

## ABSTRACT

BERRY, TRISTAN KENDRICKS. Foaming Properties, Interfacial Properties, and Foam Microstructure of Egg White Protein and Whey Protein Isolate, Alone and in Combination. (Under the direction of E. Allen Foegeding.)

Egg white protein (EWP) is the standard for culinary foams with its ability to form foams with high volume (overrun) and stability. Whey protein isolate (WPI), a by-product of the cheese-making process, contains at least 90% protein and produces foams with a similar overrun that could potentially replace EWP foams in food products. In this work, foaming properties, interfacial properties, and microstructure of WPI, EWP and combinations of the two, were compared in foams and food systems.

Physical properties of foams (overrun, drainage time, and yield stress), cakes (volume), and air/water interfaces (surface tension) were investigated, prepared with 10% (w/v) protein of EWP, WPI, or combinations of the two. Progressive substitution of WPI with EWP did not cause major changes in foam overrun, but the incorporation of 12.8% (w/v) sucrose reduced the amount of air incorporated into foams. The addition of sucrose showed a large increase in drainage time for foams made with 100% EWP, but exhibited little impact on drainage time in foams made with WPI and WPI/EWP combinations. This increase cannot be explained by an increase in viscosity, as all protein combination solutions showed similar viscosities. Surface tension measurements calculated using a contact angle goniometer showed that WPI/EWP combinations followed the temporal pattern of 100% WPI, while 100% EWP decreased to a lower surface tension, indicating that WPI may be dominating the interface when both proteins are present. Angel food cakes

produced with and without sugar resulted in high volume cakes only in the presence of sugar and at levels of 25% WPI/ 75% EWP or 100% EWP, leading to the hypothesis that sugar and at least 75% of EWP needs to be present to stabilize cake volume. However, although 25% WPI/75 % EWP cakes had volumes similar to 100% EWP, addition of WPI showed a change in cake texture, resulting in a coarser bubble structure and concave shape. This corresponds with the hypothesis that WPI dominates the bubble interface in mixtures of WPI and EWP.

Confocal microscopy was used to characterize foam microstructure and quantify bubble size distributions of foams produced from WPI, EWP, and combinations of the two. Images taken with a confocal microscope were analyzed for bubble area and bubble shape factor using image analysis software. Bubble count per unit area was used as a parameter estimating total bubble surface area. The yield stress model suggested by Princen and Kiss (1989) fit bubble count per unit area, surface tension, and yield stress data values for foams made with EWP, WPI, and combinations of the two. WPI and WPI/EWP combination foams showed a linear relationship, while EWP foams deviated from the pattern, exhibiting a higher yield stress. This confirmed that WPI/EWP mixtures behaved similarly to WPI and differently than EWP. Microstructural examination using a confocal microscope equipped with a heating stage showed that EWP foams containing sucrose formed a network at a lower temperature (~55°C) than WPI and WPI/EWP combination foams containing sucrose. Also, angel food cake batters containing 100% EWP formed a stable network during heating, while WPI and WPI/EWP combination cake batters showed similar destabilization, which resulted in large air bubbles. These results

suggest that foams produced from a combination of WPI and EWP more closely resemble WPI foams, indicating that WPI may dominate the interface in foams where both proteins are present.

Foaming Properties, Interfacial Properties, and Foam Microstructure  
of Egg White Protein and Whey Protein Isolate,  
Alone and in Combination

by  
Tristan Kendricks Berry

A thesis submitted to the Graduate Faculty of  
North Carolina State University  
In partial fulfillment of the  
requirements for the Degree of  
Master of Science

Food Science

Raleigh, NC

2008

Approved by:

---

Jack P. Davis, Ph.D.

---

Nina S. Allen, Ph.D.

---

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

## DEDICATION

For my parents, who always emphasized the importance of education and supported me no matter what.

## BIOGRAPHY

Tristan Kendricks Berry was born to Maurice and Faye Berry on January 30, 1982. She grew up on the family farm in Elizabeth City, NC along with her two sisters, Meredith and Shannon. After showing an interest in the nutritional and culinary aspects of food during high school, her father (an NC State alumnus and avid NC State ice cream consumer) suggested she look at NC State's food science program for college. She entered NC State as a freshman majoring in Food Science in 2000, and after many taking many interesting classes, meeting some great friends (and her future fiancé), and doing an internship at Kraft Foods, she graduated with her B.S. in 2004. After a year-long internship with Nestle R&D, she returned to NC State to pursue her Master's in Food Science in Dr. Allen Foegeding's lab. Her graduate school years were filled with Food Science Club activities (including serving as President for a year) and yet another internship (a summer internship with Frito Lay). Tristan was excited when her boyfriend, Russell, proposed during her final semester of graduate school and looks forward to getting married!

## ACKNOWLEDGEMENTS

In no particular order, I would like to thank:

- Dr. Allen Foegeding for being the best advisor I could ever ask for! Your passion for research was inspiring to me, especially on days when I felt research was frustrating. Your class changed the way I read journal articles, and drastically improved my critical thinking skills. Thanks for all your guidance and for pushing me a little farther than I thought I could go.
- Dr. Jack Davis and Dr. Nina Allen for serving on my committee. I am grateful for your support and feedback throughout this whole process.
- Dr. Eva Johannes for her help with microscopy and image analysis.
- The entire Foegeding lab (all versions!). Paige (and Jeab, while Paige was on maternity leave), you kept the lab organized and were always willing to answer questions (no matter how silly they seemed). Xin, I've learned so much doing research with you! You've always pushed me to work harder! Paula, Jason, Neal, Mallory, Esra, Jeab, Yie Hui, and Tookta, you've all been such fun to work with over the last couple of years! I've learned more about protein, food, and different cultures from ya'll than I ever expected!
- All the other food science students—I have wonderful memories of hanging out, partying, and going on trips with ya'll! I hope to see everyone in the future. I have to especially thank Kristin Price, with whom I shared both undergraduate and graduate classes, as well as the whole graduate school timeline. I loved working on group projects together, which is a good thing because it seems like we've been grouped together a dozen times!

- My wonderful office mates in room 204—Paula, who patiently answered a ridiculous number of questions and entertained us with her singing; Neal, who always had a good story or could at least reach the things on the very high shelves; Mallory Kelly, who was great company and enjoyed chatting as much as I did; and of course, Mallorye Lovett, my original office companion, who always had an encouraging word, a smile, or advice ready for me. The company made the small, slightly claustrophobic windowless office a place where I have many fond memories!
- My family including my parents, Maurice and Faye, for always supporting me, even when I disappeared into my schoolwork for months at a time; my sister Meredith and brother-in-law Brian, who have been wonderful in sharing in the ups and downs of graduate school; and my sister and roommate, Shannon, for sharing in the daily struggles, rarely getting mad at me, making me smile, and joining in the living room dance parties. My extended family also deserves thanks for their support and love!
- Last, but not least, the two constants in my graduate school life: my beautiful cat, Raven, and my fiancé. Raven, thanks for staying with me through many late nights, sleeping by my side (or on top of journal articles), always showing me that there are more important things in life than whatever I was worried about. Russell, my wonderful boyfriend and now fiancé, thanks for being so patient with me through all my frustrations and so excited for me in all my milestones! I only hope that I can be as loving and supportive as you have been! You'll always be my former First Man!

## TABLE OF CONTENTS

LIST OF TABLES.....	ix
LIST OF FIGURES .....	x
REVIEW OF LITERATURE .....	1
General foam applications.....	1
Food foam applications .....	2
Foam generation .....	6
Foam stabilization and destabilization .....	7
Measuring foaming properties .....	11
Drainage time/foam stability.....	12
Overrun.....	13
Yield stress .....	14
Bubble size distributions and image analysis .....	15
Confocal microscopy .....	19
Egg white protein.....	24
Whey protein .....	26
Functional properties of whey protein.....	27
Sugar effects on the foaming properties of proteins .....	32
Research objective.....	33
References.....	34

# FOAMING AND INTERFACIAL PROPERTIES OF EGG WHITE PROTEIN AND WHEY PROTEIN ISOLATE, ALONE AND IN COMBINATION

Abstract .....	43
Introduction.....	44
Materials and methods .....	47
Materials.....	47
Methods .....	48
Solution preparation .....	48
Viscosity measurement .....	50
Foam generation .....	50
Overrun .....	51
Yield stress measurement .....	51
Foam stability/drainage time.....	52
Interfacial tension .....	52
Density determination.....	53
Angel food cake preparation.....	53
Data normalization and statistical analysis .....	54
Results and discussion .....	55
Conclusions.....	67
Acknowledgements .....	68
References.....	69

MICROSTRUCTURAL COMPARISON OF FOAMS AND CAKE BATTERS  
PREPARED FROM EGG WHITE PROTEIN AND WHEY PROTEIN ISOLATE,  
ALONE AND IN COMBINATION

Introduction.....	72
Materials and methods .....	76
Egg white and whey protein isolate.....	76
Protein solutions.....	77
Sodium fluorescein.....	77
Foam generation .....	77
Protein foam microscope slide preparation .....	78
Confocal microscopy .....	78
Image analysis .....	79
Yield stress measurement.....	81
Angel food cake preparation .....	82
Angel food cake batter preparation for microscope.....	83
Protein-sucrose foams or cake batter microscope slide preparation.....	83
Confocal microscopy .....	83
Statistical analysis.....	84
Results and discussion.....	84
Conclusion.....	106
References .....	107

## LIST OF TABLES

### Review of Literature

Table 1: Definitions of some culinary food foams .....4

Table 2: Foods involved in the microstructural imaging using  
confocal microscopy .....23

### Foaming and Interfacial Properties of Egg White Protein and Whey Protein Isolate, Alone and in Combination

Table 1: Composition of no sugar cakes and normal cakes.....54

Table 2: Overrun, yield stress, drainage time, viscosity, density,  
surface tension, and cake volume of WPI/ EWP foams .....56

## LIST OF FIGURES

### Review of Literature

Figure 1: Confocal microscope pathways .....21

### Foaming and Interfacial Properties of Egg White Protein and Whey Protein Isolate, Alone and in Combination

Figure 1: Foaming properties (A: Overrun; B: Yield stress; C: Drainage time) of foams prepared from 10% (w/v) protein solutions at pH 7 with or without soluble particles. All foams were prepared from Provon WPI. .... 49

Figure 2: Overrun of foams prepared from 10% (w/v) protein solutions at pH 7, both in the presence and absence of 12.8% (w/v) sucrose. A: BiPro WPI; B: Provon WPI. ....58

Figure 3: Drainage time vs. solution kinematic viscosity of 10% (w/v) protein solutions at pH 7, both in the presence (open symbols) and absence (closed symbols) of 12.8% w/v sucrose. .... 59

Figure 4: Typical dynamic surface tension measurements of 10% (w/v) protein solutions with (open symbols) and without (closed symbols) 12.8% w/v sucrose..... 61

Figure 5: Surface tension at 600 s of 10% (w/v) protein solutions at pH 7, both in the presence and absence of 12.8% (w/v) sucrose. A: Bipro WPI; B: Provon WPI. .... 64

Figure 6: Volume of angel food cake prepared from 10% (w/v) protein solutions at pH 7. A: Bipro WPI; B: Provon WPI. .... 65

Figure 7: Cross-section images of EWP (left) and 25WPI: 75EWP (right) angel food cakes. .... 66

Microstructural comparison of foams and cake batters prepared from egg white protein and whey protein isolate, alone and in combination

Figure 1: Images prepared for analysis: measured objects shown in green, de-selected objects (not measured, less than 50% of bubble area visible) shown in orange, and background (aqueous protein phase) shown in magenta. Time images shown for 100% WPI 1 with time 0 (left), 5 minutes (center) and 10 minutes (left). ..... 81

Figure 2: Confocal images of WPI, EWP and combination foams. Field size is shown below each image. .... 85

Figure 3: Bubble shape factor histograms for WPI 1 foam (top) and EWP foam (bottom). Shape factor of 1 represents a perfect sphere. . 87

Figure 4: Mean bubble area (top) and median bubble area (bottom) for foam prepared with combinations of EWP and WPI. Data shown is the average of 5 random images throughout the foam and error bars represent one standard deviation above and below the mean... 89

Figure 5: Bubble count per unit area for a single frame over time for WPI, EWP, and combination foams. .... 90

Figure 6: Yield stress of foams made with WPI, EWP, or combinations of the two plotted against the product of bubble count per unit area and surface tension of the foaming solution. Linear regressions and  $R^2$  values are calculated and displayed for WPI and WPI/EWP combinations as well as for EWP foams. .... 93

Figure 7: Volumes of angel food cakes made with combination of EWP and WPI 1 (top) or WPI 2 (bottom). .... 95

Figure 8: Cross-sections of angel food cakes prepared using 100% EWP (left) and 75% EWP: 25% WPI (right). .... 96

Figure 9: Confocal images of WPI, EWP and WPI/EWP combination foams (10% w/v protein and 12.8% w/v sucrose) with heating. Field size is 3.6 mm x 3.6 mm. .... 98

Figure 10: Confocal images of WPI, EWP and WPI/EWP combination foams mixed into an angel food cake batter and heated. Field size is 3.6 mm x 3.6 mm. .... 101

Figure 11: Confocal images of 100% EWP angel food cake batter with heating (continuation of images of EWP cake batter seen in Figure 10). Field size is 3.6 mm x 3.6 mm. .... 103

# CHAPTER 1

## REVIEW OF LITERATURE

### **General Foam Applications**

Foams are defined as the incorporation of gas into a solid or liquid phase. Although structurally complex, foams are commonly encountered in every day life. Foods are filled with foams, from cakes and breads to champagne, beer, meringues and ice cream. Shaving cream, foaming hand soap, bubble bath foam, hairstyling mousse, and the foam created with dishwashing liquid in a sink full of water are several non-food foams observed on a daily basis (Schramm, 2005). In addition, foams can function as insulation applied inside walls or on roofs. These foams generally last much longer than the more transient personal hygiene related foams, with applicators guaranteeing the foam insulation for up to 10 years. NASA also utilizes foam as insulation for the external fuel tanks of its space shuttles. The foam protects fuel from the extreme heat of the accelerating space shuttle and prevents ice from building up on the outside of the tank (Zarembo, 2005). Foams used as temporary sanitary landfill “blankets” not only prevent erosion and wind distribution of landfill content, but also stifle odor emission and deter scavenging pests. Since foams are collapsible, incorporation into landfill layers minimizes volume occupied by foam compared to a traditional soil cover (Schramm, 2005).

Foam has more noble applications as well. Foams have been used for fire-fighting for at least a century and are especially utilized in fires fueled by petroleum-based liquids (Schramm, 2005). By creating a barrier between the flame and oxygen, foams are effective at quenching fires (Alsaati et al, 2003). Fire-fighting

foams are generally composed of a concentrated surfactant solution in water, which is mixed with about 75 to 97% air (Schramm, 2005). Various surfactants are utilized depending on the type of fire. Proteins have been studied for their potential to replace ozone-depleting halons widely used in foam fire extinguishers. Research showed that egg albumin solutions with a low pH and high protein concentration produced foams that quickly stifled fires (Alsaati et al, 2003). Also, the prospect of using masks (similar to gas masks) to allow people to break a fire-fighting foam, using breathable air trapped inside to foam for respiration, is another advantage for using protein foams for fire fighting (Ackermann et al, 2003).

“Anti-terrorism” foams are developed to neutralize dangerous agents of chemical and biological warfare, such as anthrax. The unique properties of foam, such as being sprayed from a small canister, incorporating air and expanding to fill each gap, and draining back down to a liquid in a few hours, shows this system to be a logical choice for anti-terrorism purposes (Ouellette, 2002). Also, non-toxic foams can be used immediately, even before evacuation of an infected area. Anti-terrorism foams usually have very specific detergents, oxidizing agents, and/or enzymes to deactivate the warfare agent of interest (Schramm, 2005).

### **Food Foam Applications**

Food foams are commonly encountered in everyday life, yet little thought generally goes into the complexity of these systems. In fact, most consumers do not realize ice cream contains up to 50% air, and therefore is considered a foam (Campbell and Mougeot, 1999)! Champagne and sparkling wines have air bubbles

incorporated into the liquid due to carbonation from a second fermentation process. Proteins and polysaccharides present in the wine allow foam formation. Foam is significant in the consumer acceptance of beer, as the “head” formed during pouring should have certain characteristics. Some characteristics important to consumers are foam stability, quantity, and “creaminess” (bubble texture), and much research into the complex beverage system has gone into controlling these characteristics (Schramm, 2005). Breads and cakes have air incorporated into their structure, which is then baked to extend the foam’s lifetime. Meringues are also baked to add stability. Ice cream has air incorporated to give the desired texture. Many confectionary products are aerated, such as nougat, marshmallows, aerated chocolate bars, and whipped cream products. Including air helps modify texture, add volume, and alter appearance in confections (Cooke, 2006).

Egg white has been a mainstay for many culinary aerated products. The identity of the foaming agent is even incorporated into the culinary definition of some food foams. Table 1 shows the culinary definition of several food foams as defined by Webster’s New World Dictionary of Culinary Arts (Labensky et al, 1997).

Table 1: Definition of some culinary food foams (Labensky et al, 1997)

Culinary foams		Definition
soufflé		"a dish made with a custard base lightened with whipped egg whites then baked"
mousse		French for "foam", "a soft, creamy food...lightened by adding whipped cream, beaten egg whites or both"
angel food cake		"a light, airy cake made without egg yolks for other fats; its structure is based on the air whipped into the egg whites"
meringue		"mixture of stiffly beaten egg whites and sugar"
meringue	soft	meringue made with equal parts egg white and sugar; used to top a cake or pie
	hard	meringue made with at least two parts sugar to egg white; can be shaped and baked into cookies or nests
	Italian	incorporates hot sugar syrup into egg white; can be used as a shiny topping for cakes
	Swiss	combination of egg white and sugar, heated up to 140°F then whipped

These are just some examples of aerated food products, which are met with high regard in the culinary field. A complex, fragile dish, such as a soufflé or meringue, is a testament to the skill of the chef (Campbell and Mougeot, 1999).

While food foams are associated with upper tier quality, the main purpose of aerating a food product is usually to alter the product's texture. The incorporation of air decreases density, transforming its mouthfeel (Campbell and Mougeot, 1999). Adding air gives creaminess to mousse, lightens a soufflé's texture, and gives angel food cake its characteristic light-as-a-feather quality; all properties so essential to the food product that they are included in the culinary definition. The transformation from a solution to a foam also often alters the rheology of a food product. Rheology

focuses on the relationship between a force acting on a substance and its resulting deformation; simply put, it is the study of how systems behave when work is applied (liquids might flow, solids may bend) (Walstra, 2003). A mixture of egg whites and sugar flows easily, but after whipping into a hard meringue, it can be shaped into cookies, nests, or other desired shapes (Campbell and Mougeot, 1999). Variation in the rheology of hard and soft meringues specially suits each type for its unique purpose.

Aerated confectionary products are often created utilizing a foaming agent in sugar syrup, which is whipped to form a frappé. Several different foaming agents can be used, including egg white, milk proteins (including milk proteins, whey proteins, and caseins), soy proteins, and gelatin (Campbell and Mougeot, 1999; Johnson, 2006). A foaming agent is selected for confectionary products based on its individual benefits and desired function in the product. Egg white protein allows a great volume of air to be incorporated into the product, but is best for products with a shorter shelf life as it does not last well over time. Milk protein can be used to produce stable confections, although less air may be incorporated. Soy protein is heat stable and versatile, allowing it to work in a wide variety of confections. Gelatin generates the most stable foams and yields an assortment of foam textures (Johnson, 2006). The optimal confectionary whipping agent should be selected based on processing parameters and desired end product texture and shelf-life.

## Foam Generation

Foam formation can utilize a variety of methods to incorporate air into an aqueous solution. Mechanical dispersion techniques, such as whipping and shaking, are used to create foams (Pugh, 1996). This involves forcing a liquid phase around an external gas, and is used in food products such as egg foams, cake batter, and whipped cream (Campbell and Mougeot, 1999). Whipping initially produces large bubbles, which are subsequently elongated and broken into smaller bubbles with continued whipping (Prins, 1988). Mechanical dispersion techniques are the most commonly used foaming method in the food industry but involve little control over the amount of air incorporated into the foam (Pugh, 1996; Wilde and Clark, 1996). A method called sparging allows more accurate quantification of air flow. Sparging utilizes a flow of gas passing through an orifice and producing bubbles in solution. The bubbles rise to the top of the solution forming a foam (Pugh, 1996). Gas injection is a type of sparging, and is used in food products such as injecting carbon dioxide in soda or air into ice cream. A third method used for aerating food products involves generation of gas *in situ*. Gas can be generated through a fermentation process (such as in yeast breads or wine) or leavening may be used to encourage gas bubbles (such as in some cakes) (Campbell and Mougeot, 1999). The food industry uses a pressure change to produce foams in a solution supersaturated with gas. When pressure is lowered, the gas is freed from solution, a phenomenon observed when opening a bottle of soda or beer (Pugh, 1996).

Several factors can affect the lifetime of foams, which are inherently thermodynamically unstable. Some foams, such as the foam in a glass of champagne, may only last for a lifetime of seconds, while other aerated products like ice cream can last for a year or more. The rheology of the food product has a significant impact on the lifetime of a foam, with low viscosity liquid foams having much less stability than solid foams. Therefore, one way to preserve foams and add greater stability is to modify the flow characteristics of the foam. For example, whipped egg whites survive as a foam for a matter of hours. However, with the addition of sugar before whipping and drying the foam in an oven, the foam is transformed into a solid meringue state with a lifetime of months or years. In angel food cakes, a foam batter (with a short lifetime) is transformed into a solid structure during baking through protein denaturation and starch gelatinization. An angel food cake can last for up to weeks, with its structure outlasting its sensory qualities (Campbell and Mougeot, 1999).

### **Foaming Stabilization and Destabilization**

While foams are quite complex, the basic questions to understand foams are simple, no matter what type of foam is involved. The most important aspects of foaming are how foams are formed, how the bubbles are stabilized, and how foams are destabilized (Murray, 2007). A variety of molecules are able to stabilize foams. Some of the major properties necessary for foam formation and stabilization include the ability to adsorb rapidly at the air-water interface, go through a quick

conformational change at the interface, and interact with neighboring molecules to form a cohesive film (Campbell, 2003).

Low molecular weight surfactants (LMWS) are small molecules that include both hydrophobic and hydrophilic portions. When involved in foam formation, these small molecules can quickly migrate to the air-water interface and orient themselves with the hydrophobic portion towards the air bubbles. If the film between two bubbles begins to thin, LMWS can rapidly diffuse to the thin areas, thickening the film and stabilizing the bubbles (Wilde and Clark, 1996).

Proteins are easily the most utilized foaming agents in the food industry. They adsorb strongly at the air-water interface, where the protein unfolds. Hydrophobic amino acid residues within the protein orient themselves toward the gas phase while hydrophilic portions of amino acids locate themselves in the aqueous phase. Because proteins usually have multiple hydrophobic sites, they adsorb strongly at the interface with little chance of spontaneous desorption, which is a risk for LMWS. Unfolded proteins can also interact with other protein molecules, forming a film at the interface. Measuring the strength of the film using surface rheology has been helpful in predicting stability against foam destabilization mechanisms such as coalescence and disproportionation (Murray, 2007).

