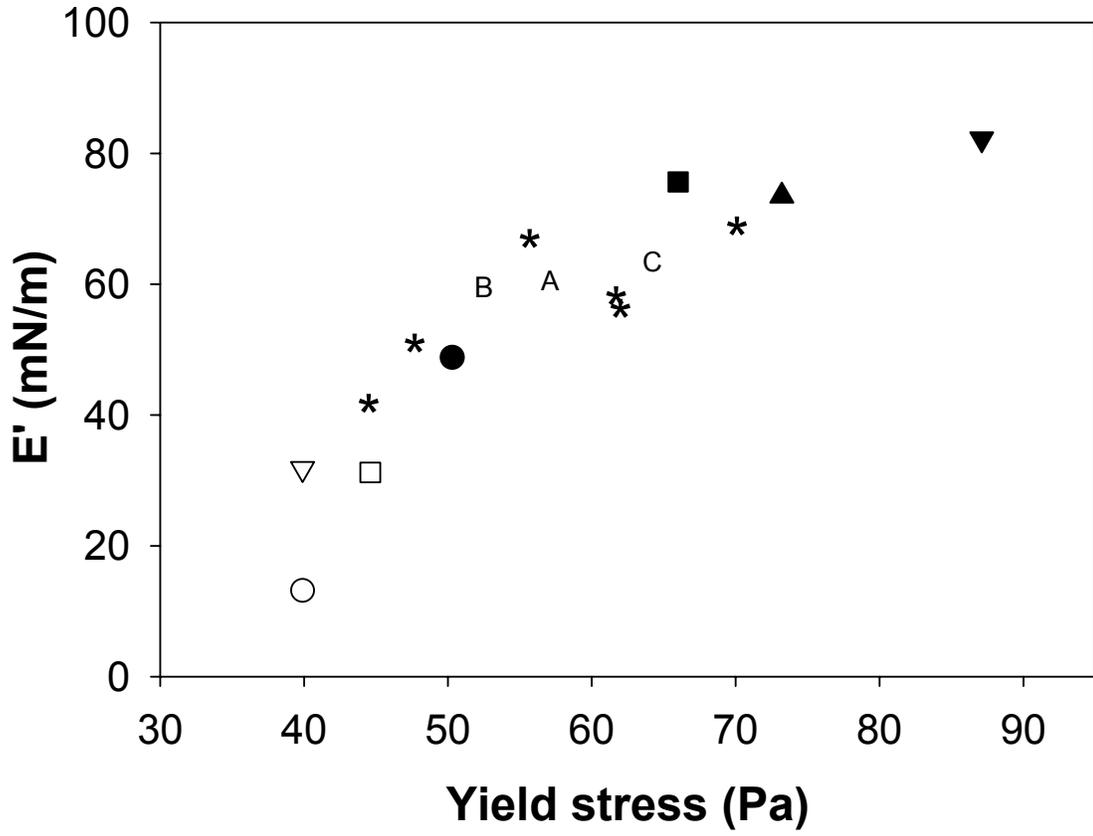


Figure 5.