Previous research on foaming surfactants assembled a peptide that switched between behaving like a LMWS (such as sodium dodecyl sulfate) and a protein (similar to  $\beta$ -lactoglobulin) depending on the condition of the solution (pH, metal ions available). Initially, the surfactant-type foam showed higher quality than the protein-type foam with smaller bubbles and more liquid held in the foam. The small

molecules reached the interface and lowered surface tension quickly during foam formation. However, the surfactant-type foam was only stable for minutes, compared to the protein-type foam, which had a lifetime of hours. The protein type foam had the ability for form elastic films at the air-water interface, which were necessary for foam stability (Malcolm et al, 2006).

Two main types of foams exist based on the volume fraction of gas incorporated into the liquid. One type is a bubbly foam, which has a lower amount of gas and spherical bubbles. The other type of foam that occurs with higher gas volume fractions is a polyhedral foam. In a polyhedral foam, the bubbles are so closely packed together that they become non-spherical, forming a honeycomb-like structure. The geometry of polyhedral foams has a big impact on the foam stability (Prins, 1988; Murray, 2007). Polyhedral foams consist of thin films between bubbles, with the area where three films intersect called a Plateau border. Capillary pressure keeps liquid inside the Plateau border. Drainage is the destabilization process that results from the pull of gravity on the liquid found in the thin films and Plateau borders of the foam (Prins, 1988). When the film between two bubbles drains, the film thins and eventually ruptures. This leads to two bubbles becoming one bubble, a destabilization process called coalescence (Damodaran, 2005).

Another type of destabilization found in foams is disproportionation. Disproportionation is the movement of gas from small bubbles to larger bubbles. The Laplace equation for pressure difference between a dispersed and continuous phase is given in Equation 1 (Damodaran, 2005):

$$\Delta P = \frac{2\gamma}{r} \quad \text{Equation 1}$$

where  $\Delta P$  is pressure difference,  $\gamma$  is interfacial tension, and  $r$  is gas bubble radius. The Laplace equation indicates that smaller bubbles have a greater pressure difference than larger bubbles (Damodaran, 2005). Since gas solubility is proportional to pressure, gas is more soluble around smaller bubbles in the foam. Disproportionation occurs when gas diffuses from small bubbles to large bubbles, and the self-accelerating process continues as the small bubbles shrink (Prins, 1988).

Disproportionation is difficult to quantify in polyhedral foams due to difficulty in measuring bubble size distributions (Ettelaie et al, 2003). Instead, disproportionation has been studied in systems observing individual bubbles (Dickinson et al, 2002) and pairs of bubbles (Ettelaie et al, 2003) near a planar air-aqueous phase interface. While these systems may not be representative of actual foams and food products, research found that initial size differences in neighboring bubbles resulted in different bubble shrinkage rates. Observing various bubble starting positions showed that diffusion between neighboring bubbles influenced changes in bubble size distribution (Ettelaie et al, 2003). Research has found that disproportionation occurs between bubbles in the same region but also results in the transfer of gas from bubbles in lower regions to higher regions of the foam (Jang et al, 2006). Attributes such as a high viscosity or solid bulk phase (such as a cake after baking) can mechanically inhibit disproportionation (Dickinson et al, 2002). Theoretically, a

strong interfacial film formed by protein around air bubbles should be able to prevent or at least greatly reduce disproportionation (Damodaran, 2005). However, research has found that even highly viscoelastic films do not stop disproportionation but only slow it down slightly (Dickinson et al, 2002; Du et al, 2003). Disproportionation has been observed as a destabilization mechanism in foams produced from a combination of egg white and invert sugar (Lau and Dickinson, 2005).

### **Measuring Foaming Properties**

Protein foams are complex structures which fluctuate depending on countless variables. The concentration of protein in solution and the method of hydration can impact foaming properties (Phillips et al, 1990). Previous research found a decrease in the rate of drainage in both ovalbumin and WPI foams when protein concentration increased (up to 16% protein). Also, an increase in overrun up to a maximum (at about 8% protein) was shown when protein concentration increased for both types of protein foams. Increased viscosity with increasing protein concentration could explain the changes, as the overrun decrease at high protein concentrations could be due to lower protein solubility (Britten and Lavoie, 1992). When whipping a foam, the initial volume of protein solution (especially in relation to bowl volume and geometry) affects the amount of air incorporated. The mixer used to produce the foam is also very important. Number and type of beaters, type and size of bowl, beating speed, and even mixer model should be kept constant to produce consistent foams. Also, foams tend to change rapidly with time, so subsequent measurements should be made in a consistent, prompt fashion (Phillips et al, 1990). The need for

standardized methods becomes obvious if data is to be compared between researchers or laboratories. The following are some common methods used for characterizing foams.

### **Drainage Time/ Foam Stability**

Foam stability (or drainage time) can be quantified in several ways. Generally, a more stable foam has a longer drainage time, which quantifies the destabilization process of gravity pulling on the solution present in the bubble lamellae. One method of quantifying foam stability involves measuring the time necessary for half of the foam's mass to drain (Phillips et al, 1987; Wilde and Clark, 1996). A foam is whipped in a bowl equipped with a 0.6 cm hole. Immediately after whipping, the hole is uncovered and the bowl is placed on a ring stand above a scale. Over time, liquid drains into a weigh boat on the scale and the amount of time necessary for half the mass to drain is recorded as drainage time (Phillips et al, 1987). Another expression of foam stability is the foam to drainage liquid volume ratio after a certain period of time. A simple measurement of the amount of liquid in a foam by monitoring the liquid-foam boundary over time quantifies foam stability (Wilde and Clark, 1996). Conductivity can also be used for measuring foam drainage. The initial conductivity of the liquid is recorded, and as the foam is formed, the conductivity decreases as the liquid level is reduced. Conductivity measurements over time should increase with increasing liquid level, providing a measure of foam stability (Wilde and Clark, 1996).

Whipped egg white protein has been found to show a decrease in foam stability with increasing whip times. It is thought that the ovomucin-lysozyme complex that aids in the initial foam formation in the initial 5 to 10 minutes of whipping is insoluble and thus greater complex formation after 15 minutes of whipping increases the rate of drainage (Phillips et al, 1987).

### **Overrun**

Overrun is the measure of the amount of air incorporated into a foam. It can be quickly calculated using the equation: Equation 2

$$\text{Overrun (\%)} = \frac{(\text{weight of 100 mL protein solution} - \text{weight of 100 mL of foam})}{(\text{weight 100 mL of foam})} \times 100$$

A standardized method of overrun calculation simply involves a weigh boat holding 100 mL. The weight of protein solution required to fill the weigh boat is measured, and after whipping, foam is gently scooped out of the bowl and placed in the weigh boat. Incorporation of large air bubbles is avoided and the top of the weigh boat is leveled off. The weight of the foam is then entered into overrun calculations (Phillips et al, 1987).

Overrun is dependent on the foam whipping time. Overrun of a high sugar egg white mix increased steeply in the first few minutes of whipping due to large air bubbles incorporated into the foam. The large air bubbles were broken down into smaller bubbles during 5 to 10 minutes of whipping, which caused a decrease in overrun. After 10 minutes, the overrun hit a plateau, showing little change in total air incorporated (Lau and Dickinson, 2004). Generally relatively long whipping times

allow more unfolding of the egg white proteins, which leads to greater interactions (and a more elastic film) at the air bubble interface. However, extended whipping times can lead to overbeating of egg white, which occurs when proteins coagulate excessively at the interface, form insoluble aggregates, and disrupt the foam structure. This can lead to a less stable foam and reduction in overrun (Lau and Dickinson, 2004).

### **Yield Stress**

While overrun and foam stability are the most common properties measured to characterize a foam, yield stress is also of importance. Yield stress is a type of rheological measurement, and is related to the “peak stage” mentioned in culinary recipes. When whipping egg whites, a recipe may state to whip until hard (or soft or medium) peaks are formed, this reflects the ability of a foam to hold its shape. If the correct stage is not obtained, the finished product will probably be of low quality (Pernell et al, 2000). In scientific terms, yield stress is defined as the point at which the foam transitions from behaving like an elastic solid to behaving like a liquid when subjected to a deformation (Princen and Kiss, 1989). An everyday illustration of yield stress is found when ketchup takes a few minutes to begin flowing from a bottle. Initially the product is behaving like a solid, but under enough stress it flows like a viscous liquid.

A quick, easy, and reliable method for measuring yield stress of protein foams utilizes a vane attached to a rotational rheometer (Pernell et al, 2000). The yield stresses of other food products, including mayonnaise, mustard, ketchup,

applesauce, and tomato puree, have been successfully measured using the vane method (Genovese and Rao, 2005). For measurement of foam yield stress, a vane is carefully inserted until the top is level with the foam, then vane rotation begins. The maximum torque is recorded and using the dimensions of the vane, yield stress can be calculated according to the following equation:

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

where  $\tau_0$  is yield stress,  $M_0$  is maximum torque, and  $h$  and  $d$  are vane height and diameter. Some advantages of using the vane method for determining foam yield stress are minimal disruption to the foam structure, elimination of wall slip, and rapid measurements, which are important for time dependent foams (Pernell et al, 2000).

Yield stress has been modeled based on the following equation (Princen and Kiss, 1989):

$$\tau_0 = \frac{\gamma}{R_{32}} \phi^{1/3} Y(\phi) \quad \text{Equation 4}$$

where  $\gamma$  is interfacial tension,  $\phi$  is gas volume fraction,  $R_{32}$  is surface-volume mean drop radius and  $Y(\phi)$  is an experimentally derived parameter. Of these variables, overrun measures the gas volume fraction of the foam, but quantifying bubble size distributions is important to fully understand the mechanisms behind yield stress.

### **Bubble Size Distributions and Image Analysis**

Quantifying bubble size is important to understanding the structure of a foam. Bubble size influences mechanical flow properties, foam stability, and even appearance of foams. Measuring bubble size distributions is not as common as

measuring gas volume fraction (overrun) mainly due to difficulty in acquiring the needed microstructural data (Labbafi et al, 2007). Routine bubble size measurements are difficult for several reasons, including the fact that diluting foams does not work well, foams are thermodynamically unstable, and foams tend continuously change through creaming, coalescence, or disproportionation (Murray, 2007). The fact that foams continuously change in both space and time make bubble size distributions particularly difficult to quantify (Dickinson et al, 2002).

Numerous methods have been explored to tackle the complex problem of quantifying foam structure. Methods such as magnetic resonance imaging (MRI) and multiple light scattering using fiber optics have been used to view foams over time (Pugh, 2005; Rouimi et al, 2005; Wang and Narsimhan, 2006). Optical tomography, a technique involving imaging thin slices of a translucent foam then using software to combine the images into a 3-D reconstruction, has been used to compare wet and dry foam structures (Pugh, 2005). X-ray tomography is similar in method, employing x-rays to pick up density differences within a sample. Samples of aerated food products were reconstructed in three dimensions slice by slice, then parameters such as bubble size distribution and cell wall thickness were analyzed successfully (Lim and Barigou, 2004).

Optical microscopy is the most prevalent method for foam image analysis and bubble size quantification in the food industry (Labbafi et al, 2007). Confocal microscopy is a useful technique for imaging foams (Murray, 2007). Images acquired using confocal microscopy have been analyzed for object dimensions (such as area, length, or perimeter) or shape (like shape factor) by thresholding the

image so the objects stand out against a background. Then commercial image analysis software can be used to calculate desired parameters (Ferrando and Speiss, 2000). Confocal microscopy has been used to image whipped cake batter, with measurements of air bubble area, diameter, area fraction of air, and estimates of surface area per unit volume (Richardson et al, 2002). Egg white and invert sugar foams were imaged using confocal microscopy focusing on a single frame over time to track the changes in selected individual bubble diameters (Lau and Dickinson, 2005). Because these foams had a high solids content, they were stable over long periods of time and suitable for time lapse imaging.

Other imaging methods utilize optical microscopy other than confocal. An on-line image capturing method for food foams (white sauce, fresh cheese, ice cream, whipped cream) has been developed which involves foams passing through a quartz visualization cell under a phase contrast microscope. The images were subsequently analyzed using software, and the bubble diameter (assuming a spherical shape), elongation ratio (length to width relationship), and circularity (irregularity from a perfect circle) were collected (Labbafi et al, 2007). Denatured  $\beta$ -lactoglobulin foams were viewed using a reversed phase light microscope and processed with image analysis software (Bals and Kulozik, 2003). Sponge cake batter was imaged using a light microscope, with a batter layer flattened between a slide and cover slip. The equivalent circle diameter of the flattened air bubbles was calculated along with the equivalent sphere volume (Sahi and Alava, 2003). A light microscope equipped with a heating stage was used to image cake batter while heating (simulating a baking process) with similar image analysis (Pateras et al,

1994). Digital and video cameras have been used to capture foam images, which were then transferred to a computer and analyzed using image analysis software. The foam images were enhanced, transformed into two dimensions, and the bubble size distributions were measured (Hicasmaz et al, 2003; Pugh, 2005; Murray et al, 2005). Foams produced from ice cream mixes in a glass column were analyzed for mean bubble diameter at different heights. The thicknesses of plateau borders touching the glass column were also analyzed. This was used to determine the size of fat particles that could exist within the film without inducing bubble coalescence (Eisner et al, 2007). A unique method for measuring bubbles in an ovalbumin solution utilized a capillary tube with a slight vacuum placed in the foam. When liquid and gas bubbles traveled into the tube, bubbles passed through two pairs of infrared phototransistors, which detected the length of the deformed bubbles. Corrected bubble size and gas volume fractions were calculated (Du et al, 2003).

Several challenges exist in relation to analyzing foam images from microscopy. Foam bubbles obtained from optical microscopy and used in image analysis are often not spherical but polyhedral in shape. However, previous work has assigned the polyhedra an average diameter as if spherical (Pugh, 1994). Other dimensions previously quantified using image analysis on cake batter are area of air bubbles and shape factor (which measures deviation from a perfect sphere) (Hicasmaz et al, 2003). Another debatable issue is the number of bubbles or frames that need to be analyzed for strong data. Previous published work has imaged 5 images per treatment (Pateras, 1994); 5 images per treatment, for a total of at least 100 bubbles (Hicasmaz et al, 2003); 16 images per sample (Sahi and Alava, 2003);

5-10 images per sample, with an average of 300 bubbles (Bals and Kulozik, 2003); 5 images per treatment, with an average of at least 500 bubbles total (Labbafi et al, 2007); and 200 to 300 bubbles per treatment (Wang and Narsimhan, 2006). Others have repeated experiments in duplicate (Eisner et al, 2007) or in triplicate (Richardson et al, 2002). Other challenges associated with confocal microscopy image analysis have been met by excluding “bubbles” with an area of less than 50 pixels (noise) and only including bubbles touching the border on two sides, but not those touching the other two sides of the image (Richardson et al, 2002).

Bubble size distributions can be useful when trying to identify the main destabilization mechanism in foams over time. Coalescence generally shows larger bubbles appearing. Disproportionation results in a bimodal distribution as the large bubbles grow at the expense of smaller bubbles (Pugh, 1994).

### **Confocal Microscopy**

Confocal laser scanning microscopy (CSLM) is a type of light microscopy that can image very thin optical sections of a thick specimen. A scanning laser beam illuminating the sample and pinhole which eliminates light from areas of the specimen other than the focal plane are two unique components of this microscope system. The resulting images are sharp and allow for 3-D reconstruction of samples (Foster, 1997). Other advantages of using a confocal microscope include the ability to image live cells non-invasively using optical sections, high sensitivity to fluorescence, high resolution, ability to label multiple structures, and digital images suitable for further image processing or analysis (Hibbs, 2004).

The idea for a confocal microscope was initially patented by Marvin Minsky in 1957, but it took several decades, and the invention of lasers and computers, before confocal microscopy made an impact (Hibbs, 2004; Sheppard and Shotton, 1997). The first commercial confocal microscopes appeared in the late 1980s and were met with excitement from the biological research community, as this type of microscope could image live cells. These early microscopes were quite slow in image processing, but today numerous improvements (including more fluorescent probes and computer upgrades) have brought confocal microscopy to the forefront (Hibbs, 2004).

In essence, CSLM is light microscopy with the addition of lasers and a pinhole. A laser scans a specimen in a raster pattern (across horizontally, and then down). The laser is reflected from a dichroic mirror (which reflects light of a certain wavelength, but allows longer wavelengths to pass through) to an objective lens, which focuses the laser into the sample. The fluorescence in the sample is excited by the laser, and emitted light (which is of a longer wavelength) passes through the dichroic mirror, passes through a pinhole, and travels to a detector (a photomultiplier) which then sends the information to a computer to produce an image (see Figure 1).

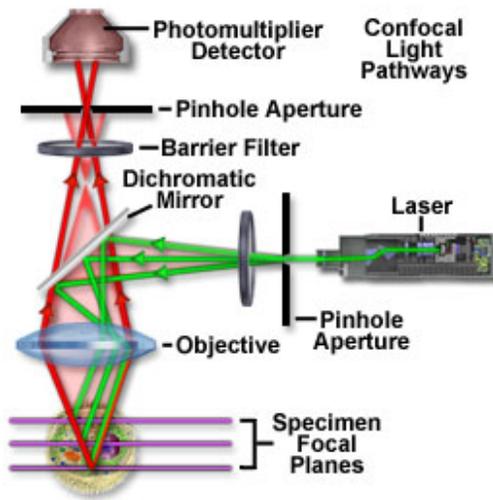


Figure 1: Confocal Microscope Pathways (Fellers and Davidson, 2007)

The pinhole is of utmost importance. Only light emitted from the focal plane will pass through the pinhole to reach the detector; light from planes above or below the focus will fall short or long of passing through. The elimination of out-of-focus light is the reason for the clear optical sectioning obtained from confocal microscopy (Ferrando and Spiess, 2000).

Visualizing the microstructure of food products is useful to the food industry when improving existing products, creating novel foods, and altering properties of products derived directly from plant and animal tissue. Confocal microscopy is especially useful in visualizing microstructure because, unlike many techniques, the non-invasive preparation produces few artifacts and can be used on physiologically active structures. Emulsions are good specimen for confocal microscopy, and visualizing the emulsion droplet size and structure is helpful in developing low fat products (such a margarines, cheeses, and meat emulsions). Plant tissues (such as potato, apple, and onion) can be imaged to give more information about the cells

and distribution of structural elements and macromolecules (Ferrando and Spiess, 2000).

Confocal microscopy has been used to further understand the microstructure of many food products in the last decade. Confocal microscopy was used to visualize bubbles in an aerated high sugar egg white system, showing changes in bubble density and bubble size distributions as well as indicating the extent of protein associated with the bubbles (Lau and Dickinson, 2004). Some previous products whose microstructure has been imaged using confocal microscopy include those listed in Table 2. The popularity and wide ranging utility of confocal microscopy is evident from the microstructural imaging of these extremely diverse food products.

Table 2: Foods involved in microstructural imaging using confocal microscopy  
**Foods imaged using confocal microscopy** **Reference**

	<b>Foods imaged using confocal microscopy</b>	<b>Reference</b>
Fresh fruits and vegetables	carrots, celery, cucumbers, and apples	Alvarez et al, 2000
	yams	Durrengerger et al, 2001
Bread products	wheat dough and fresh wheat bread	Durrengerger et al, 2001
	wheat gluten and sourdough bread model systems	Schober et al, 2003
	gluten-free sorghum bread	Schober et al, 2007
Cheese products	processed cheese	Mounsey and O’Riordan, 2008
	cheddar cheeses with differing fat contents	Gunasekaran and Ding, 1999
	Mozzarella cheese	Auty et al, 2001
Meat products	ostrich meat product “Yor” (Thai sausage)	Utaiwan et al, 2007
	chicken meat gels	Trespalacios and Pla, 2007
	rice-flour battered chicken drumsticks	Mukprasirt et al, 2000
Starch	starch granules, both native and after gelatinization	Velde et al, 2002
	granule-associated proteins within starches	Han and Hamaker, 2002
Gels	WPI and xanthan gum gels	Bertrand and Turgeon, 2007
	$\beta$ -lactoglobulin stabilized emulsion gels	Kerstens et al, 2006
	$\beta$ -lactoglobulin/ $\beta$ -carrageenan mixed gels	Ould-Eleya et al, 2006
	$\beta$ -lactoglobulin/ amylopectin gels	Olsson et al, 2002
Other	cooked spaghetti	Durrengerger et al, 2001
	milk chocolate	Auty et al, 2001
	mayonnaise	Langton et al, 1999

## **Egg White Protein**

Eggs are the historical gold standard used in many culinary applications. The three most recognized uses of eggs are heating of eggs so they coagulate or solidify as in cakes and breads, utilizing egg yolk components as an emulsifier for mayonnaise, sauces, and dressings, and whipping egg white to incorporate air into products such as angel food cakes and meringues (Lomakina and Mikova, 2006). Many different proteins make up egg white, and lend to its unique functional properties, namely foaming ability. The major protein, ovalbumin, makes up about 54% of egg white proteins by weight. Ovalbumin has a molecular weight of about 45,000 Da and an isoelectric point at a pH of 4.6-4.8. It possesses four free sulfhydryls and one disulfide bond. Ovalbumin easily denatures with heat treatment or with shaking or whipping (Ternes, 2001). Ovalbumin alone produces foams after a longer beating time and with a coarse structure (Dickinson, 1992). An angel food cake prepared with ovalbumin showed a texture slightly tougher than a control prepared with egg white (Johnson and Zabik, 1981b). If ovalbumin is removed from egg white, the resulting foam collapses upon baking, indicating the role of the main protein in providing thermal stability in the transition from a liquid to a solid foam (Dickinson, 1992).

Despite widespread use and research on egg whites, knowledge on the minor proteins in egg white is far from complete. Guerin-Dubiard and others (2006) recently identified 16 unique proteins in egg white and found 3 unidentified proteins, while an even more up-to-date study found 78 egg white proteins (Mann, 2007). Several of the main minor egg white proteins are conalbumin, ovomucoid, globulins,

lysozyme, and ovomucin (Mine, 1995). Conalbumin makes up about 13% of egg white proteins, has the ability to bind ions, and has the lowest heat denaturation temperature of egg white proteins (60°C) (Ternes, 2001). Using conalbumin alone to produce angel food cakes resulted in a low volume cake with a tough texture. Ovomuroid had the greatest capacity for lowering surface tension, but showed poor foaming ability and low volume cakes (Johnson and Zabik, 1981b). However, ovomucoid is very thermostable, and thus is thought to assist in stabilization of thermal transitions during baking (Ternes, 2001). Globulins alone exhibited the greatest foaming capacity, which resulted in cakes with the highest volume and a texture similar to an egg white control cake. Lysozyme had low foaming ability on its own and makes low volume, tough cakes. Ovomucin showed the highest viscosity of all the egg white proteins, but showed little foaming ability on its own (Johnson and Zabik, 1981b).

Previous research by Johnson and Zabik (1981a) focused on various combinations of egg white proteins analyzed using a response surface methodology. Foaming ability increased with increasing ovomucin and decreasing lysozyme concentration. However, angel food cake volume showed the opposite effect, increasing with increasing lysozyme and decreasing ovomucin content. The optimal levels of ovomucin and lysozyme were found to be 0.2-1% and 0-1.8% respectively, as opposed to the normal levels in egg white of about 1.5% and 3.5% (Johnson and Zabik, 1981a). Ultrastructural examination of foams revealed that lysozyme strengthens egg white foams, resulting in less rupturing and a more flexible film. Ovomucin appeared to increase viscosity of the foaming solution but refrains from

forming a continuous layer, indicating that ovomucin does not form intermolecular associations (Johnson and Zabik, 1981c). Overall, the outstanding foaming properties of egg white appear to be the result of interactions between the individual proteins (Damodaran, 2005).

## **Whey Protein**

Whey is a by-product of the cheese-making process. Whey proteins are specifically defined as milk proteins soluble in whey at pH 4.6 and 20°C, after casein precipitates during cheese production (Farrell et al, 2004). If acid is added during casein precipitation, such as in cottage cheese production, then the resulting product is acid whey. If enzymes or microorganisms are added to milk to aid precipitation, then sweet whey is the end product. Sweet whey is the major type of whey produced in the United States, claiming about 94% of the market, while acid whey represents only 6% of whey production (Kilara and Vaghela, 2004).

The main component of whey is water (about 94%), with other minor components such as lactose (4.5%), protein (0.8%), and minerals (0.7%). When whey is dried and undergoes filtration processes to concentrate components, a high protein powder is the result. Whey protein concentrates (WPC) contain at least 25% protein, and whey protein isolates (WPI) are more purified by-products, containing at least 90% protein. Whey proteins have found a niche as an ingredient in the food industry due to their distinctive solubility over a broad range of pH, unique functional properties, and relatively low cost compared to other protein products (Kilara and Vaghela, 2004).

Several different proteins make up whey proteins. The major protein is  $\beta$ -lactoglobulin (approximately 10% of the total protein in milk and about 58% of whey proteins), composed of 162 amino acids, 2 disulfide bonds and a free thiol, and a molecular weight of 18,277 Da (Kilara and Vaghela, 2004; Farrell et al, 2004). The second most common whey protein is  $\alpha$ -lactalbumin, which represents about 2% of the total milk protein and about 13% of all whey proteins (Kilara and Vaghela, 2004). This 14,178 Da molecular weight globular protein contains 123 amino acids and 4 disulfide bonds (Farrell et al, 2004). Of the two main components of whey protein,  $\beta$ -lactoglobulin shows better foaming characteristics, with high yield stress and higher overrun values than  $\alpha$ -lactalbumin. When mixtures of solely these two proteins were combined, overrun showed a linear increase with increasing  $\beta$ -lactoglobulin content. In contrast, yield stress showed a non-linear increase, indicating a different mechanism for this property (Luck et al, 2001). Bovine serum albumin is the third most abundant whey protein, making up about 8% of total whey proteins. BSA includes 583 amino acids, 17 disulfide bonds, and has a molecular weight of 66,399 Da. Various immunoglobulins make up about 6% of total whey proteins (Farrell et al, 2004).

### **Functional Properties of Whey Protein**

Whey proteins have numerous functional properties which allow them to improve a wide range of food products. Increasing viscosity in soups and sauces, providing emulsion stability in dressings, boosting water-holding ability in meat and seafood products, altering texture in meats, seafood, and cake products, and

enhancing foam stability in ice creams, whipped toppings, and meringues are just several of the various applications of whey proteins as food ingredients (Holt and Roginski, 2001).

The functional properties of whey have been identified for decades. In 1930, Peter and Bell's research noted that whey protein may be a suitable, economical replacement for egg white in foaming applications, except for those applications involving heating or baking. While various whey protein treatments investigated produced foams equal to or greater than egg white in foam stability and strength, the heat coagulative properties of whey proteins fell short of egg white (Peter and Bell, 1930). Richert (1979) also confirmed whey protein's foaming capabilities as comparable to egg white in overrun, whipping time, and foam stability, but also observed the lack of ability to incorporate additional solids into the foam and retain stability during heating.

Subsequent investigations into whey protein substitutions for egg white in angel food cakes confirmed this theory. DeVilbiss and others (1974) conducted experiments comparing control (egg white) angel food cakes to those produced with whey protein concentrate. During baking, the whey protein cakes rose quickly to a maximum volume and then collapsed, while the control cakes held their shape (DeVilbiss et al, 1974). Further experiments on angel food cakes made with WPI showed similar expansion followed by collapse during baking. Egg white and WPI behaved similarly during initial baking expansion. However, around the protein denaturation temperatures (75°C to 85°C), elasticity of the cake batter increased for egg white but showed a decrease for WPI. During subsequent starch gelatinization,

the egg white batter possessed enough strength, elasticity, or both to sustain the cake network while WPI batters collapsed (Pernell et al, 2002b).

Whey protein isolates can vary in composition based on cheese-making production parameters. However, research found that despite differences in composition between acid WPI and sweet WPI, no significant differences were found in foaming properties tested (overrun and foam stability). Also, the presence of high levels of glycomacropeptide in the sweet WPI did not affect foaming properties, as these treatments exhibited excellent foaming properties comparable to acid WPI with low levels of glycomacropeptide (Hawks et al, 1993). However, this research utilized batches of whey protein which were separated then processed, not using commercial products available from different suppliers.

The opportunity to modify whey proteins to produce foaming capabilities greater or equal to egg white has led to a body of research. Foaming properties of WPC solutions significantly increased with a heat treatment of 65 to 70°C before foaming. However, higher heat treatments exhibited negative effects on foaming properties, leading to the hypothesis that heat treatments below the denaturation temperature modify the solution's structure to favor absorption at the air-water interface (Richert et al., 1974). Later research investigating the effect of pre-heating protein solutions prior to foaming found that a mixture of native whey proteins as well as denatured whey proteins produced the most desirable foaming characteristics of high overrun and great foam stability. The foam with a combination of high overrun, rigidity, and stability had about 75% of whey proteins denatured (a heat treatment of 90°C for 12 minutes) (Bals and Kulozik, 2003). Other research used whey protein

polymers produced from heat treatment of 80°C for 30 minutes. These were mixed with native WPI, and the resulting foaming properties were quantified. Overrun decreased with increasing polymerized content, while yield stress increased to a maximum around 50% polymerized WPI then decreased (Davis and Foegeding, 2004).

The effects of other modifications on the foaming properties of WPI have been explored. Hydrolyzed whey proteins (portions of whey protein cut by enzymes) could theoretically increase foaming properties as a smaller size molecule may more adsorb more rapidly at the air-water interface. Various hydrolysates have shown mixed foaming properties. Hydrolysates of WPI showed a decrease in foam stability compared to native WPI. WPI hydrolysate foaming ability was correlated with molecular weight, with sufficient peptides greater than 3 kDa in size necessary for high foaming ability (van der Ven et al, 2002). Hydrolyzing  $\beta$ -lactoglobulin increased the yield stress of foams compared to native  $\beta$ -lactoglobulin, but the addition of heat treatments to inactivate the hydrolyzing enzymes decreased yield stress. Compared to native  $\beta$ -lactoglobulin, all hydrolysates (with and without heat treatment) adsorbed more quickly at the air-water interface (Davis et al, 2005).

Egg white foams differ from WPI foams in yield stress. EWP foams usually are higher in yield stress. Pernell et al (2002a) found that EWP foams increased in yield stress with longer whipping times until reaching a plateau around 10 minutes. WPI foams, on the other hand, retained lower, relatively stable yield stress measurements through whipping time trials. Investigations into the electrostatic

effects on WPI foams found that pH, salt type, and salt concentration all affected the yield stress (Davis et al, 2004).

Although egg white protein is the traditional gold standard for culinary foams, whey protein is a readily available alternative for some aerated food applications. Replacement of egg white protein with whey protein in an angel food cake application has been investigated. While cakes containing higher proportions of whey protein showed lower volumes and a much coarser bubble structure, cakes containing up to 75% egg white, 25% whey protein were not significantly different from control egg white cakes in physical and sensory cake evaluations (Arunepanlop et al, 1996). This study also investigated the effect of various hydrocolloids to help improve cake texture. Other research found that foams produced with WPC had a much lower stability than egg white foams. However, statistical analysis showed that combinations of WPC and egg white protein had a synergistic effect on foaming stability. These two proteins increased stability more than expected than if the two proteins interacted in an additive fashion (Aryana et al, 2002). WPI combined with sugar syrup produced a foam with very small air bubbles that showed no signs of destabilization during the first 24 hours after formation (Rouimi et al, 2005). A confectionary nougat application in peppermint patties found those produced with WPI resulted in a similar color, flavor, and texture to control peppermint patties made with egg white protein (Baker, 2006).

## **Sugar Effects on Foaming Properties of Proteins**

Interactions of sugars and proteins are important in food products, especially given that sugars are some of the most abundant taste additives in a wide range of foods (Semenova et al, 2002). Culinary foams, such as those used in confectionary products, meringues, and angel food cakes, often contain high ratios of sugar. Sugar has been shown to delay overbeating in protein foams (Kinsella, 1981). Previous research on sugar effects has shown high levels of sugar to increase foam stability, probably due to an increase in solution viscosity (Damodaran, 2005; Raikos et al, 2007). An increase in viscosity could also explain findings showing a decrease in foam overrun with increasing sugar concentration (Raikos et al, 2007). However, Davis and Foegeding (2007) found that solutions containing 25% sucrose increased yield stress and stability for egg white protein foams, but decreased yield stress and had little effect on stability for WPI foams. The surface tension of ovalbumin increased in the presence of sucrose, indicating less activity at the air-water interface. This phenomenon can be attributed to the theory of preferential protein hydration. Preferential protein hydration stems from the exclusion of sugar from the region immediately around the protein due to steric exclusion. This may affect protein-sucrose solutions by increasing protein diffusion to the air-water interface and decreasing protein surface activity, both due to reduced protein unfolding (Semenova et al, 2002).

## **Research Objective**

While whey proteins exhibit superb foaming properties, they currently fall short of the foaming properties shown by egg white. This research investigates the foaming properties of combinations of egg white and whey protein with the purpose of identifying an additive, synergistic, or antagonistic effect on foaming properties such as stability, overrun, and yield stress. In addition to bulk foam properties, foam microstructure will be observed using confocal microscopy. Quantifying bubble size distributions and examining destabilization mechanisms over time will offer greater insight into the mechanism behind combination foams. In addition, a comparison of foams both with and without sugar will be tested to give a greater understanding of how foams behave in a food system. Angel food cakes will be used as a model system to test the function of combination foams in a food matrix with heating. Confocal microscopy utilizing a heating stage will be used to examine the microstructure of these model systems under increasing temperatures. Overall, this research seeks to understand how foams produced from a combination of egg white and whey protein behaves alone and in a model angel food cake system.

## REFERENCES

- Ackermann Jr DM, Jewell DN, Stedman ML, Burapatana V, Atukorale PV, Pinson ML, Wardle AE, Zhu W, Tanner RD. 2003. Breathing air from protein foam. *Applied Biochem and Biotech* 105-108: 659-671.
- Alsaatie HA, Ditzler D, Burapatana V, Tanner RD. 2003. Using egg albumin foam to extinguish fires. *Brazilian Archives Biology and Tech* 46 (4): 735-740.
- Alvarez MD, Saunders DEJ, Vincent JFV, Jeronimidis G. 2000. An engineering method to evaluate the crisp texture of fruit and vegetables. *J Texture Stud* 31(4): 457-473.
- Arunepanlop B, Morr CV, Karleskind D, Laye I. 1996. Partial replacement of egg white proteins with whey proteins in angel food cakes. *J Food Sci* 61: 1085-1093.
- Aryana KJ, Haque ZZ, Gerard PD. 2002. Influence of whey protein concentrate on the functionality of egg white and bovine serum albumin. *Int J Food Sci and Tech* 37: 643-652.
- Auty MAE, Twomey M, Guinee TP, Mulvihill DM. 2001. Development and application of confocal scanning laser microscopy methods for studying the distribution of fat and protein in selected dairy products. *J Dairy Research* 68(3): 417-427.
- Baker L. 2006. Whey proteins in nutritional confections. Page 62 in *Proc of the 60<sup>th</sup> Annual Production Conf of the PMCA*, Hershey, PA.
- Bals A, Kulozik U. 2003. Effect of pre-heating on the foaming properties of whey protein isolate using a membrane foaming apparatus. *Int Dairy J* 13: 903-908.
- Bertrand ME, Turgeon SL. 2007. Improved gelling properties of whey protein isolate by addition of xanthan gum. *Food Hydrocolloids* 21(2): 159-166.
- Britten M, Lavoie L. 1992. Foaming properties of proteins as affected by concentration. *J Food Sci* 57: 1219-1222, 1241.
- Campbell GM, Mougeot E. 1999. Creation and characterization of aerated food products. *Trends Food Sci and Tech* 10: 283-296.
- Campbell L, Raikos V, Euston SR. 2003. Review: Modification of functional properties of egg-white proteins. *Nahrung/Food* 6: 369-376.

- Cooke JI. 2006. Back to basics – aerated confections: Introductory remarks. Page 24 in Proc of the 60<sup>th</sup> Annual Production Conf of the PMCA, Hershey, PA.
- Damodaran S. 2005. Protein stabilization of emulsions and foams. *J Food Sci* 70: R54-R66.
- Davis JP, Doucet D, Foegeding EA. 2005. Foaming and interfacial properties of hydrolyzed  $\beta$ -lactoglobulin. *J Colloid and Interface Sci* 288: 412-422.
- Davis JP, Foegeding EA. 2004. Foaming and interfacial properties of polymerized whey protein isolate. *J Food Sci* 69: C404-C410.
- Davis JP, Foegeding EA. 2007. Comparisons of the foaming and interfacial properties of whey protein isolate and egg white proteins. *Colloids and Surfaces B: Biointerfaces* 54: 200-210.
- Davis JP, Foegeding EA, Hansen FK. 2004. Electrostatic effects on the yield stress of whey protein isolate foams. *Colloids and Surfaces B: Biointerfaces* 34: 13-23.
- DeVilbiss ED, Holsinger VH, Posati LP, Pallansch MJ. 1974. Properties of whey protein concentrate foams. *Food Tech* March 1974: 40-48.
- Dickinson E. 1992. An introduction to food colloids. New York: Oxford Univ Press. 216 p.
- Dickinson E, Ettelaie R, Murray BS, Du Z. 2002. Kinetics of disproportionation of air bubbles beneath a planar air-water interface stabilized by food proteins. *J Colloid and Interface Sci* 252: 202-213.
- Du L, Prokop A, Tanner RD. 2003. Variation of bubble size distribution in a protein foam fractionation column measured using a capillary probe with photoelectric sensors. *J Colloid and Interface Sci* 259: 180-185.
- Durrenberger MB, Handschin S, Conde-Petit B, Escher F. 2001. Visualization of food structure by confocal laser scanning microscopy (CLSM). *Lebensm.-Wiss. U-Technol* 34: 11-17.
- Eisner MD, Jeelani SAK, Bernhard L, Windhab EJ. 2007. Stability of foams containing proteins, fat particles and nonionic surfactants. *Chem Eng Sci* 62: 1974-1987.

- Ettelaie R, Dickinson E, Du Z, Murray BS. 2003. Disproportionation of clustered protein-stabilized bubbles at planar air-water interfaces. *J Colloid and Interface Sci* 263: 47-58.
- Farrell Jr HM, Jiminez-Flores R, Bleck GT, Brown EM, Butler JE, Creamer LK, Hicks CL, Hollar CM, Ng-Kwai-Hang KF, Swaisgood HE. 2004. Nomenclature of the proteins in cows' milk—sixth revision. *J. Dairy Sci* 87: 1641-1674.
- Fellers TJ, Davidson MW. 2007. Theory of confocal microscopy. Olympus Corp. Available: <http://www.olympusconfocal.com/theory/index.html>. Accessed 11/17/07.
- Ferrando M, Spiess WEL. 2000. Review: Confocal scanning laser microscopy. A powerful tool in food science. *Food Sci and Tech Int* August 2000: 267-284.
- Foster B. 1997. Optimizing light microscopy for biological and clinical laboratories. Dubuque, IA: Kendall/Hunt Pub Co. 187 p.
- Genovese DB, Rao MA. 2005. Components of vane yield stress of structure food dispersions. *J Food Sci* 70: E498-E504.
- Guerin-Dubiard C, Pasco M, Molle D, Desert C, Croguennec T, Nau F. 2006. Proteomic analysis of hen egg white. *J Agric and Food Chem* 54: 3901-3910.
- Gunasekaran S, Ding K. 1999. Three-dimensional characteristics of fat globules in Cheddar cheese. *J Dairy Sci* 82(9): 1890-1896.
- Han XZ, Hamaker BR. 2002. Location of starch granule-associated proteins revealed by confocal laser scanning microscopy. *J Cereal Sci* 35: 109-116.
- Hawks SE, Phillips LG, Rasmussen RR, Barbano DM, Kinsella JE. 1993. Effects of processing treatment and cheese-making parameters on foaming properties of whey protein isolates. *J Dairy Sci* 76: 2468-2477.
- Hibbs AR. 2004. Confocal microscopy for biologists. New York: Kluwer Academic/Plenum Pub. 467 p.
- Hicasmaz Z, Yazgan Y, Bozoglu F, Katnas Z. 2003. Effect of polydextrose-substitution on the cell structure of the high-ratio cake system. *Lebensm-Wiss. U-Technol* 36: 441-450.
- Holt C, Roginski H. 2001. Milk proteins: Biological and food aspects of structure and function. In: Sikorski ZE, editor. *Chemical and functional properties of food proteins*. Lancaster PA: Technomic Publishing Co Inc. p 271-334.

- Jang,W, Nikolov A, Wasan DT. 2006. Research note: The destabilization of aerated food products. *J Food Eng* 76: 256-260.
- Johnson GC. 2006. Back to basics – aerated confections: Nougat and marshmallow. Page 32 in Proc of the 60<sup>th</sup> Annual Production Conf of the PMCA, Hershey, PA.
- Johnson TM, Zabik ME. 1981a. Response surface methodology for analysis of protein interactions in angel food cakes. *J Food Sci* 46: 1226-1230.
- Johnson TM, Zabik ME. 1981b. Egg albumen proteins interactions in an angel food cake system. *J Food Sci* 46: 1231-1236.
- Johnson TM, Zabik ME. 1981c. Ultrastructural examination of egg albumen protein foams. *J Food Sci* 46: 1237-1240.
- Kerstens S, Mugnier C, Murray BS, Dickinson E. 2006. Influence of ionic surfactants on the microstructure of heat-set beta-lactoglobulin-stabilized emulsion gels. *Food Biophysics* 1(3): 133-143.
- Kilara A, Vaghela MN. 2004. Whey proteins. In: Yada RY, editor. *Proteins in food processing*. Washington DC: CRC Press. p 72-99.
- Kinsella JE. 1981. Functional properties of proteins: Possible relationships between structure and function in foams. *Food Chem* 7: 273-288.
- Labbafi M, Thakur RK, Vial C, Djelveh G. 2007. Development of an on-line optical method for assessment of the bubble size and morphology in aerated food products. *Food Chem* 102: 454-465.
- Labensky S, Ingram GG, Labensky SR. 1997. *Webster's new world dictionary of culinary arts*. Upper Saddle River, NJ: Prentice Hall. 443 p.
- Langton M, Jordansson E, Altskar A, Sorenson C, Hermansson AM. 1999. Microstructure and image analysis of mayonnaises. *Food Hydrocolloids* 13(2): 113-125.
- Lau CK, Dickinson E. 2004. Structural and rheological properties of aerated high sugar systems containing egg albumen. *J Food Sci* 69: E232-E239.
- Lau CK, Dickinson E. 2005. Instability and structural change in an aerated system containing egg albumen and invert sugar. *Food Hydrocolloids* 19: 111-121.
- Lim KS, Barigou M. 2004. X-ray micro-computed tomography of cellular food products. *Food Research Int* 37: 1001-1012.

- Lomakina K, Mikova K. 2006. A study of the factors affecting the foaming properties of egg white—a review. *Czech J Food Sci* 24: 110-118.
- Luck PJ, Bray N, Foegeding EA. 2001. Factors determining yield stress and overrun of whey protein foams. *J Food Sci* 67: 1677-1681.
- Malcolm AS, Dexter AF, Middelberg APJ. 2006. Foaming properties of a peptide designed to form a stimuli-responsive interfacial films. *Soft Matter* 2: 1057-1066.
- Mann K. 2007. The chicken egg white proteome. *Proteomics Journal* 7: 3558-3568.
- Mine Y. 1995. Recent advances in the understanding of egg white protein functionality. *Trends in Food Sci and Tech* 6: 225-232.
- Mounsey JS, O’Riordan ED. 2008. Influence of pre-gelatinised maize starch on the rheology, microstructure, and processing of imitation cheese. *J Food Eng* 84(1): 57-64.
- Mukprasirt A, Herald TJ, Boyle DL, Rausch KD. 2000. Adhesion of rice flour-based batter to chicken drumsticks evaluated by laser scanning confocal microscopy and texture analysis. *Poultry Sci* 79(9): 1356-1363.
- Murray B. 2007. Stabilization of bubbles and foams. *Current Opinion in Colloid and Interface Sci* 12: 232-241.
- Murray BS, Dickinson E, Lau CA, Nelson PV, Schmidt E. 2005. Coalescence of protein-stabilized bubbles undergoing expansion at a simultaneously expanding planar air-water interface. *Langmuir* 21: 4622-4630.
- Olsson C, Langton M, Hermansson AM. 2002. Microstructures of beta-lactoglobulin/ amylopectin gels on different length scales and their significance for rheological properties. *Food Hydrocolloids* 16(2): 111-126.
- Ouellette J. 2002. The physics of ... foam. *Discover* 23 (16). Available online: <http://www.discover.com/issues/jun-02/departments/featphysics/>. Accessed 4 Jan 2006.
- Ould-Eleya MM, Leng XJ, Turgeon SL. 2006. Shear effects on the rheology of beta-lactoglobulin/beta-carrageenan mixed gels. *Food Hydrocolloids* 20: 946-951.

- Pateras IMC, Howells KF, Rosenthal AJ. 1994. Hot-stage microscopy of cake batter bubbles during simulated baking: sucrose replacement by polydextrose. *J Food Sci* 59: 168-170, 178.
- Pernell CW, Foegeding EA, Daubert CR. 2000. Measurement of the yield stress of protein foams by vane rheometry. *J Food Sci* 65: 110-114.
- Pernell CW, Foegeding EA, Luck PJ, Davis JP. 2002a. Properties of whey and egg white protein foams. *Colloids and Surfaces A: Physiochem and Eng Aspects*. 204: 9-21.
- Pernell CW, Luck PJ, Foegeding EA, Daubert CR. 2002b. Heat-induced changes in angel food cakes containing egg-white protein or whey protein isolate. *J Food Sci* 67: 2945-2951.
- Peter PN, Bell RW. 1930. Normal and modified foaming properties of whey-protein and egg-albumin solutions. *Industrial and Eng Chem* 22 (10): 1124-1128.
- Phillips LG, German JB, O'Neill TE, Foegeding EA, Harwalkar VR, Kilara A, Lewis BA, Mangino ME, Morr CV, Regenstein JM, Smith DM, Kinsella JE. 1990. Standardized procedure for measuring foaming properties of three proteins, a collaborative study. *J Food Sci* 55: 1441-1444, 1453.
- Phillips LG, Hague Z, Kinsella JE. 1987. A method for the measurement of foam formation and stability. *J Food Sci* 52: 1074-1077.
- Princen HM, Kiss AD. 1989. Rheology of foams and highly concentrated emulsions IV: An experimental study of the shear viscosity and yield stress of concentrated emulsions. *J Colloid and Interface Sci* 128(1): 176-187.
- Prins A. 1988. Principles of foam stability. In: Dickinson E, Stainsby G, editors. *Advances in Food Emulsions and Foams*. New York: Elsevier Applied Sciences. p 91-122.
- Pugh RJ. 1996. Foaming, foam films, antifoaming and defoaming. *Adv Colloid and Interface Sci* 64: 67-142.
- Pugh RJ. 2005. Experimental techniques for studying the structure of foams and froths. *Adv Colloid and Interface Sci* 114-115: 239-251.
- Raikos V, Campbell L, Euston SR. 2007. Effects of sucrose and sodium chloride on foaming properties of egg white proteins. *Food Research Int* 40: 347-355.

- Richardson G, Langton M, Faldt P, Hermansson AM. 2002. Microstructure of  $\alpha$ -crystalline emulsifiers and their influence on air incorporation in cake batter. *Cereal Chem* 79(4): 546-552.
- Richert SH. 1979. Physical-chemical properties of whey protein foams. *J Agric Food Chem* 27: 665-668.
- Richert SH, Morr CV, Cooney CM. 1974. Effect of heat and other factors upon foaming properties of whey protein concentrates. *J Food Sci* 39: 42-48.
- Rouimi S, Schorsch C, Valentini C, Vaslin S. 2005. Foam stability and interfacial properties of milk protein-surfactant system. *Food Hydrocolloids* 19: 467-478.
- Sahi SS, Alava JM. 2003. Functionality of emulsifiers in sponge cake production. *J Sci Food and Agric* 83: 1419-1429.
- Schober TJ, Bean SR, Boyle DL. 2007. Gluten-free sorghum bread improved by sourdough fermentation: biochemical, rheological, and microstructural background. *J Agric and Food Chem* 55: 5137-5146.
- Schober TJ, Dockery P, Arendt EK. 2003. Model studies for wheat sourdough systems using gluten, lactate buffer, and sodium chloride. *European Food Research and Tech* 217 (3): 235-243.
- Schramm LL. 2005. Emulsions, foams, and suspensions: Fundamentals and applications. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA. 448 p.
- Semenova MG, Antipova AS, Belyakova LE. 2002. Food protein interactions in sugar solutions. *Current Opinion in Colloid and Interface Sci* 7: 438-444.
- Sheppard CJR, Shotton DM. 1997. Confocal laser scanning microscopy. New York: Springer-Verlag. 106 p.
- Ternes W. 2001. Egg proteins. In: Sikorski ZE, editor. Chemical and functional properties of food proteins. Lancaster, PA: Technomic Publishing Co Inc. p 335-71.
- Trespalacios P, Pla R. 2007. Simultaneous application of transglutaminase and high pressure to improve functional properties of chicken meat gels. *Food Chem* 100: 264-272.
- Utaiwan-Chatpong, Arunee-Apichartsrangkoon, Bell AE. 2007. Effects of hydrocolloid addition and high pressure processing on the rheological properties and microstructure of a commercial ostrich meat product 'Yor' (Thai sausage). *Meat Sci* 76: 548-554.

- Van de Velde F, van Riel J, Tromp RH. 2002. Visualisation of starch granule morphologies using confocal scanning laser microscopy (CSLM). *J Sci of Food and Agric* 82 (13): 1528-1536.
- Van der Ven C, Gruppen H, de Bont DBA, Voragen AGJ. 2002. Correlations between biochemical characteristics and foam-forming and –stabilizing ability of whey and casein hydrolysates. *J Agric and Food Chem* 50: 2938-2946.
- Walstra P. 2003. *Physical chemistry of foods*. New York: Marcel Dekker Inc. 832 p.
- Wang Z, Narsimhan G. 2006. Model for Plateau border drainage of power-law fluid with mobile interface and its application to foam drainage. *J Colloid and Interface Sci* 300: 327-337.
- Wilde PJ, Clark DC. 1996. Foam formation and stability. In: Hall GM, editor. *Methods for testing protein functionality*. New York: Blackie Academic and Professional. p 110-52.
- Zarembo A. 2005. "The Fine Art of Foam." *Los Angeles Times*. Accessed 21 Sept. 2005.

## CHAPTER 2

Manuscript to be submitted for publication

Foaming and Interfacial Properties of Egg White Protein and Whey Protein Isolate,  
Alone and in Combination

Authors: Tristan Berry, Xin Yang, and Allen Foegeding

## **Abstract**

Egg white protein (EWP) is the standard for culinary foams with its ability to form foams with high volume (overrun) and stability. Whey protein isolate (WPI) foams have similar overrun and could potentially replace EWP in food products. In this work, foaming properties of WPI, EWP and combinations of the two, were compared in model and food systems. Physical properties of foams (overrun, drainage time, and yield stress), cakes (volume) and air/water interfaces (surface tension) were investigated. Progressive substitution of WPI with EWP did not cause major changes in foam overrun. In contrast, addition of WPI decreased foam stability (drainage time) and yield stress. Incorporation of 12.8% (w/v) sucrose decreased foam overrun but greatly increased EWP foam stability. However, the enhancing effect of sucrose on foam stability was less significant for WPI and WPI/EWP combination foams. Changes in surface tension showed that even the lowest level of WPI substitution (25% WPI) was enough to cause the temporal pattern of surface tension decrease to be similar to WPI, suggesting that whey proteins dominated the interface. The presence of sugar increased angel food cake volume when the proportion of EWP was higher than 50%. Cakes with 25% WPI substitution resulted in nearly the same volume as EWP cakes but with a coarser bubble structure. It appears that whey proteins dominate the air-water interface in protein mixtures. While it does not alter overrun, substitution of WPI causes a decrease in foam stability, yield stress and angel food cake quality.

**Key words:** whey proteins, foam, yield stress, surface tension, angel food cake

## Introduction

Foams are defined as the incorporation of gas into a solid or liquid phase. Although structurally complex, foams are commonly encountered in every day life. Foods are filled with foams, from cakes and breads to champagne, beer, meringues and ice cream. Many food products utilize foams either during processing or as the finished product, with the main benefit of aeration giving a desirable texture. The incorporation of air decreases density, transforming the mouthfeel of a food product (Campbell and Mougeot, 1999).

Foams, which are inherently thermodynamically unstable, can be modified to increase stability. The rheology of the food product has a significant impact on the lifetime of a foam, with low viscosity liquid foams having much less stability than a solid foam. Therefore, one way to preserve foams and add greater stability is to modify the flow characteristics of the foam. Angel food cakes are of particular interest among food foam products because the protein foam is generated independently then combined with other ingredients. This allows for separate investigation of the properties of the foam and food product. The initial foam (with a lifetime of hours) is transformed into a solid structure during baking through protein denaturation and starch gelatinization. An angel food cake can last for up to weeks, with its structure outlasting its sensory qualities (Campbell and Mougeot, 1999).

Although egg white protein is the traditional standard for culinary foams, whey protein (a cheese-making by-product) shows a comparable foaming functionality. In 1930, Peter and Bell's research noted that whey protein may be a suitable, economical replacement for egg white in foaming applications, except for those

applications involving heating or baking. While various whey protein treatments investigated produced foams equal to or greater than egg white in foam stability and strength, the heat coagulative properties of whey proteins fell short of egg white (Peter and Bell, 1930). Richert (1979) also confirmed whey protein's foaming capabilities as comparable to egg white in overrun, whipping time, and foam stability, but also observed the lack of ability to incorporate additional solids into the foam and retain stability during heating.

Subsequent investigations into whey protein substitutions for egg white in angel food cakes confirmed this theory. DeVilbiss et al. (1974) conducted experiments comparing control (egg white) angel food cakes to those produced with whey protein concentrate. During baking, the whey protein cakes rose quickly to a maximum volume and then collapsed, while the control cakes held their shape (DeVilbiss et al., 1974). Further experiments on angel food cakes made with WPI showed similar expansion followed by collapse during baking (Pernell et al, 2002). Egg white and WPI behaved similarly during initial baking expansion. However, around the protein denaturation temperatures (75°C to 85°C), elasticity of the cake batter increased for egg white but showed a decrease for WPI. During subsequent starch gelatinization, the egg white batter possessed enough strength, elasticity, or both to sustain the cake network while WPI batters collapsed (Pernell et al, 2002).

Mixtures of egg white protein and whey proteins in an angel food cake application have been investigated (Arunepanlop et al, 1996). Up to 25% whey protein could be substituted without resulting in a significant change in cake texture (springiness, hardness, and cohesiveness) or crust color. However, increasing

substitution of whey protein showed progressively lower cake volumes and coarser bubble structures. Even 25% substitution of whey protein resulted in angel food cakes with a broader bubble size distribution compared to egg white cakes (Arunepanlop et al, 1996). The mechanisms responsible for the effect of whey proteins in the cakes were not established.

The role of individual proteins in the foaming functionality of egg white and whey protein has been a topic of study. Johnson and Zabik (1981) investigated angel food cakes made with individual egg white proteins and found that some protein-protein interactions had positive effects and some had negative effects in foaming and angel food cake applications. Overall, the foaming functionality of egg white appears to be the result of complex interactions between the individual proteins rather than attributable to a single protein component and the interactions are still not completely understood (Damodaran, 2005). Previous research has investigated the effect of mixing of individual whey proteins in various ratios. The two major components of whey protein,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin, showed a linear increase in overrun and a non-linear increase in yield stress with greater proportions of  $\beta$ -lactoglobulin (Luck et al, 2002). Foaming properties of various combinations of WPC, egg white protein, and bovine serum albumen showed that combinations of WPC and egg white protein had a synergistic effect on foaming stability (Aryana et al, 2002).

Characterizing protein foams is a complicated matter. Two common measures of foaming properties are overrun, which measures the air incorporated into the foam, and foam stability, which measures the time necessary for half the

mass of solution to drain (Phillips et al, 1987). Also, the functionality of proteins at the air-water interface is important in relation to foaming properties. The protein's ability to reduce interfacial tension and the rheological properties of its films can have an effect on foam formation and subsequent destabilization (Richert, 1979). Film dilatational elasticity has been tied to foam stability, as theoretically the elastic interfacial films would be less susceptible to bubble destabilization such as coalescence and disproportionation (Damodaran, 2005; Rouimi et al, 2005).

This research focused on investigating the foaming functionality of combinations of EWP and WPI foams. Additive and synergistic effects in combinations of individual component proteins and major proteins have been observed, but the mechanism is not known. This study determined the foaming properties of combinations of EWP and WPI, evaluating foam overrun, stability, yield stress, and surface tension. These combinations were additionally examined in an angel food cake system. The objective of this research was to establish a mechanism of how combinations of WPI and EWP affect foaming functionality.

## **Materials and Methods**

### **Materials**

Two commercial samples of whey protein isolate were obtained. (1) WPI 1: BiPro (93% protein, dry basis) was supplied by Davisco Foods International, Inc. (Le Sueur, MN). (2) WPI 2: Provon 190 (91% protein, dry basis) was supplied by Glanbia Foods, Inc. (Twin Falls, ID). Spray dried egg white protein (P-18J, 80% protein, dry basis) was provided by Henningsen Foods, Inc. (Omaha, NE). The

whey protein isolates (WPI) and egg white protein (EWP) were stored at refrigeration temperatures. Sodium hydroxide and sucrose were obtained from Fisher Scientific (Fair Lawn, NJ). Hydrochloride acid was procured from Mallinckrodt Baker Inc. (Phillipsburg, NJ). Cake flour and 10× powdered sugar were purchased from a local grocery store.

## **Methods**

### **Solution Preparation**

Protein solutions were made at a level of 10% w/v protein. About 80% of the total amount of deionized water to be used was added to a beaker with a magnetic stirrer. Protein was added and hydrated while slowly stirring overnight (10 to 12 hours). Before the final volume adjustment, the pH of all solutions was adjusted to 7.0 using 1N NaOH. Protein solutions of five EWP/WPI ratios (100/0, 75/25, 50/50, 25/75 and 0/100) were prepared by mixing EWP and WPI solutions. When required, sucrose was added to the protein solutions on a 12.8% w/v basis.

Since insoluble particles were present in EWP solutions, EWP and EWP/WPI combination solutions were centrifuged and supernatants were obtained. No significant difference was observed between the foaming properties of supernatants and whole solutions (Figure 1). All remaining data presented in this work represents whole solutions containing insoluble EWP particles.

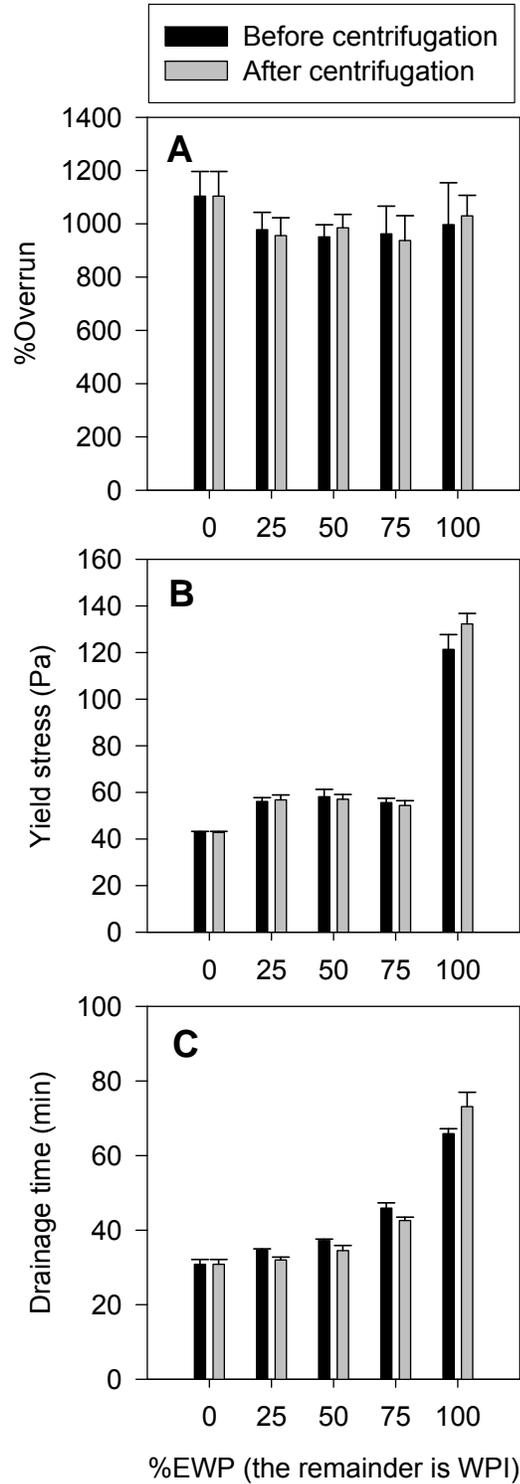


Figure 1: Foaming properties (A: Overrun; B: Yield stress; C: Drainage time) of foams prepared from 10% (w/v) protein solutions at pH 7 with or without insoluble particles. All foams were prepared from Provon WPI.

## Viscosity Measurement

Viscosity of 10% (w/v) protein solutions were measured on a controlled stress rheometer (StressTech; Reologica Instruments AB, Lund, Sweden). The measuring system, CCE25, utilized cup and bob geometry. After a pre-shear at 50 1/s for 30 s, viscosity was tested over a range of shear rates (0.5 1/s to 225 1/s). The rheological behavior of protein solutions is described by a power law model as shown in

Equation 1:

$$\sigma = K\gamma^n \quad \text{Equation 1}$$

where  $\sigma$  and  $\gamma$  are shear stress and shear rate, and  $K$  and  $n$  are consistency constant and flow behavior index, respectively. Microsoft Excel was used to fit shear stress and shear rate data to a power law model and the consistency constant and flow behavior index were calculated. All measurements were carried out at room temperature (23°C) and repeated at a minimum of three times.

## Foam Generation

A Kitchen Aid Ultra Power Mixer (Kitchen Aid, St. Joseph's, MI) with a 4.5 qt (4.3L) stationary bowl and rotating beaters was used to generate the foams from protein solutions. Two hundred mL of 10% (w/v) protein solutions were whipped for 20 min at a speed setting of 8 (planetary rpm of 225 and beater rpm of 737), both in the presence and absence of 12.8% (w/v) sucrose. The 20 min whip time for all solutions was utilized to prevent underbeating of WPI and EWP/WPI combinations, although slightly overbeating of EWP solutions resulted. After the foam generation was complete, the beaters were gently lifted out of the foam.

## Overrun

Overrun was measured according to the method of Phillips et al (1987). For overrun measurements, foam was gently scooped into a standard weigh boat (100 mL) and leveled using a rubber spatula. The weight was recorded and this process was repeated a minimum of 10 times for each foam. This measurement was completed within 20 minutes after the foam was removed from the mixer. The mean of all the weights recorded was used for overrun calculations according to the following equation:

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

Each treatment was replicated a minimum of three times.

## Yield stress measurement

Yield stress was measured using a vane attachment to a Brookfield 25xLVTDV-ICP viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) according to the method of Pernell et al (2000). Immediately after foam generation, the vane (10 mm in diameter and 40 mm in length) was gently inserted into the foam until the top edge was even with the foam surface. The vane was then rotated at a speed of 0.3 rpm. Maximum torque response ( $M_0$ ) was recorded and used to calculate yield stress according to the following equation:

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

where  $\tau_0$  is yield stress,  $M_0$  is maximum torque, and  $h$  and  $d$  are vane height and diameter, respectively. Three consecutive measurements throughout the bowl were

completed within five minutes after the foam generation. The average of three measurements was recorded as yield stress for one foam. A minimum of three replications were conducted for each treatment.

### **Foam Stability/Drainage Time**

The stability of the foams was measured by recording the length of time required for half of the pre-foam solution weight to drain according to the method of Phillips et al (1987). Special bowls with a 6-mm diameter hole were used for stability measurements. The hole was covered during whipping. A stopwatch began recording time immediately after foam generation was complete. The bowl was placed in a ring stand directly over a weight boat on a scale. The hole was uncovered and liquid was allowed to drip into the weigh boat. The time necessary for half of the pre-foam mass to drain were recorded as drainage time. A longer drainage time corresponds to greater foam stability. Each treatment was replicated a minimum of three times.

### **Interfacial tension**

Interfacial tension of pre-foam solutions and air was measured through pendant drop method (Myrvold and Hansen, 1998). An automated contact angle goniometer along with the DROPimage computer program (Ramé-Hart Inc., Mountain Lakes, NJ) was used for both data collection and calculations. A 16 $\mu$ L pendant drop of pre-foam solution was generated by a computer-controlled syringe. The drop extended from a stainless-steel capillary into an environmental chamber with standing water at its bottom to minimize evaporation. A digital camera captured the image of the pendant drop every 2 s for a total of 600 s and interfacial tension

was calculated according to shape parameters. All samples were equilibrated to room temperature (23°C) before measurement. Values reported are the averages of a minimum of three replications.

### **Density Determination**

DROPimage software requires inputs of densities of the component phases to calculate interfacial tension from the shape analysis of drops. A Mettler-Toledo DE40 density meter (Mettler-Toledo, Columbus, OH) equipped with a viscosity correction card measured the density of each solution at 23°C. The accuracy of the instrument was  $1 \times 10^{-4} \text{ g/cm}^3$ . Each solution was evaluated in triplicate and averaged.

### **Angel Food Cake Preparation**

Angel food cakes were prepared based on the method of Pernell et al (2002). Solutions at 10% (w/v) protein were prepared as described above with 12.8% (w/v) 10× powdered sugar for normal cakes or without sugar for no-sugar cakes. Foams were generated by the same method stated above except allowed to whip only for 15 min. To prepare cake batters, 66.0 g of cake flour was sifted 3 times. For normal cakes, 151.6 g of 10x powdered sugar was added to the cake flour before sifting (see Table 1). The dry blend was gently folded into the foam using a balloon whisk in 3 installments and no more than 20 strokes total. The batter was poured into 3 cake pans (size 6.5 cm × 13 cm × 5.5 cm) with 75.0 g batter in each pan. If necessary, the batter was gently pushed into the corners of the pan using a spatula. The cakes were baked in a conventional oven at 400°F (204°C) for 14 min. After baking, the cakes were cooled upside down on a wire rack for 30 min at room

temperature. After cooling, cake volume was measured using a rapeseed displacement method. Cake batters were made in duplication and volumes were averaged from six cakes.

Table 1. Composition of no sugar cakes and normal cakes

Pre-foam solutions (200mL)	Flour (g)	10× powdered sugar (g)	Cake
10% (w/v) protein solution	66.0	0	No-sugar
10% (w/v) protein solution with 12.8% (w/v) 10× powdered sugar	66.0	151.6	Normal

### Data Normalization and Statistical Analysis

Two batches of EWP were utilized in experiments, with slight differences in foaming properties. To remove the systematic error due to ingredient differences, the data for overrun, drainage time, yield stress, and cake volume were normalized prior to statistical analysis. The data was normalized by dividing each data point by the value of the EWP data, resulting in a value of one for EWP. The normalized foam data (overrun, drainage time, yield stress, and cake volume), solution apparent viscosity, and surface tension data were analyzed using the General Linear Model procedure of the SAS statistical software package (Version 9.1; SAS Institute, Inc., Cary, N.C., U.S.A.). Analysis of variance was conducted with means separation to determine differences between treatments. Significant differences were established at  $p \leq 0.05$ .

## **Results and Discussion**

The foaming properties of whey protein isolates (WPI), egg white protein (EWP) and their combinations (10% w/v protein, pH 7.0) are summarized in Table 2. While the data shown in the table are the original data obtained, the statistical analyses shown for overrun, yield stress, drainage time, and cake volume was performed on normalized data.

Table 2: Overrun, Yield Stress, Drainage Time, Viscosity, Density, Surface Tension, and Cake Volume of WPI/ EWP foams

		WPI	75WPI:25EWP	50WPI:50EWP	25WPI:75EWP	EWP
%Overrun	WPI 1	1229 ± 138 <sup>aa</sup>	1126 ± 45 <sup>aa</sup>	1250 ± 67 <sup>aa</sup>	1308 ± 109 <sup>aa</sup>	1321 ± 7 <sup>aa</sup>
	WPI 1*	882 ± 88 <sup>ba</sup>	848 ± 58 <sup>ba</sup>	899 ± 66 <sup>ba</sup>	912 ± 72 <sup>ba</sup>	993 ± 80 <sup>ba</sup>
	WPI 2	1104 ± 92 <sup>aa</sup>	978 ± 65 <sup>aa</sup>	951 ± 45 <sup>aa</sup>	946 ± 79 <sup>aa</sup>	997 ± 156 <sup>aa</sup>
	WPI 2*	965 ± 41 <sup>ba</sup>	1025 ± 37 <sup>ba</sup>	947 ± 39 <sup>ba</sup>	1032 ± 87 <sup>ba</sup>	1121 ± 83 <sup>ba</sup>
Yield Stress (Pa)	WPI 1	73.6 ± 1.9 <sup>aa</sup>	110.0 ± 3.7 <sup>ab</sup>	151.5 ± 1.9 <sup>ac</sup>	133.4 ± 2.2 <sup>ad</sup>	143.3 ± 6.6 <sup>ac</sup>
	WPI 1*	66.1 ± 3.7 <sup>ba</sup>	99.6 ± 6.6 <sup>bb</sup>	119.0 ± 9.3 <sup>bc</sup>	108.0 ± 6.6 <sup>bd</sup>	120.1 ± 9.6 <sup>bc</sup>
	WPI 2	42.7 ± 0.5 <sup>aa</sup>	56.1 ± 1.6 <sup>ab</sup>	58.1 ± 3.1 <sup>ab</sup>	55.6 ± 1.9 <sup>ab</sup>	121.3 ± 6.4 <sup>ac</sup>
	WPI 2*	44.9 ± 4.0 <sup>ba</sup>	58.5 ± 0.1 <sup>bb</sup>	51.0 ± 3.7 <sup>bb</sup>	61.9 ± 6.9 <sup>bb</sup>	102.8 ± 1.5 <sup>bc</sup>
Drainage 1/2 life (min)	WPI 1	36.5 ± 2.4 <sup>aa</sup>	57.6 ± 1.9 <sup>ab</sup>	78.9 ± 1.2 <sup>ac</sup>	89.7 ± 9.7 <sup>ac</sup>	91.1 ± 5.9 <sup>ad</sup>
	WPI 1*	39.7 ± 3.1 <sup>ba</sup>	55.1 ± 6.1 <sup>bb</sup>	72.4 ± 11.8 <sup>bc</sup>	86.8 ± 13.9 <sup>bc</sup>	140.9 ± 16.4 <sup>bd</sup>
	WPI 2	30.8 ± 1.3 <sup>aa</sup>	34.6 ± 0.3 <sup>ab</sup>	37.2 ± 0.44 <sup>ab</sup>	45.5 ± 1.3 <sup>ac</sup>	65.8 ± 1.3 <sup>ad</sup>
	WPI 2*	46.3 ± 0.7 <sup>ba</sup>	56.6 ± 2.0 <sup>bb</sup>	63.7 ± 1.4 <sup>bb</sup>	73.2 ± 3.2 <sup>bc</sup>	133.6 ± 10.9 <sup>bd</sup>
Apparent viscosity (mPa.s)	WPI 1	1.94 ± 0.38 <sup>aa</sup>	2.39 ± 0.18 <sup>ab</sup>	2.43 ± 0.07 <sup>ab, ac</sup>	2.76 ± 0.10 <sup>ac</sup>	2.59 ± 0.25 <sup>ac</sup>
	WPI 1*	3.25 ± 0.10 <sup>ba</sup>	4.00 ± 0.30 <sup>bb</sup>	4.39 ± 0.38 <sup>bb, bc</sup>	4.51 ± 0.26 <sup>bc</sup>	4.77 ± 0.21 <sup>bc</sup>
	WPI 2	2.69 ± 0.07 <sup>aa</sup>	2.77 ± 0.10 <sup>aa</sup>	2.67 ± 0.03 <sup>aa</sup>	2.74 ± 0.05 <sup>aa</sup>	2.59 ± 0.25 <sup>aa</sup>
	WPI 2*	4.20 ± 0.00 <sup>ba</sup>	4.14 ± 0.11 <sup>ba</sup>	4.30 ± 0.29 <sup>ba</sup>	4.67 ± 0.37 <sup>ba</sup>	4.77 ± 0.21 <sup>ba</sup>
Density (g/mL)	WPI 1	1.0243	1.0260	1.0273	1.0291	1.0309
	WPI 1*	1.0263	1.0276	1.0288	1.0296	1.0309
	WPI 2	1.0712	1.0726	1.0742	1.0752	1.0764
	WPI 2*	1.0744	1.0747	1.0754	1.0762	1.0764
Surface tension (mN/m)	WPI 1	50.0 ± 1.0 <sup>aa</sup>	49.6 ± 0.5 <sup>aa</sup>	49.5 ± 0.9 <sup>aa</sup>	49.7 ± 0.4 <sup>aa</sup>	42.6 ± 1.0 <sup>ab</sup>
	WPI 1*	49.1 ± 1.5 <sup>aa</sup>	50.2 ± 0.9 <sup>aa</sup>	49.8 ± 1.0 <sup>aa</sup>	49.7 ± 0.8 <sup>aa</sup>	43.3 ± 0.1 <sup>ab</sup>
	WPI 2	47.3 ± 1.1 <sup>aa</sup>	48.1 ± 0.6 <sup>aa</sup>	47.5 ± 0.8 <sup>aa</sup>	48.1 ± 0.5 <sup>aa</sup>	43.5 ± 0.6 <sup>ab</sup>
	WPI 2*	46.5 ± 0.2 <sup>aa</sup>	46.9 ± 1.4 <sup>aa</sup>	47.1 ± 0.5 <sup>aa</sup>	47.1 ± 0.2 <sup>aa</sup>	43.2 ± 0.2 <sup>ab</sup>

Table 2: Continued

		WPI	75WPI:25EWP	50WPI:50EWP	25WPI:75EWP	EWP
Cake volume (mL)	WPI 1	249 ± 42 <sup>aa</sup>	246 ± 25 <sup>aa</sup>	253 ± 21 <sup>aa</sup>	271 ± 13 <sup>aa</sup>	260 ± 52 <sup>aa</sup>
	WPI 1*	248 ± 15 <sup>ba</sup>	242 ± 10 <sup>ba</sup>	248 ± 17 <sup>ba</sup>	401 ± 22 <sup>bb</sup>	409 ± 32 <sup>bb</sup>
	WPI 2	177 ± 15 <sup>aa</sup>	200 ± 29 <sup>ab</sup>	196 ± 16 <sup>ac</sup>	229 ± 5 <sup>ad</sup>	290 ± 41 <sup>ad</sup>
	WPI 2*	138 ± 18 <sup>ba</sup>	167 ± 6 <sup>bb</sup>	265 ± 23 <sup>bc</sup>	359 ± 26 <sup>bd</sup>	376 ± 15 <sup>bd</sup>

1. \* is marked for protein solutions contain 12.8% (w/v) sucrose or normal cakes with sugar.
2. Apparent viscosities of pre-foam solutions are calculated at a shear rate of 8.5/s based on the power law model.
3. Densities of pre-foam solutions are measured at room temperature (23 °C).
4. Statistical analysis was conducted on normalized data for overrun, drainage time, yield stress, and cake volume. Original data was analyzed for surface tension and viscosity. The first letter indicates significant difference between protein samples and protein samples with 12.8% (w/v) sucrose or between normal cakes and no sugar cakes. The second letter notifies significant difference due to WPI/EWP ratio.

Overrun data is presented in Figure 2.

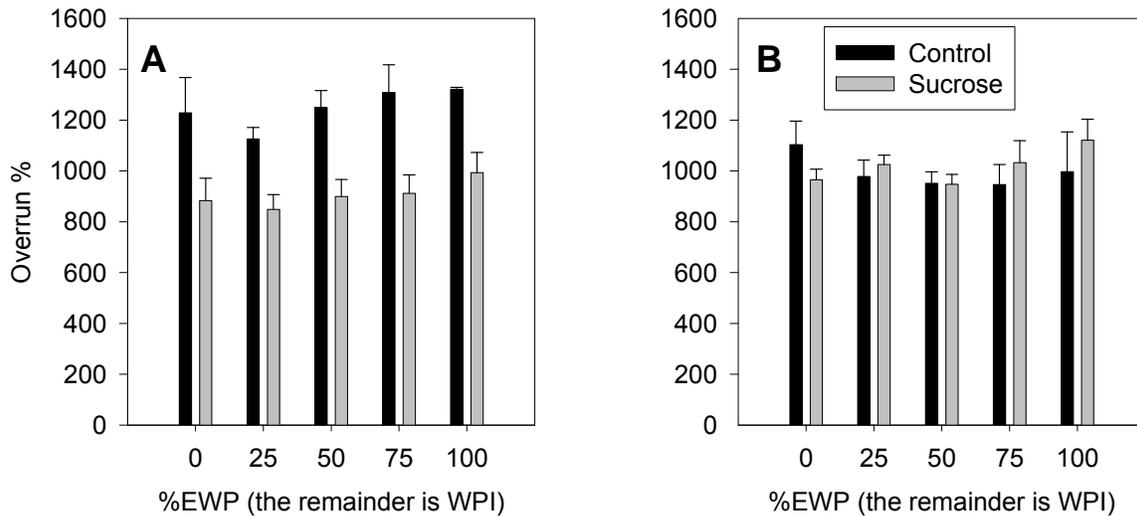


Figure 2: Overrun of foams prepared from 10% (w/v) protein solutions at pH 7, both in the presence and absence of 12.8% (w/v) sucrose. A: BiPro WPI; B: Provon WPI

Statistical analysis on overrun data normalized to account for different batches of EWP showed that WPI, EWP and combination foams were not significantly different ( $p > 0.05$ ) from each other (Table 2). However, foams containing sucrose showed a significant decrease in overrun compared to foams without sucrose. This has been previously observed in egg white foams containing invert sugar which showed a decrease in overrun with increasing concentrations of sugar (Lau and Dickinson, 2005), EWP foams containing 12% sugar (Raikos et al, 2007) and EWP and WPI foams containing 25% sucrose (Davis and Foegeding, 2007). The addition of sucrose increases the solution density and viscosity and reduces the amount of air incorporated into the foam.

Solution viscosity can affect foam stability. Polyhedral foams are composed of air bubbles separated by thin films; Plateau borders are the intersection of three

thin films. Gravity causes liquid to move through the network of Plateau borders, resulting in foam drainage (Wang and Narsimhan, 2006). A high viscosity continuous phase prior to foaming can slow the rate of drainage (Pugh, 1996). Gravity induced drainage generally results in shear rates ranging from 0.1/s to 10/s (Barnes et al, 1989). Dynamic viscosity data of all solutions were calculated at 8.5/s based on power law models (Equation 1). Kinematic viscosity (dynamic viscosity divided by solution density) represents gravity-induced flow and is plotted against drainage time in Figure 3.

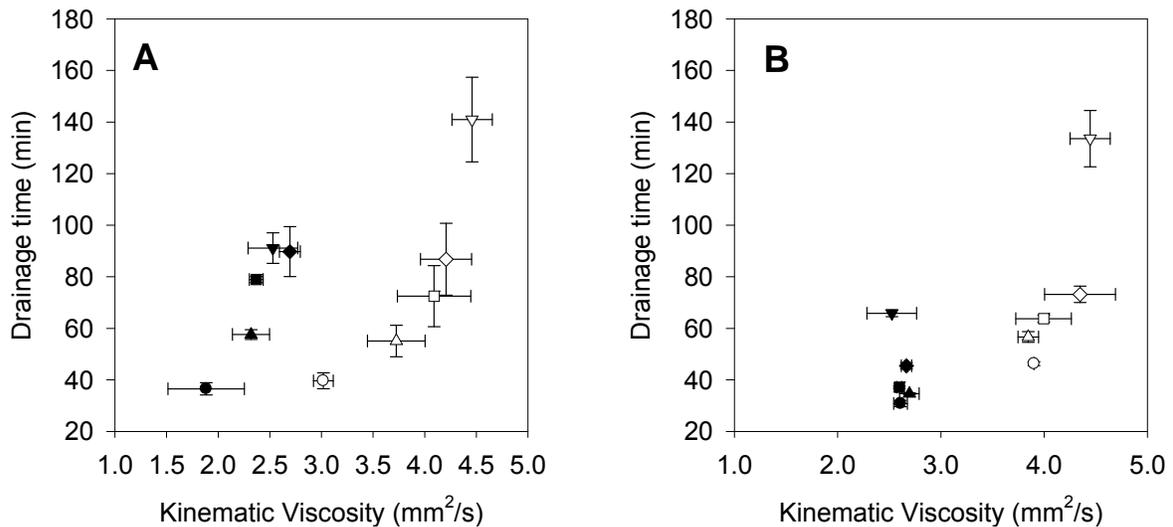


Figure 3: Drainage time vs. solution kinematic viscosity of 10% (w/v) protein solutions at pH 7, both in the presence (open symbols) and absence (closed symbols) of 12.8% (w/v) sucrose. ● WPI; ▲ 75WPI:25EWP; ■ 50WPI:50EWP; ◆ 25WPI:75EWP; ▼ EWP. A: BiPro WPI; B: Provon WPI. Viscosity data are calculated from equation 1 at a shear rate of 8.5/s divided by solution density.

The addition of sucrose showed a systematic viscosity increase in all treatments (Table 2). Drainage time was lowest for WPI and increased with progressive substitution of EWP. Most treatments followed the general trend of sucrose addition increasing viscosity but showing little effect on drainage time.

100% EWP was the exception to this trend. The addition of sucrose nearly doubled the drainage time for 100% EWP, although the viscosity did not increase more for EWP than other treatments. This observation suggests the sucrose enhancing effect on EWP foam stability was not due to the increasing of solution viscosity since the WPI and WPI/EWP combination solution viscosities also increased. With incorporation of WPI into EWP solutions, even 25% replacement, the sucrose enhancing effect on foam stability was lowered compared to EWP alone. One hypothesis to explain this observation is that WPI may dominate the bubble interface and govern the foam properties when two proteins are mixed.

Adsorption rates at the air/water interface of WPI, EWP and WPI/EWP combination solutions were qualitatively assessed by the rate of surface tension decline for freshly formed pendant drops (Myrvold and Hansen, 1998).

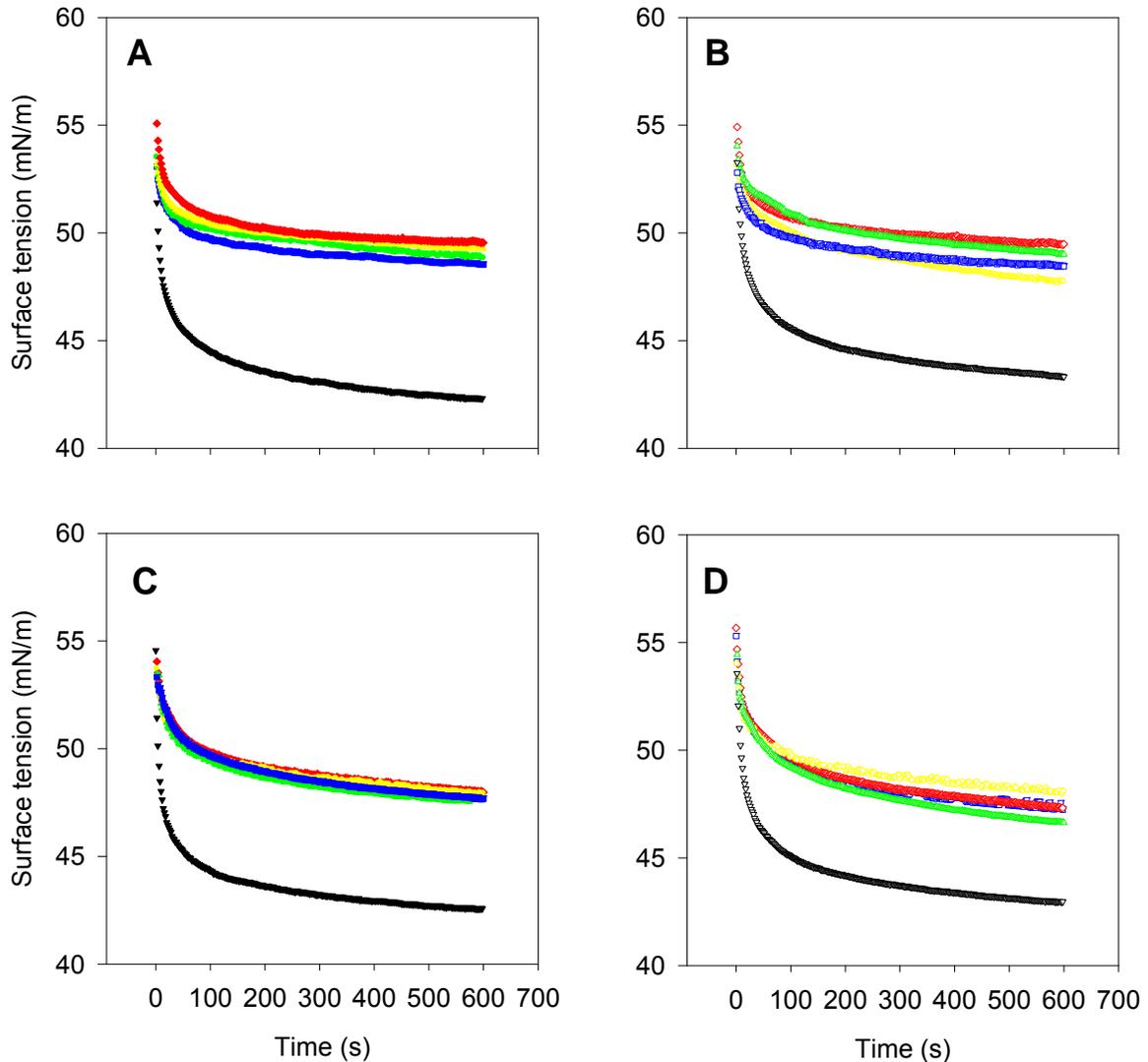


Figure 4: Typical dynamic surface tension measurements of 10% (w/v) protein solutions with (open symbols) and without (closed symbols) 12.8% (w/v) sucrose.

● WPI; ▲ 75WPI:25EWP; ■ 50WPI:50EWP; ◆ 25WPI:75EWP; ▼ EWP.  
 A: BiPro WPI; B: BiPro WPI with sucrose; C: Provon WPI; D: Provon WPI with sucrose

The EWP solutions exhibited the most rapid adsorption rates while all WPI/EWP combination solutions followed the pattern of WPI, both in the presence and absence of 12.8% (w/v) sucrose (Figure 4). The more rapid rates and lower surface tensions of EWP than WPI were reported previously (Davis and Foegeding,

2007). Similar surface tension decline patterns of WPI/EWP combinations and WPI solutions suggest that surface active molecules in WPI rather than those in EWP adsorb at the interface when two proteins are mixed. A similar relationship was observed in mixtures of  $\beta$ -casein and  $\beta$ -lactoglobulin at the air-water interface, where  $\beta$ -casein tended to dominate surface properties of the mixed system (Ridout et al, 2004). Although EWP exhibited a more rapid adsorption rate, surface active molecules in WPI appeared to occupy the interface. Competitive adsorption at an interface depends on factors beyond surface activity. If the protein reaching the interface first does not have a chance to unfold, it could be displaced by competing proteins (Dickinson, 1999). As a result, the WPI/EWP combination interfaces demonstrate similar interfacial properties to WPI, in agreement with observations that bulk foam properties of WPI/EWP foams tend to follow those of WPI.

Incorporation of 12.8% (w/v) sucrose showed little effect on the adsorption pattern for WPI and WPI/EWP combination protein solutions, but did slightly raise the surface tension of EWP at the first stage of adsorption. Previous research found that the presence of 25% (w/w) sucrose slowed the rate of surface tension decline for WPI and EWP (Davis and Foegeding, 2007). However, a mixture of bovine serum albumin (a component of whey protein) and sucrose concentrations up to 1M (~34% w/v) adsorbed more rapidly at the air/water interface than BSA in water. This rapid rate was seen during the first stage of adsorption, in which diffusion to the interface dominates the process (Nino and Patino, 2002). Other research found that the presence of sucrose decreased the adsorption rate of BSA, with 40% w/w

sucrose solutions showing an adsorption rate two orders of magnitude lower than a control BSA solution (Guzey et al, 2003).

An explanation for these diverse findings could be in the balance of two different sugar effects on a protein solution. Preferential hydration is the phenomenon of proteins remaining more compact in sugar solutions (Lee and Timasheff, 1981). A more compact protein could move more quickly through solution to an interface, resulting in a faster rate of adsorption. However, the presence of sugar increases solution viscosity, which could result in slower movement of proteins to the interface (Nino and Patino, 2002). The unchanged adsorption patterns observed both in presence and absence of sucrose may be a result from the two opposite effects. In addition, the sucrose concentration (12.8%, w/v) in this study may not be high enough to detect changes of protein adsorption at interfaces. Previous studies also investigated protein concentrations higher than the 10% w/v used in this research.

The major component in EWP, ovalbumin was found to adsorb less rapidly at the air/water interface in the presence of sucrose (Davis and Foegeding, 2007). In aqueous solutions, ovalbumin has been shown to form hydrogen bonds with sucrose, increasing the hydrophilic nature of the protein and decreasing its activity at the air-water interface (Antipova et al, 1999). The decreased hydrophobicity of ovalbumin with sucrose may explain the slightly higher surface tension of EWP with sucrose at the initial adsorption stage. As the protein concentration (10%, w/v) was very high, the surface tension decreased rapidly after the initial stage due to the large amount of molecules available for adsorption. The surface tension reached a

more slowly declining value after 600 s of aging. The surface tension at 600s was recorded and the average of three replications of data is shown in Figure 5.

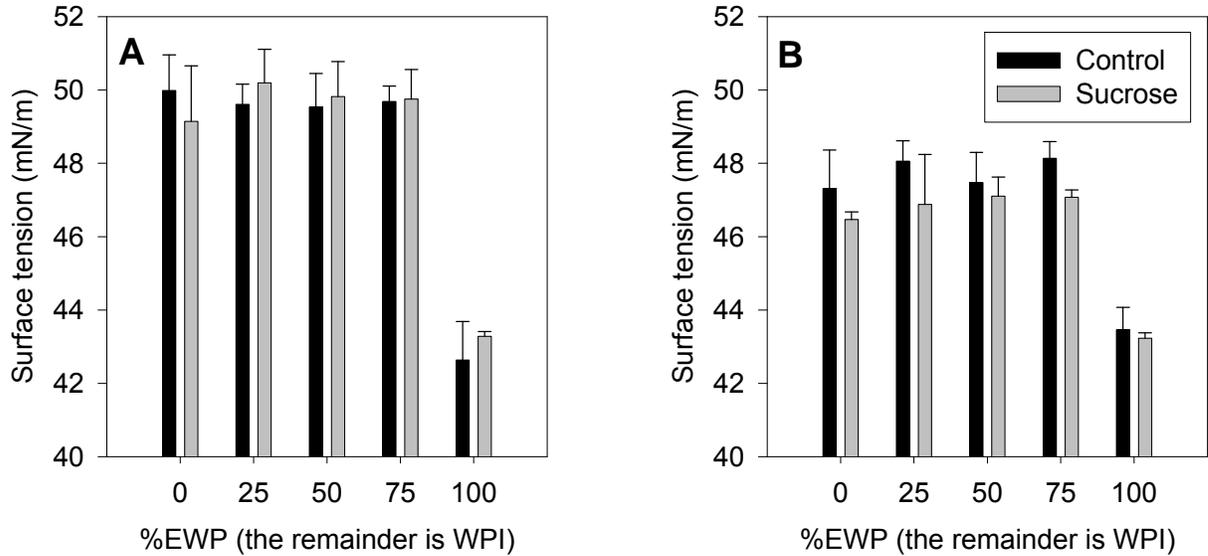


Figure 5: Surface tension at 600 s of 10% (w/v) protein solutions at pH 7, both in the presence and absence of 12.8% (w/v) sucrose. A: Bipro WPI; B: Provon WPI

There were no significant differences ( $p > 0.05$ ) in surface tension values at 600s among WPI and WPI/EWP combination solutions within a type of WPI. In contrast, the EWP solutions were significantly lower than all other treatments of the same type of WPI, both in the presence and absence of sucrose. No significant difference ( $p > 0.05$ ) was observed in surface tension at 600s between protein solutions and protein solutions with 12.8% (w/v) sucrose.

To study the heating effect on foams, angel food cakes were prepared from the same protein solutions (formulations listed in Table 1). Cake volumes, quantified using a rapeseed displacement method, are compared in Figure 6.

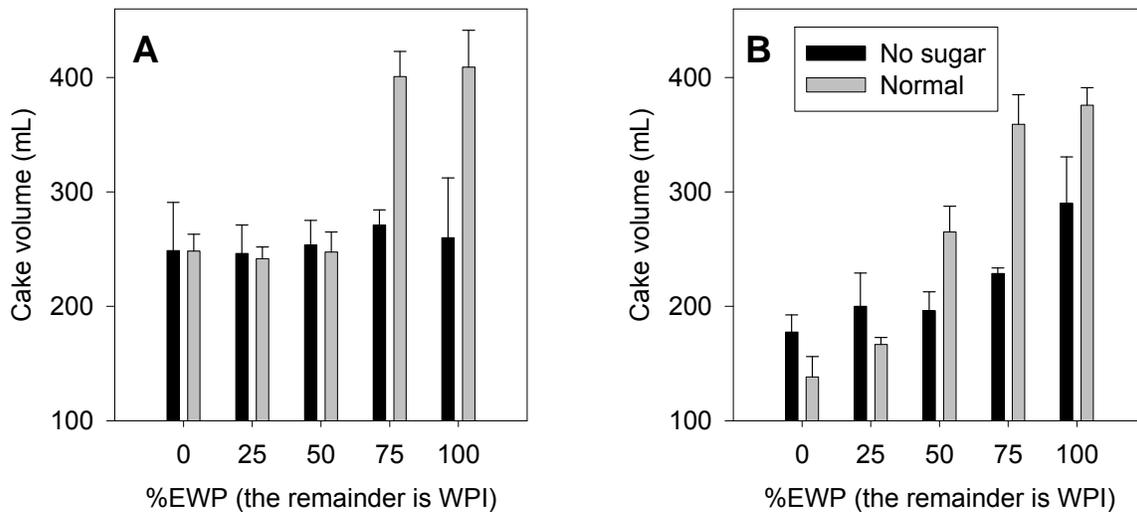


Figure 6: Volume of angel food cake prepared from 10% (w/v) protein solutions at pH 7, both with and without sugar. A: BiPro WPI; B: Provon WPI

The main trend seen in angel food cake volumes was the increased volume of normal cakes made with 100% EWP and 75% EWP/ 25% WPI. Within each type of WPI, these two treatments were not significantly different ( $p > 0.05$ ) from each other, but were statistically different from cakes produced with 50% or more WPI. The addition of sugar showed an enhancing effect on cake volume when cake contained a majority of EWP in the protein ratio. As stated above, sucrose incorporation contributed to the stability of EWP foams, but had no significant effects on foam stability of WPI and WPI/EWP combinations. The interfacial properties were seen to be dominated by WPI rather than EWP in WPI/EWP combinations. A cake matrix is built on a protein foam structure as a result of thermal denaturation of proteins and starch gelatinization during baking (Pernell et al, 2002). Sugar incorporation may assist the liquid phase, rich in EWP, to stabilize and build the cake matrix or resist the drainage of the liquid phase during baking.

As seen in Figure 6, 25WPI/75EWP and EWP normal cakes exhibit almost same volume for both WPI systems. However, images of freshly cut cake sections reveal differences in internal structural features of two cakes (Figure 7).

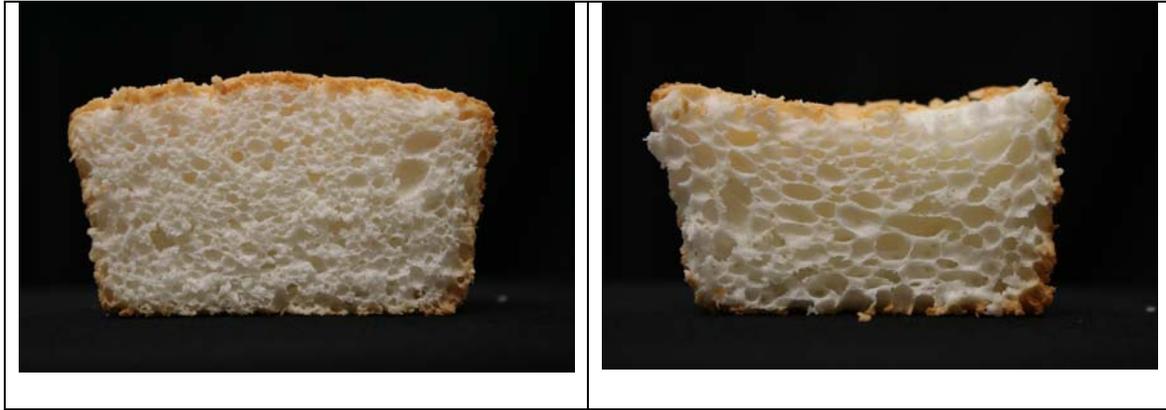


Figure 7: Cross sections of angel food cakes prepared with 100% EWP (left) and 25%WPI:75%EWP (right).

The EWP cake displays a fine structured matrix with many small, uniformly sized air cells and only a few larger air cells. The 25WPI/75EWP cake shows coarser structures with a predominance of larger air cells. This observation is in agreement with previous reports by Arunepanlop et al (1996). In the baking process, air bubbles expand due to heating. The 25WPI/75EWP foam may have expanded faster than the network could stabilize the structure, resulting in a collapse. Previous work on angel food cakes made with whey proteins found that cakes expanded to a maximum height then collapsed during baking (DeVilbiss et al, 1974; Pernell et al, 2002). This is observed in Figure 7 by the concave shape of the 25WPI:75EWP cake, which indicates some collapse. Foam destabilization processes such as coalescence and disproportionation caused an increase in air bubble size and a decrease in cake quality. With the incorporation of sugar, the EWP cake expands and the cake matrix is reinforced, resulting in a high cake

volume. This reinforcing effect is also seen in cakes composed of 25WPI/75EWP, probably due to large amount of EWP in the liquid phase. However, as WPI molecules dominate the interface in WPI/EWP combinations, the cake structure followed that of WPI and displayed a coarse texture with large air cells.

## **Conclusions**

Combinations of WPI and EWP did not show an additive effect in foaming properties, interfacial measurements, or cake volume. The addition of 12.8% (w/v) sucrose showed a large increase in foam stability for foams made with 100% EWP, but exhibited little impact on stability in foams made with WPI and WPI/EWP combinations. This increase cannot be explained by an increase in viscosity, as all protein combination solutions showed similar viscosities. Surface tension measurements found that WPI/EWP combinations followed the pattern of 100% WPI, while 100% EWP decreased to a lower surface tension, indicating that WPI may be dominating the interface when both proteins are present. Angel food cakes produced with and without sugar resulted in high volume cakes only in the presence of sugar and at levels of 25% WPI/ 75% EWP or 100% EWP, leading to the hypothesis that sugar and at least 75% of EWP needs to be present to stabilize cake volume. However, although 25% WPI/75 % EWP cakes had volumes similar to 100% EWP, addition of WPI showed a change in cake texture, resulting in a coarser bubble structure and concave shape. Further research is needed to characterize the differences in bubble size distributions and its effect on foaming functionality between foams produced from WPI, EWP, and combinations of the two.

## **Acknowledgements**

Support from the North Carolina Agriculture Research Service, Dairy Management Inc. and the Southeast Dairy Foods Research Center is gratefully acknowledged.

## REFERENCES

- Antipova AS, Semenova MG, Belyakova LE. 1999. Effect of sucrose on the thermodynamic properties of ovalbumin and sodium caseinate in bulk solution and at air-water interface. *Colloid and Surfaces B: Biointerfaces* 12: 261-270.
- Arunepanlop B, Morr CV, Karleskind D, Laye I. 1996. Partial replacement of egg white proteins with whey proteins in angel food cakes. *J Food Sci* 61(5):1085-1093.
- Aryana KJ, Haque ZZ, Gerard PD. 2002. Influence of whey protein concentrate on the functionality of egg white and bovine serum albumin. *Int J Food Sci and Tech* 37: 643-652.
- Barnes HA, Hutton, JF, Walters K. 1989. *An introduction to rheology*. Amsterdam: Elsevier. 199 p.
- Campbell GM, Mougeot E. 1999. Creation and characterization of aerated food products. *Trends in Food Sci and Tech* 10: 283-296.
- Damodaran S. 2005. Protein stabilization of emulsions and foams. *J Food Sci* 70: R54-R66.
- Davis JP, Foegeding EA. 2007. Comparisons of the foaming and interfacial properties of whey protein isolate and egg white proteins. *Colloids and Surfaces B: Biointerfaces* 54: 200-210.
- DeVilbiss ED, Holsinger VH, Posati LP, Pallansch MJ. 1974. Properties of whey protein concentrate foams. *Food Tech* March 1974: 40-48.
- Dickinson E. 1999. Review: Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology. *Colloids and Surfaces B: Biointerfaces*. 15: 161-176.
- Dickinson E, Ettelaie R, Murray BS, Du Z. 2002. Kinetics of disproportionation of air bubbles beneath a planar air-water interface stabilized by food proteins. *J Colloid and Interface Sci* 252: 202-213.
- Du L, Prokop A, Tanner RD. 2003. Variation of bubble size distribution in a protein foam fractionation column measured using a capillary probe with photoelectric sensors. *J Colloid and Interface Sci* 259: 180-185.
- Foegeding EA, Luck PJ, Davis JP. Factors determining the physical properties of protein foams. *Food Hydrocolloids* 20: 284-292.

- Guzey D, McClements DJ, Weiss J. 2003. Adsorption kinetics of BSA at air-sugar solution interfaces as affected by sugar type and concentration. *Food Research Int* 36: 649-660.
- Johnson TM, Zabik ME. 1981. Egg albumen proteins interactions in an angel food cake system. *J Food Sci* 46: 1231-1236.
- Lau C, Dickinson E. 2005. Instability and structural change in an aerated system containing egg albumen and invert sugar. *Food Hydrocolloids* 19: 111-121.
- Lee JC, Timasheff SN. 1981. The stabilization of proteins by sucrose. *J Biological Chem* 256: 7193-7201
- Luck PJ, Bray N, Foegeding EA. 2002. Factors determining yield stress and overrun of whey protein foams. *J Food Sci* 67: 1677-1681.
- Myrvold R, Hansen FK. 1998. Surface elasticity and viscosity from oscillating bubbles measured by automatic axisymmetric drop shape analysis. *J Colloid and Interface Sci* 207: 97-105.
- Nino MRR, Patino JMR. 2002. Effect of the aqueous phase composition on the adsorption of bovine serum albumin to the air-water interface. *Industrial Eng Chem Research* 41: 1489-1495.
- Pernell CW, Foegeding EA, Daubert CR. 2000. Measurement of the yield stress of protein foams by vane rheometry. *J Food Sci* 65: 110-114.
- Pernell CW, Luck PJ, Foegeding EA, Daubert CR. 2002. Heat-induced changes in angel food cakes containing egg-white protein or whey protein isolate. *J Food Sci* 67: 2945-2951.
- Peter PN, Bell RW. 1930. Normal and modified foaming properties of whey-protein and egg-albumin solutions. *Industrial and Eng Chem* 22: 1124-1128.
- Phillips LG, Haque Z, Kinsella JE. 1987. A method for the measurement of foam formation and stability. *J Food Sci* 52: 1074-1077.
- Pugh RJ. 1996. Foaming, foam films, antifoaming and defoaming. *Adv in Colloid and Interface Sci* 64: 67-142.
- Raikos V, Campbell L, Euston SR. 2007. Effects of sucrose and sodium chloride on foaming properties of egg white proteins. *Food Research Int* 40: 347-355.
- Richert SH. 1979. Physical-Chemical properties of whey protein foams. *J Agric Food Chem* 27: 665-668.

Ridout MJ, Mackie AR, Wilde PJ. 2004. Rheology of mixed  $\beta$ -casein/  $\beta$ -lactoglobulin films at the air-water interface. *J Agric Food Chem* 52: 3930-3937.

Rouimi S, Schorsch C, Valentini C, Vaslin S. 2005. Foam stability and interfacial properties of milk and protein-surfactant systems. *Food Hydrocolloids* 19: 467-478.

## CHAPTER 3

### **Microstructural Comparison of Foams and Cake Batters Prepared from Egg White Protein and Whey Protein Isolate, Alone and in Combination**

#### **Introduction**

Foams are defined as the incorporation of gas into a solid or liquid phase. Although structurally complex, foams are commonly encountered in every day life. Foods are filled with foams, from cakes and breads to champagne, beer, meringues and ice cream. Two main types of foams exist based on the volume fraction of gas incorporated into the liquid. The first type is a bubbly foam, which has a lower amount of gas and spherical bubbles. The other type of foam that occurs with higher gas volume fractions is a polyhedral foam. In a polyhedral foam, the bubbles are so closely packed together that they become non-spherical, forming a honeycomb-like structure. The geometry of polyhedral foams has a big impact on the foam stability (Prins, 1988; Murray, 2007). Polyhedral foams consist of thin films between bubbles, with the area where three films intersect called a Plateau border. Capillary pressure keeps liquid inside the Plateau border. Drainage is the destabilization process that results from the pull of gravity on the liquid found in the thin films and Plateau borders of the foam (Prins, 1988). When the film between two bubbles drains, the film thins and eventually ruptures. This leads to two bubbles becoming one bubble, a destabilization process called coalescence (Damodaran, 2005).

Another type of destabilization found in foams is disproportionation. Disproportionation is the movement of gas from small bubbles to larger bubbles.

Laplace pressure in bubbles states that smaller bubbles are under greater pressure than larger bubbles. Since gas solubility is proportional to pressure, gas is more soluble around smaller bubbles in the foam. Disproportionation occurs when gas diffuses from small bubbles to large bubbles, and the self-accelerating process continues as the small bubbles shrink (Prins, 1988).

While overrun (gas volume fraction) and foam stability are the most common properties measured to characterize foams, yield stress is also of importance. Yield stress is a type of rheological measurement, and is related to the “peak stage” mentioned in culinary recipes. When whipping egg whites, a recipe may state to whip until hard (or soft or medium) peaks are formed, which reflects the ability of a foam to hold its shape. If the correct stage is not obtained, the finished product will probably be of low quality (Pernell et al, 2000). In scientific terms, yield stress is defined as the point at which the foam transitions from behaving like an elastic solid to behaving like a liquid when subjected to a deformation (Princen and Kiss, 1989).

Yield stress has been modeled based on the following equation (Princen and Kiss, 1989):

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

where  $\tau_0$  is the yield stress,  $\gamma$  is the interfacial tension,  $\phi$  is the gas volume fraction,  $R_{32}$  is the surface-volume mean drop radius and  $Y(\phi)$  is an experimentally derived parameter. Of these variables, overrun measures the gas volume of the foam, but quantifying bubble size distributions is important to fully understand the mechanisms behind yield stress.

Quantifying bubble size is important to understanding the structure of a foam. Routine bubble size measurements are difficult for several reasons, including the fact that diluting foams does not work well, foams are thermodynamically unstable, and foams tend continuously change through creaming, coalescence, or disproportionation (Murray, 2007). The fact that foams continuously change in both space and time make bubble size distributions particularly difficult to quantify (Dickinson et al, 2002).

Numerous methods have been explored to tackle the complex problem of quantifying foam structure. Optical microscopy is the most prevalent method for foam image analysis and bubble size quantification (Labbafi et al, 2007). Confocal microscopy is a useful technique for imaging foams (Murray, 2007). Confocal laser scanning microscopy (CSLM) is a type of light microscopy that can image thin specimen or very thin optical sections of a thick specimen. Images acquired using confocal microscopy have been analyzed for object dimensions (such as area, length, or perimeter) or shape (like shape factor) by thresholding the image so the objects stand out against a background. Then commercial image analysis software can be used to calculate desired parameters (Ferrando and Speiss, 2000). Confocal microscopy has been used to image whipped cake batter, with measurements of air bubble area, diameter, area fraction of air, and estimates of surface area per unit volume (Richardson et al, 2002). Egg white and invert sugar foams were imaged using confocal microscopy focusing on a single frame over time to track the changes in selected individual bubble diameters (Lau and Dickinson, 2005).

Although egg white protein is the traditional standard for culinary foams, whey protein (a cheese-making by-product) shows comparable foaming functionality. In 1930, Peter and Bell's research noted that whey protein may be a suitable, economical replacement for egg white in foaming applications, except for those applications involving heating or baking. While various whey protein treatments investigated produced foams equal to or greater than egg white in foam stability, the heat coagulative properties of whey proteins fell short of egg white (Peter and Bell, 1930). Richert (1979) also confirmed whey protein's foaming capabilities as comparable to egg white in overrun, whipping time, and foam stability, but also observed the lack of ability to incorporate additional solids into the foam and retain stability during heating.

Whey protein is a readily available alternative to egg white for some aerated food applications. Replacement of egg white protein with whey protein in an angel food cake application has been investigated (Arunepanlop et al, 1996). Up to 25% whey protein could be substituted without resulting in a significant change in cake texture (springiness, hardness, and cohesiveness) or crust color. However, increasing substitution of whey protein showed progressively lower cake volumes and coarser bubble structures. Even 25% substitution of whey protein resulted in angel food cakes with a broader bubble size distribution compared to egg white cakes (Arunepanlop et al, 1996). Previous research has also noted that foams made with combinations of egg white protein and whey protein isolate had properties that more closely resembled WPI in bulk properties of foam stability, overrun, and cake volume (Yang et al, 2007).

The purpose of this research was to investigate the effects of progressive substitution of WPI for EWP and the resulting changes in foam microstructure and relate microstructural changes to variation in bulk foaming properties. Confocal microscopy was used to characterize the microstructure of foams in relationship to foam yield stress as well as observe the microstructure of foams and cake batters during heating.

## **Materials and Methods**

### **Egg white and whey protein isolate**

Commercial samples of two types of whey protein isolate, WPI 1 (BiPro WPI from Davisco Foods International, Inc., Le Sueur, MN) and WPI 2 (Provon 190 from Glanbia Foods Inc., Twin Falls, ID) as well as spray dried egg white protein (P-18J from Henningsen Foods, Inc., Omaha, NE) were obtained. The Henningsen spray dried egg white was used for all experiments except for the foams and cake batters imaged while heating on the Olympus confocal microscope. These experiments utilized spray dried egg white protein sourced from Primera Foods (Cameron, WI) due to the ceased production of Henningsen egg white. The Primera egg white showed similar foaming functionality as the Henningsen egg white (data not shown). Whey protein isolates (WPI) were stored at room temperature, while the egg white protein (EWP) was refrigerated. The WPI 1 (Bipro) and WPI 2 (Provon) had protein levels of 93% and 91%, respectively. The Henningsen EWP contained 80% protein and the Primera EWP contained 82% protein. Five different combinations of WPI

and EWP were investigated: 100% WPI, 75% WPI/25% EWP, 50% WPI/50% EWP, 25% WPI/75% EWP, and 100% EWP.

### **Protein Solutions**

Protein solutions were made at a level of 10% w/v protein. About 80% of the total amount of deionized water to be used was added to a beaker with a magnetic stirrer. Protein was added and hydrated while stirring at room temperature overnight (10 to 12 hours). If a treatment involved the addition of sucrose, 12.8% w/v sucrose (Fisher Scientific, Fair Lawn, NJ) was added and allowed to dissolve. The pH was then adjusted to 7.00 using 1M NaOH. Deionized water was added to bring the solution to volume. For the solutions with two types of proteins, each protein solution was prepared separately and the solutions were combined prior to foaming.

### **Sodium Fluorescein**

Sodium fluorescein (obtained from Sigma-Aldrich, St. Louis, MO) was added at a level of 0.1 mM to the protein solutions used for microscopy directly before foaming. The fluorescein dissolved readily in the protein solutions before mixing commenced.

### **Foam Generation**

A Kitchen Aid Ultra Power Mixer (St. Joseph's, MI) with a 4.3L stationary bowl and rotating beaters was used to generate the foams from the protein solutions. Two hundred mL of protein solution was added to the bowl and allowed to whip for 20 minutes at a speed setting of 8 (planetary rpm of 225 and beater rpm of 737). After the foam generation was complete, the beaters were gently lifted out of the foam.

## **Protein Foam Microscope Slide Preparation**

A small amount of foam was loaded into a single-welled microscope slide with a #1.5 coverslip attached to the bottom using silicon grease and immediately used for microscope imaging.

## **Confocal Microscopy**

An inverted Leica DM IRBE (Heidelberg, Germany) confocal laser scanning microscope imaged the samples. An argon laser excited samples at 488nm, and light was collected at a range from 500-550 nm, with a transmitted light differential interference contrast (DIC) image recorded simultaneously. An HC PL FLUOTAR 10.0x, with a 0.30 numerical aperture, was the objective used for 10x magnification. Collected images were viewed using Leica software.

Images were collected as a series of z-stacks for each foam. Each foam sample was placed in the microscope and five series of z-stacks were imaged. A z-stack was defined as the range from where bubbles were first visible to where they no longer appeared as separate entities (generally about 80  $\mu\text{m}$  in depth). Software calculated the optimal number of section for the stack (generally around 22 sections), and the entire stack was imaged. The field of vision was then moved to another random position in the foam and repeated until five stacks were completed (total time of less than 20 minutes).

Time lapse data was also collected for all foam samples. Directly after foam preparation and slide loading, a single plane was focused into the foam. Images were taken every 5 seconds for 10 minutes, with both confocal and DIC images recorded simultaneously. This was completed once per prepared slide.

Leica software was used to convert the z-stacks into 3-D foam reconstructions and combine the 120 time lapse images per treatment into a time lapse video.

### **Image Analysis**

Image analysis of confocal images was performed using MetaMorph Imaging System software (Molecular Devices, Downington, PA). Initially, the image was thresholded to convert it to binary, with the same threshold cut-off used for each image. Thresholding allowed the objects (air bubbles) to stand out against the background (aqueous phase). The image was then enhanced with pseudocolor, which converted the green (aqueous phase) areas to magenta for easier visibility during the image analysis. After the image had thresholding and pseudocolor, the image was modified if necessary. In some images, overlapping or touching bubbles were separated using a “cut” feature that allowed manual separation of objects. In other images, especially those where some small bubbles appeared to have green aqueous phase in the center, a “join” feature was used to manually outline bubbles that were not typical objects.

An integrated morphometry analysis feature evaluated the images. Object (bubble) area and object shape factor were measured. Shape factor was determined by the equation:

$$Shape\ Factor = \frac{4\pi\ Area}{Perimeter^2} \quad \text{Equation 2}$$

A shape factor of 1 indicated a perfectly spherical bubble, while lower shape factors showed a deviation from roundness. This option was utilized due to the non-

spherical nature of many of the bubbles. Using the integrated morphometry analysis, all bubbles were selected for measurement. Then, those bubbles with less than 50% of their estimated area visible in the image frame were manually de-selected. This prevented exclusion of all bubbles touching the edge of the frame, which was a large proportion of the total number of bubbles imaged. Excluding bubbles thought to be a majority outside of the frame also prevented skewing the bubble sizes to smaller areas due to only a small portion of the bubble being analyzed.

Areas and shape factors of the remaining bubbles were transferred to an Excel spreadsheet. Area, given in pixels, was converted to  $\mu\text{m}^2$  after measurement. The bubble areas were sorted by increasing size, and bubbles with areas of less than 5 pixels were excluded from further image data analysis. Observations showed that objects this small were not actual bubbles, but generally fringe around bubbles. These were excluded to prevent skewing the data. Excel was used to create histograms of the data, as well as calculate means and medians of the bubble distributions.

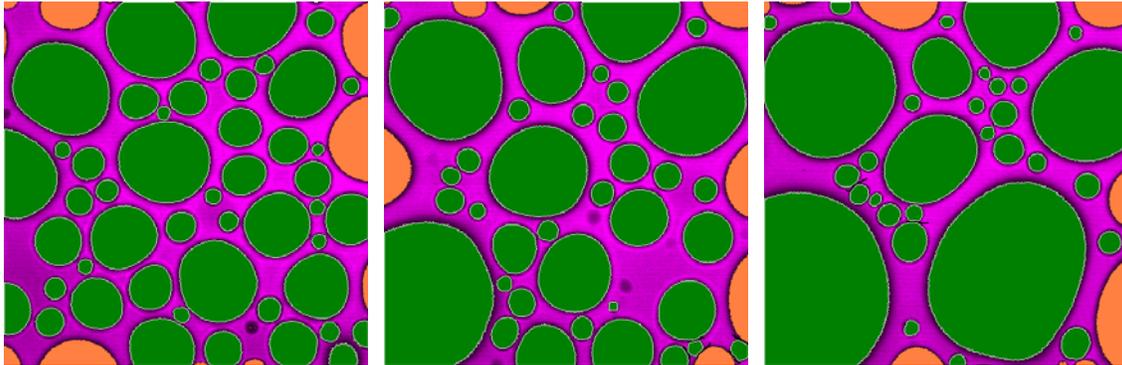


Figure 1: Images prepared for analysis: measured objects shown in green, de-selected objects (not measured, less than 50% of bubble area visible) shown in orange, and background (aqueous protein phase) shown in magenta. Time lapse images shown for 100% WPI 1 with time 0 (left), 5 min (center) and 10 min (right).

Images selected for analysis were from both z-stacks and time lapse images of foams. Z-stacks were taken at five random places within a foam. An image from the center of each stack was analyzed, which gave 5 images per foam treatment. At least 200 bubbles were analyzed per foam. For time lapse images, only one visual frame was captured over 10 minutes. The images from time 0, 2.5 minutes, 5 minutes, 7.5 minutes, and 10 minutes were analyzed for each treatment.

### **Yield stress measurement**

Yield stress of the protein foams was measured according to the method of Pernell et al (2000). A vane was attached to a Brookfield 25xLVTDV-ICP viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA). Yield stress measurements began immediately after foam generation and were completed within 5 min. The vane (10 mm in diameter and 40 mm in length) was gently lowered into the foam until the top edge was even with the foam surface in the mixing bowl. After

the vane was positioned, rotation began at a speed of 0.3 rpm and the percent maximum torque was recorded. Three consecutive measurements were taken per foam, and each foam treatment was replicated three times. The average of the three maximum torque values was used to form one data point, and the yield stress was then calculated using the following equation:

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

where  $\tau_0$  is the yield stress,  $M_0$  is the maximum torque,  $h$  is the vane height and  $d$  is vane diameter.

### **Angel Food Cake Preparation**

Angel food cake batters were prepared based on the method of Pernell et al (2002b). Solutions at 10% (w/v) protein and 12.8% (w/v) sucrose were prepared and foams were generated by the same methods stated above, except foam whipping time was only 15 minutes. To prepare cake batters, 66.0 g of cake flour and 151.6 g of 10x powdered sugar (both obtained from a local supermarket) were sifted together three times. The flour/sugar mixture was gently folded into the foam using a balloon whisk in 3 installments and no more than 30 strokes total. The batter was poured into 3 cake pans (size 6.5 cm × 13 cm × 5.5 cm) with 75.0 g batter in each pan. The batter was gently pushed into the corners of the pan using a spatula when necessary. The cakes were baked for 14 minutes in a conventional oven at 400°F (204°C). After baking, the cakes were cooled upside down on a wire rack for 30 minutes at room temperature. After cooling, cake volume was measured

by a rapeseed displacement method. Cakes were prepared in duplicate and volumes were averaged from six cakes.

### **Angel Food Cake Batter Preparation for Microscope**

Angel food cake batters were prepared as described above, except that 0.1mM sodium fluorescein added to the foaming solution prior to whipping and foams were whipped for 20 minutes. The flour and sugar were folded into foam using a rubber spatula, and the additional folding was completed within 5 minutes of whipping termination.

### **Protein-Sucrose Foams or Cake Batter Microscope Slide Preparation**

A small amount of foam or cake batter was transferred to a microscope slide, where it was placed inside a metal ring with an inner diameter of 1.5 cm and a height of 0.1 cm. A #1.5 coverslip was placed on top of the batter, which was then pressed down until level with the top edge of the metal ring, providing an even height of batter and a sample preparation similar to that of Lau and Dickinson (2005). The sample was imaged immediately.

### **Confocal Microscopy**

Samples were viewed using an Olympus BX61 research microscope equipped with an Olympus FV5 PSU confocal scanning laser (Olympus America Inc., Center Valley, PA). An argon laser excited samples at 488nm, and light was collected at a range from 500-550 nm. A UPlanFL 4x, with a 0.13 numerical aperture, was the objective used for 4x magnification. A 4x magnification was used to keep multiple bubbles in the viewing area, even with the large bubble size observed with heating. Collected images were viewed using Fluoview software.

A Linkam PE-BX120 heating/cooling stage attached to a Linkam PE 94 temperature control (Linkam Scientific Instruments, Surrey, U.K.) was used for heating. For these experiments, the temperature started at 25°C and increased to 85°C at a rate of 10°C per minute. Images of the foams or cake batters were taken at 25°C, and then the heating ramp began. At subsequent 10°C intervals, the temperature was held and images were taken until the foam structure was no longer visible. This method showed changes in a single frame with heating, and treatments were completed in triplicate.

### **Statistical Analysis**

Statistical analyses were performed using the General Linear Model procedure of the SAS statistical software package (Version 9.1; SAS Institute, Inc., Cary, N.C., U.S.A.). Analysis of variance was conducted with means separation to determine differences between treatments. Significant differences were established at  $p \leq 0.05$ .

### **Results and Discussion**

Confocal microscopy combined with image analysis is a useful tool for characterizing bubble size distributions of foams. Images taken utilizing confocal microscopy show a thin optical section of a thick sample. Figure 2 shows confocal microscopy images of WPI and EWP foams. These representative images were from the center of a z-stack, and all represent approximately the same time after foam generation.

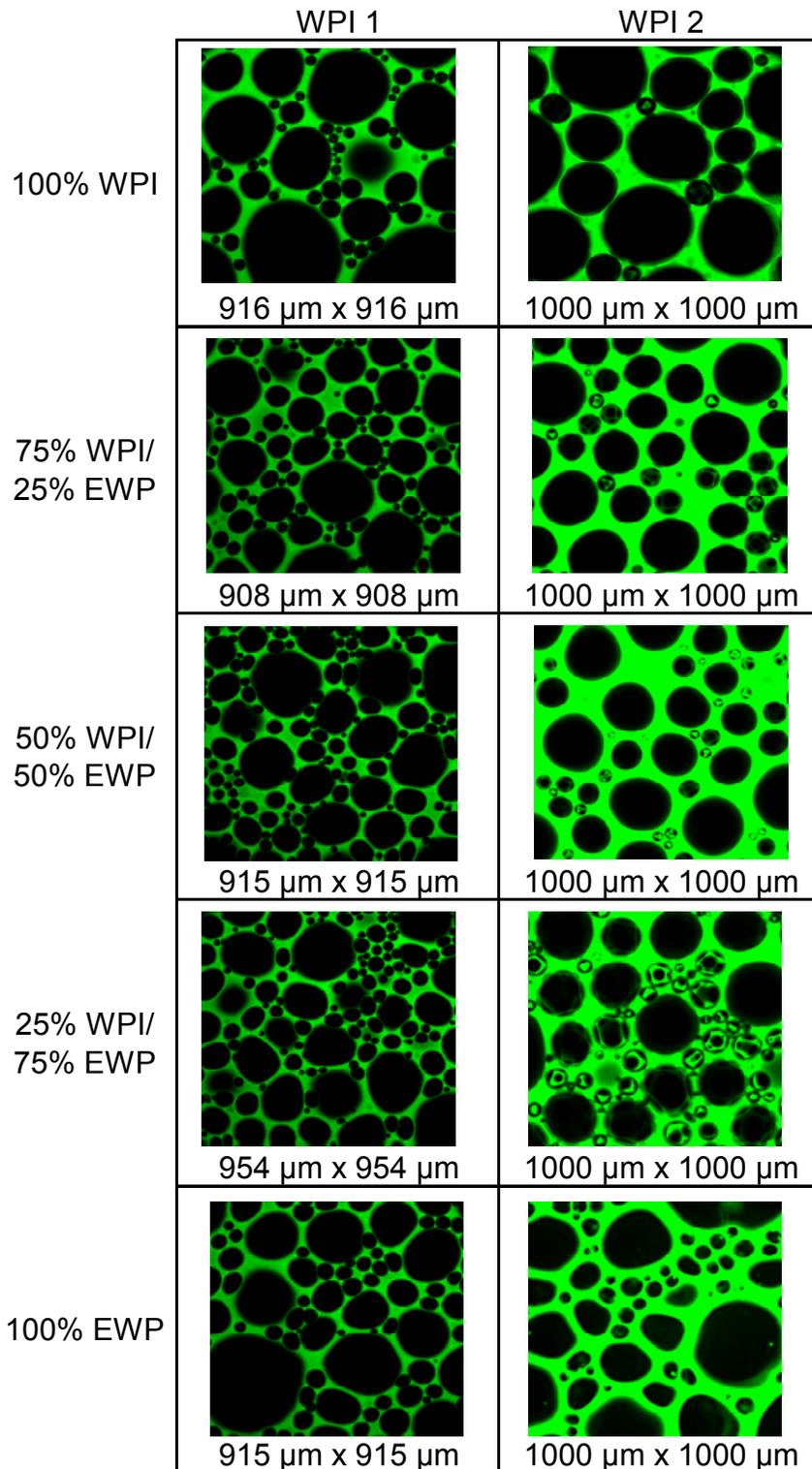


Figure 2: Confocal images of WPI, EWP and combination foams. Field size is shown below each image.

These foam images lead to several conclusions. First, WPI and combinations involving WPI 1 show numerous smaller bubbles compared to WPI 2. Also, foams prepared from EWP have a large number of more closely packed small bubbles compared to WPI, which has fewer bubbles of a larger size. Combinations appear visually similar to both WPI and EWP, containing both small and large bubbles. In addition, bubbles do not appear to be completely spherical, especially in 100% EWP foams. To further investigate the deviation of bubbles from a spherical shape, bubble shape factors were calculated using image analysis. Two representative histograms of bubble shape factors are shown in Figure 3.

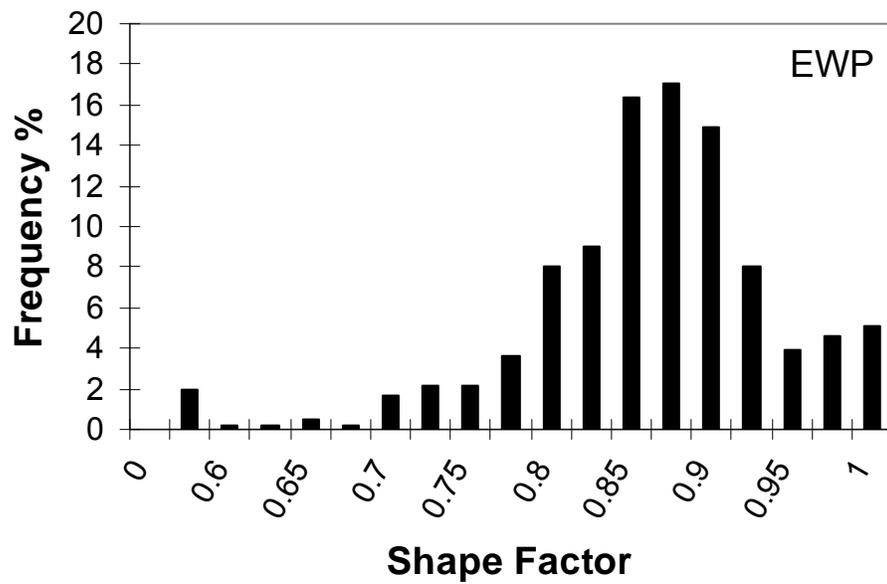
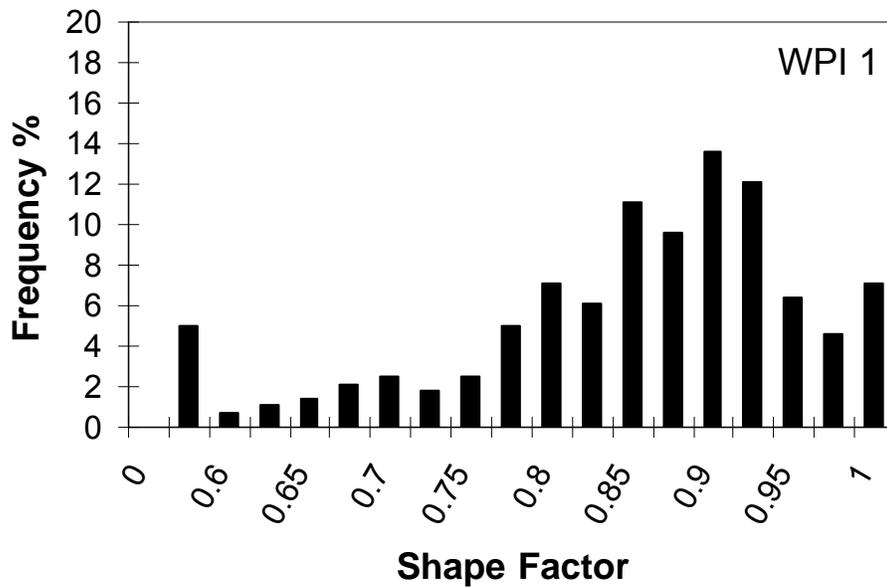


Figure 3: Bubble shape factor histograms for WPI 1 foam (top) and EWP foam (bottom). Shape factor of 1 represents a perfect sphere.

Bubble shape factor histograms (Figure 3) show that foam bubbles differ from a perfect sphere, represented by a shape factor of one. The shape factors generally formed a distribution skewed toward the lower numbers and centered around 0.85 to 0.90. From this data, it is evident that all bubbles are not spherical. Instead of assuming spherical bubble shapes and calculating bubble diameters, bubble area was calculated. Utilizing bubble shape factor and bubble area as a method of image analysis has been previously done for cake batter (Hicasmaz et al, 2003).

Mean and median bubble areas were calculated and are shown in Figure 4. The means and medians are averages of five random images taken throughout the foam. This confirmed visual observation that WPI 1 and WPI 1 combination foams show smaller bubble sizes than WPI 2. Also, WPI 2 combinations tended to show bubble sizes intermediate to WPI and EWP in a somewhat linear fashion, while foams made with WPI 1 showed a more consistent bubble size over the treatment spectrum. All foams made with combinations of WPI and EWP did not show statistically significant differences ( $p > 0.05$ ) in mean and median bubble areas when compared to other foam combinations made with the same type of WPI. This suggests that the amount of WPI substituted into combination foams has a similar impact on bubble size, whether 25% or 75% is substituted for EWP.

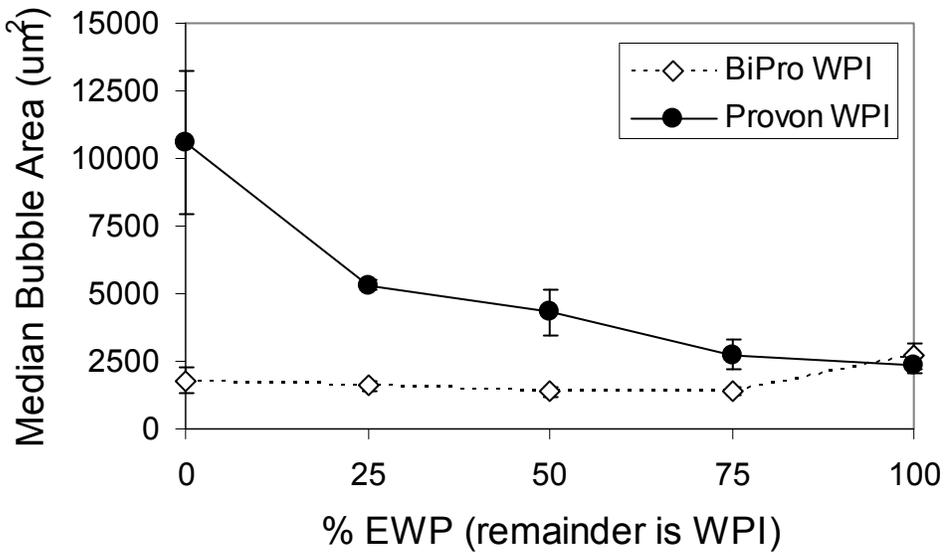
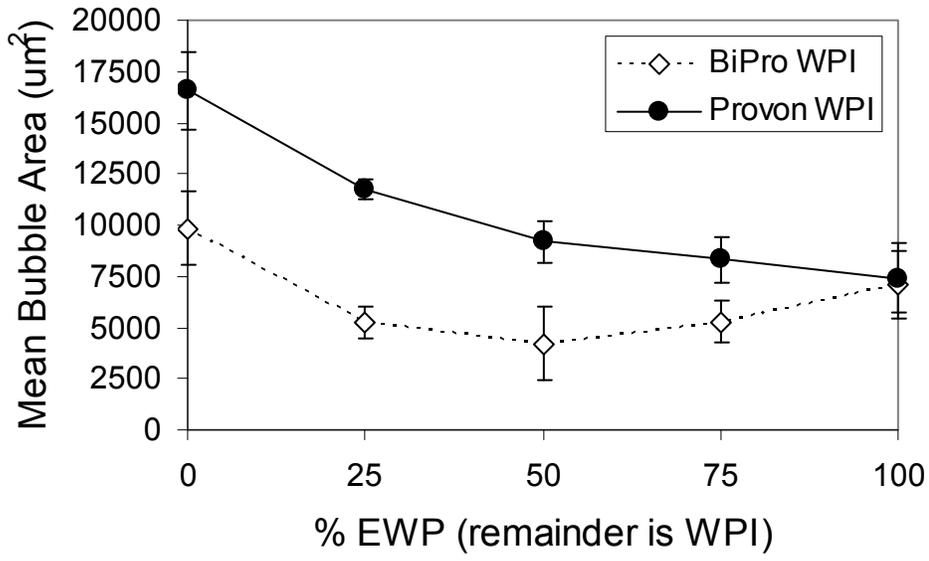


Figure 4: Mean bubble area (top) and median bubble area (bottom) for foams prepared with combinations of EWP and WPI. Data is compiled from 5 random images throughout the foam and error bars represent one standard deviation above and below the mean.

Foams were also imaged in a time lapse manner, with one frame kept in focus and imaged over 10 minutes. Analyses of time lapse images are shown in Figure 5.

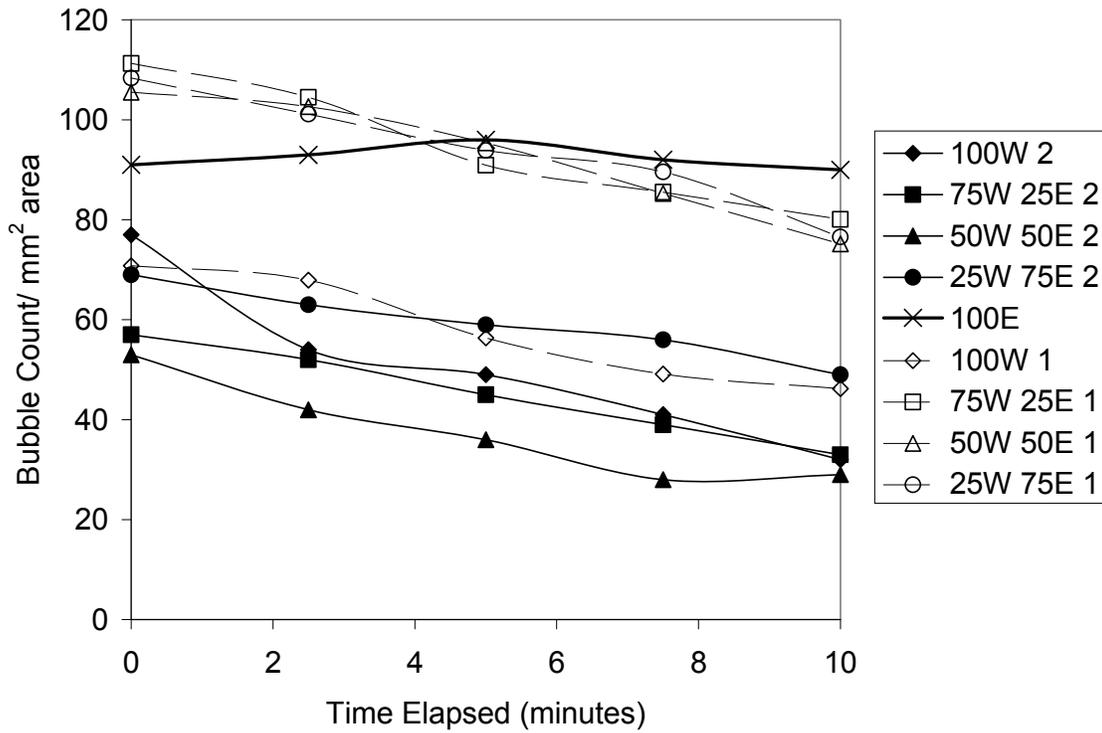


Figure 5: Bubble count per unit area for a single frame over time for WPI, EWP, and combination foams.

The parameter of bubble count per unit area over time shows several interesting features of the data. Destabilization mechanisms in foams, such as coalescence and disproportionation, result in changes in bubble size distributions. Coalescence is the rupturing of the film between two bubbles, joining them into one bubble. Disproportionation occurs when gas diffuses from small bubbles through the aqueous phase into larger bubbles, resulting in small bubbles shrinking and large

bubbles growing. Both destabilization mechanisms would result in a reduced bubble count. Figure 4 shows a decrease in bubble count over time for WPI foams as well as WPI/EWP combination foams. However, 100% EWP exhibited a consistent bubble count over time, an indicator of greater stability.

Characterizing the particular destabilization process at work is difficult simply by looking at bubble count over time. Looking at general bubble size distributions, coalescence could be shown by larger bubbles appearing. Disproportionation results in a bimodal distribution as the large bubbles grow at the expense of smaller bubbles (Pugh, 1994). Simply observing the bubbles over time is one way to determine the difference between coalescence and disproportionation. Time lapse images were processed into time lapse videos. From the movies, coalescence was not observed, while the shrinking and eventual disappearance of small bubbles and growth of large bubbles was seen. This leads to the conclusion that disproportionation is the main destabilization mechanism seen in these foams. In theory, viscoelastic films such as those formed by proteins at the air-water interface should inhibit the diffusion of gas into the aqueous phase. However, research has found that even highly viscoelastic films do not stop disproportionation but only slow it down slightly (Dickinson et al, 2002; Du et al, 2003). Disproportionation has also been observed from confocal microscopy time lapse imaging as a destabilization mechanism in foams produced from a combination of egg white and invert sugar (Lau and Dickinson, 2005).

Bubble count per unit area is also a useful parameter for estimating total surface area of bubbles in a foam. This is applicable to the Princen and Kiss (1989)

model of foam yield stress shown in Equation 1. Bubble count per area is proportional to the parameters of gas volume fraction divided by surface mean drop radius of the bubbles, also an estimate of total surface area of bubbles. Substituting bubble count per unit area for gas volume divided by drop radius gives Equation 4:

$$\tau_0 = \left( \gamma * \frac{\text{bubble count}}{\text{area}} \right) Y(\phi) \quad \text{Equation 4}$$

where  $\tau_0$  is the yield stress,  $\gamma$  is the interfacial tension, and  $Y(\phi)$  is an experimentally derived parameter. A plot of foam yield stress against the product of bubble count per area and surface tension of the foaming solution (measurements provided by Xin Yang and presented in the previous chapter) should represent the  $Y(\phi)$  experimentally derived parameter. This is shown in Figure 6.

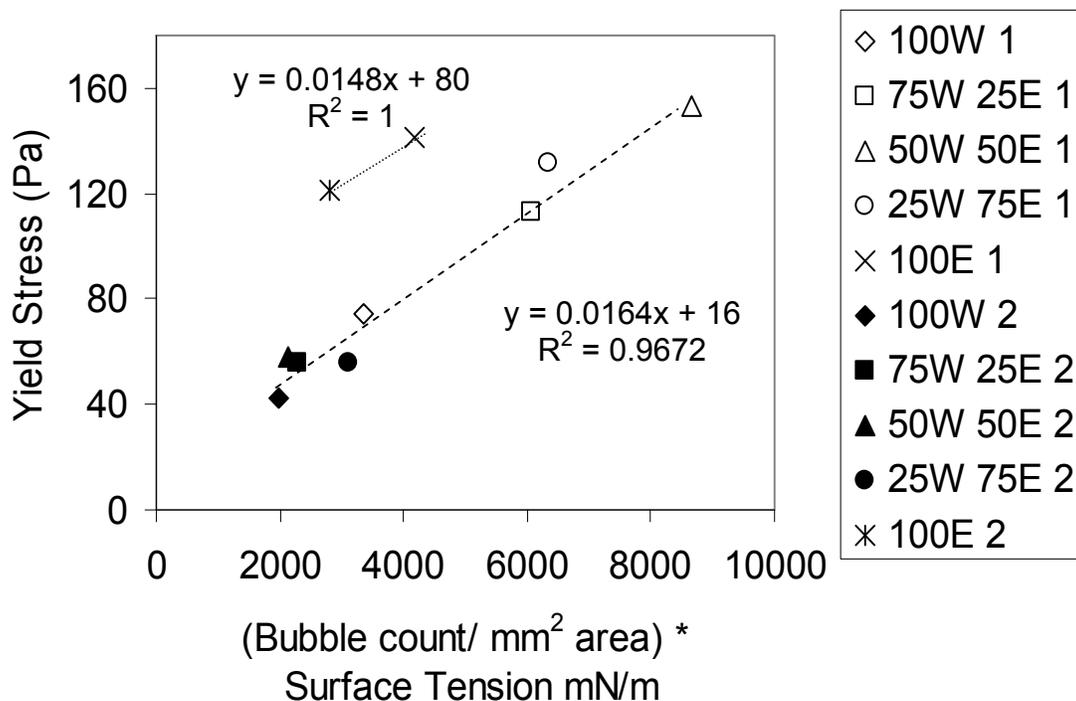


Figure 6: Yield stress of foams made with WPI, EWP, or combinations of the two plotted against the product of bubble count per unit area and surface tension of the foaming solution. Linear regressions and  $R^2$  values are displayed for WPI and WPI/EWP combinations as well as EWP foams.

The results show a distinct linear pattern for WPI foams and WPI/EWP combination foams. However, 100% EWP foams show a deviation from the linear pattern, with a higher yield stress than expected. To further quantify this difference, a linear regression was calculated for the WPI and WPI/EWP combination foam data points, shown in Figure 6. A separate linear regression line was calculated for the EWP foams, although the data for EWP foams was only two data points. While both regression lines showed similar slopes (0.016 for WPI-containing foams and 0.015 for EWP foams), the y-intercept was much higher for the EWP foams, showing that

these foams are shifted up higher in yield stress. According to equation 5, this suggests that the  $Y(\phi)$  experimentally derived parameter in the Princen and Kiss (1989) model of yield stress is higher for EWP foams compared to WPI or WPI/EWP combination foams. A higher  $Y(\phi)$  for EWP foams compared to WPI foams has been previously seen (Pernell et al, 2002a). The behavior of WPI/EWP combination foams fell in line with that of WPI, suggesting the WPI dominated the interface in foams composed of both WPI and EWP even at the lowest level of 25% WPI: 75% EWP.

Angel food cakes are a useful application for evaluating protein foaming functionality since protein foams are initially generated and then used as an ingredient in the cake. WPI has good foaming properties but has been found to perform poorly when substituted for EWP in angel food cakes. Figure 7 shows cake volumes obtained from cakes prepared with WPI, EWP, and combinations of the two.

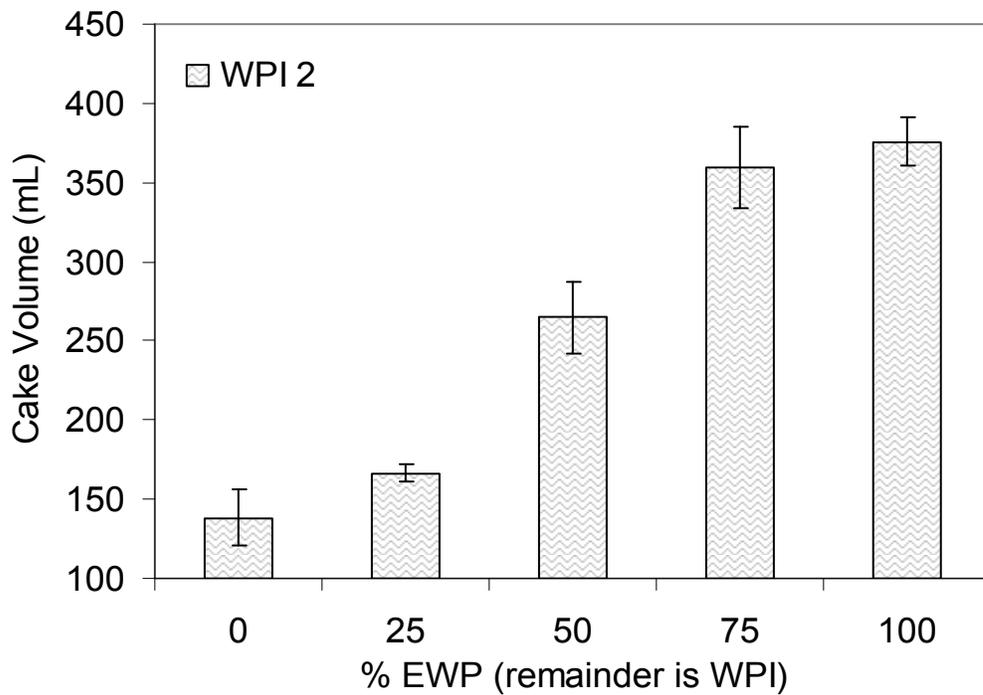
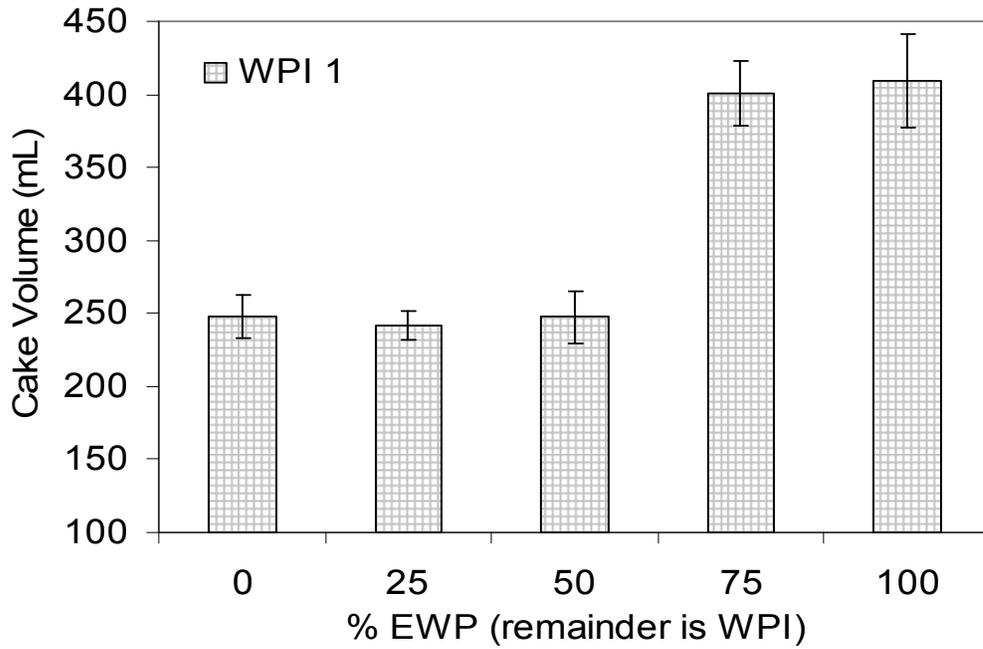


Figure 7: Volumes of angel food cakes made with combinations of EWP and WPI 1 (top) or WPI 2 (bottom).

While the two different types of WPI show slightly different cake volumes, the main trend is the same. Cakes composed of at least 50% WPI (50% or less EWP) showed volumes significantly ( $p < 0.05$ ) lower volumes than those prepared with EWP or 75% EWP: 25% WPI. The volume measurements indicate that with at least 75% EWP present, angel food cakes produce volume measurements similar to those made with 100% EWP. Statistical analysis showed no significant difference between cakes produced with 75% EWP: 25% WPI and 100% EWP ( $p > 0.05$ ). However, volume measurements do not give a comprehensive understanding of cake structure. Photos of cross-sections of angel food cakes prepared with 100% EWP and 75% EWP: 25% WPI are shown in Figure 8.

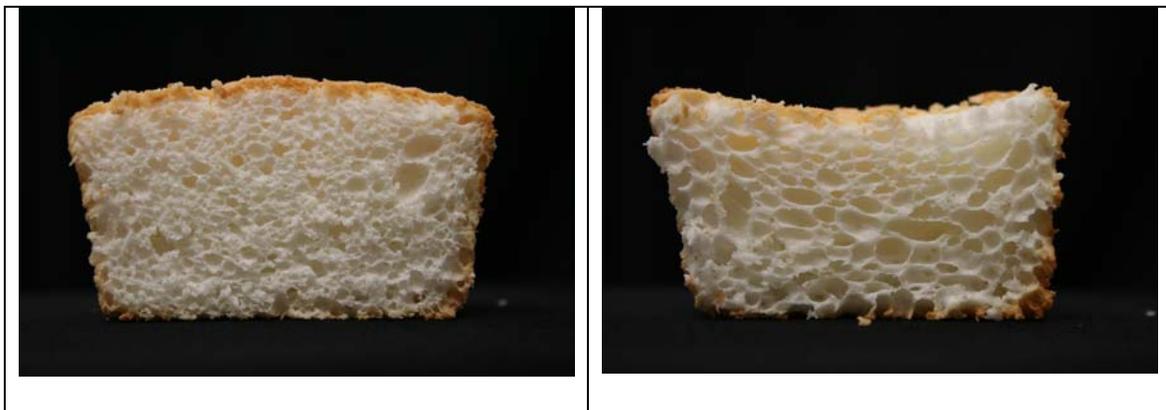


Figure 8: Cross sections of angel food cakes prepared using of 100% EWP (left) and 75% EWP: 25% WPI (right).

Differences between angel food cakes prepared with 100% EWP and those with just 25% WPI substitution are not seen in cake volume, but can be seen in cross-sectional images. EWP cakes exhibited a smaller bubble structure and finer texture. Cakes prepared with 75% EWP: 25% WPI showed a much larger bubble structure as well as a concave surface, indicating cake expansion and slight

collapse. Other cakes prepared with greater proportions of WPI showed even larger bubbles structure and more collapse, hence the lower cake volumes. The conclusion drawn from the images suggest that WPI dominates the interface when both WPI and EWP are present and affects cake stability, even in substitutions of 25% WPI.

To further investigate these differences, confocal microscopy was utilized to view the microstructure of foams prepared with EWP, WPI, and 75% EWP: 25% WPI. The foams were all produced at a level of 10% (w/v) protein and 12.8% (w/v) sucrose, which is comparable to the sucrose levels used to produce angel food cakes. These protein and sugar foams were “baked” on a heating stage attached to a confocal microscope. Each treatment was completed in triplicate, with similar results seen for each replicate. Figure 9 shows representative foam images for each treatment at temperatures from 25°C to 55°C.

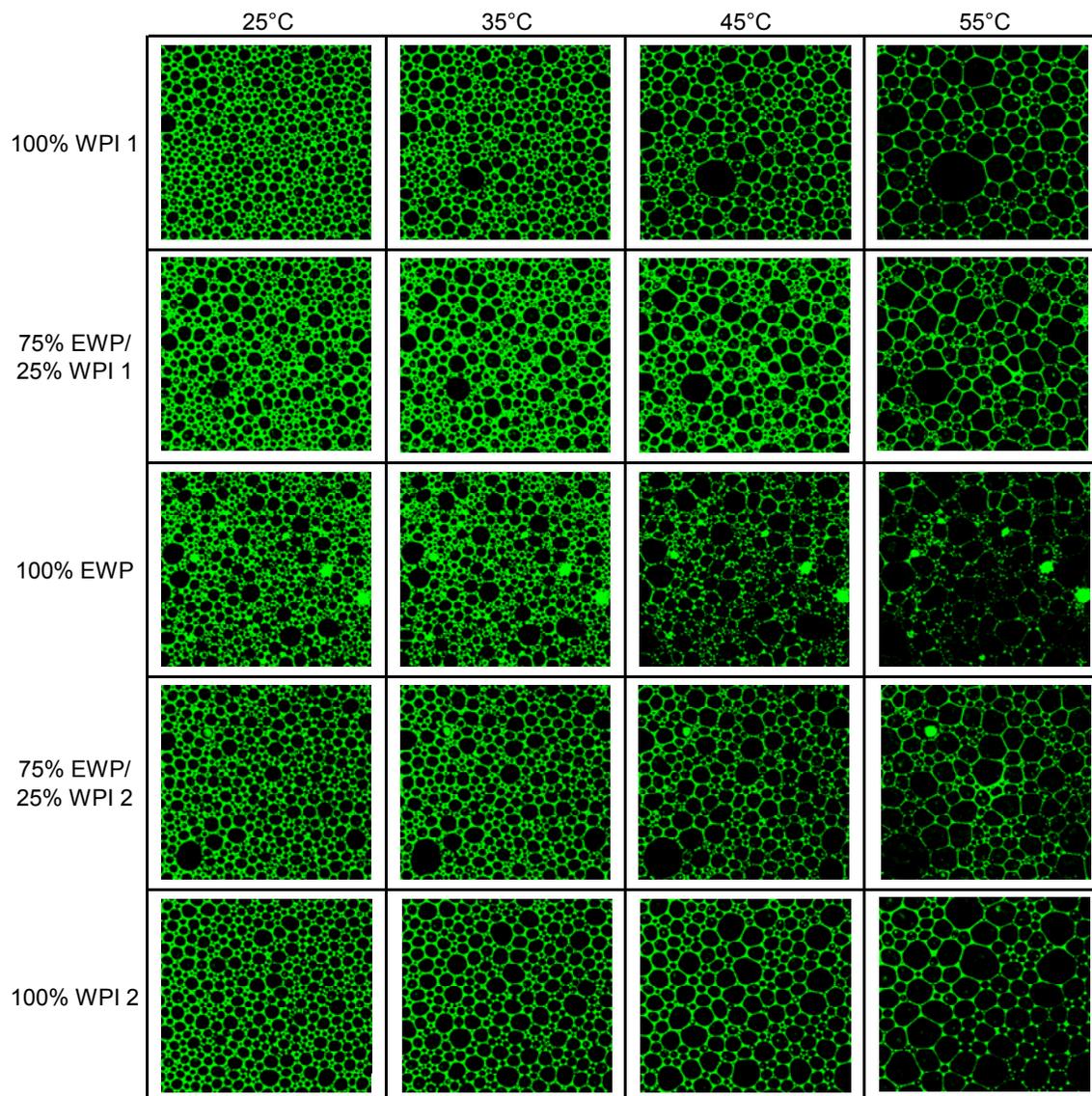


Figure 9: Confocal images of WPI, EWP and WPI/EWP combination foams (10 % w/v protein and 12.8% w/v sucrose) with heating. Field size is 3.6 mm x 3.6 mm.

Sodium fluorescein is a water soluble fluorescent dye that binds to protein. Thus, in Figure 9, the aqueous protein solution is shown as the green phase while air bubbles are black. EWP contains some insoluble particles in solution, but these were not removed as they have not been seen to make a difference in bulk foaming properties (see previous chapter). Several insoluble particles are visible in the EWP foam image as well as in the 75% EWP/25% WPI images.

At 4x magnification and 25°C, all treatments appear similar in bubble size. With heating to 35°C, all foam images still appear similar. However, at 45°C, EWP foams deviated from the other treatments. At this temperature, EWP foams started to form a network. By 55°C, EWP foams showed a network of thin films and beyond this temperature little can be seen with a confocal microscope. In contrast, both types of WPI showed similar patterns at 45°C and 55°C, with bubbles growing larger with increasing temperatures. No aqueous phase network was formed. At temperatures around 65°C, bubble expansion reduced the aqueous phase to a thin film, usually followed by film rupture. The combination of 75% EWP and 25% WPI treatments showed properties most similar to that of WPI. A slight difference was seen around 55°C, where combination foams exhibited slightly more bubble deformation than WPI although this did not result in a stronger network. The combination foams behaved like WPI at higher temperatures, showing thin film formation and subsequent rupture.

Pernell and others (2002b) investigated the thermal transitions of protein-sugar solutions using differential scanning calorimetry (DSC). The peak temperature was correlated to protein denaturation temperature and was found to be 84°C for

10% EWP and 77°C for 10% WPI (Pernell et al, 2002b). This suggests that EWP should denature at higher temperatures than WPI. However, Figure 9 shows EWP foams began network formation at lower temperatures than WPI foams and also at a temperature (~45°C) much lower than the reported protein denaturation temperature (84°C). The disparities may be due to the fact that DSC measured protein denaturation of a solution, while the microscope images show network formation of a foam. When a solution was whipped into a foam, proteins at the air-water interface unfolded and were already at least partially denatured before heating even began. Once partially denatured, EWP foams showed network formation at lower temperatures compared to the reported denaturation temperature. WPI foams showed little network formation.

Angel food cake batters produced from protein-sucrose foams were also imaged using confocal microscopy. The batters were imaged in triplicate for each treatment, and a representative image of each is shown in Figure 10.

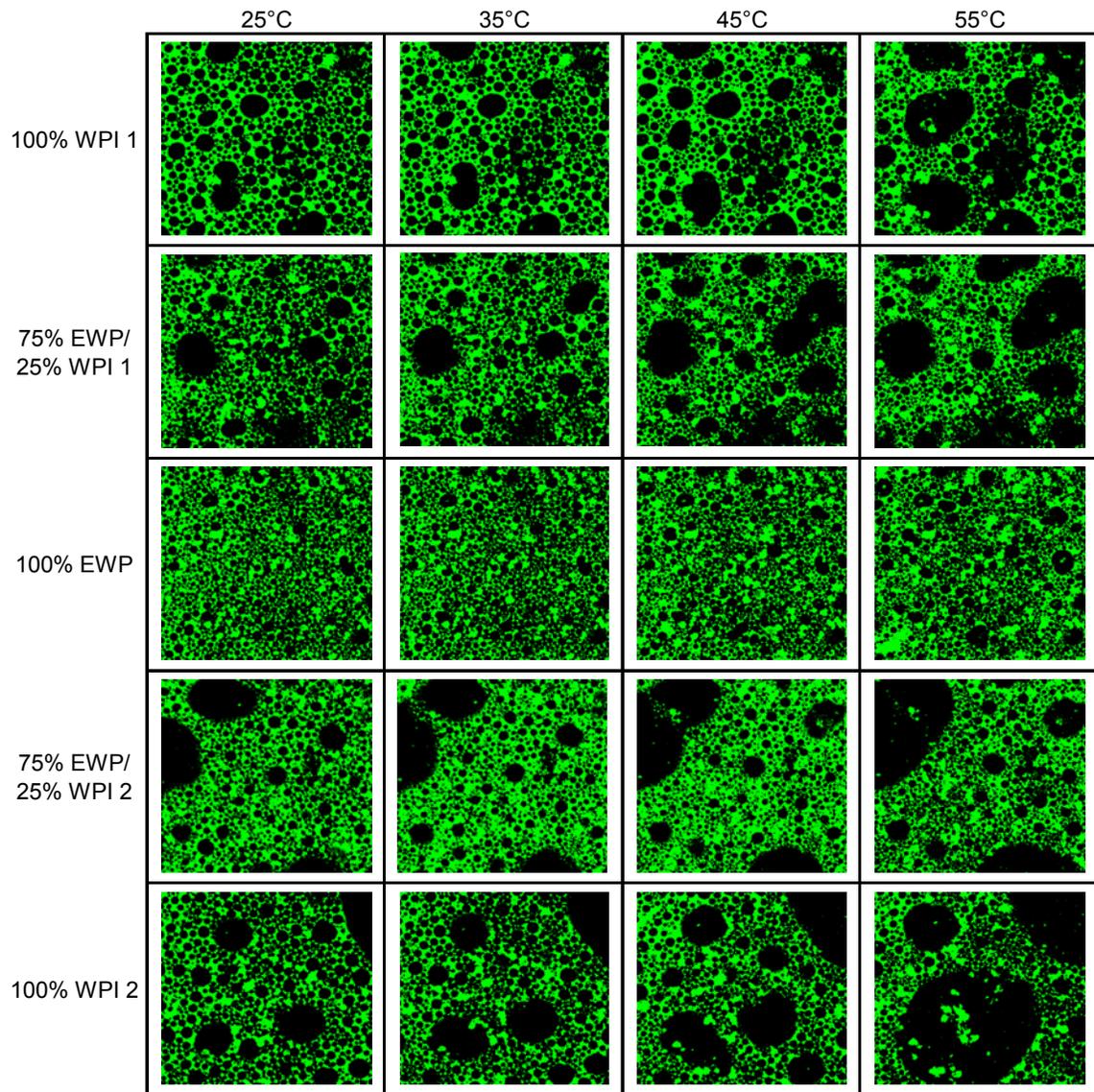


Figure 10: Confocal images of WPI, EWP and WPI/EWP combination foams mixed into an angel food cake batter and heated. Field size is 3.6 mm x 3.6 mm.

Angel food cake batters showed a pattern similar to those observed in the protein-sucrose foams upon heating. However, for cake batters, the initial image at 25°C exhibited larger bubbles for WPI and WPI/EWP combination treatments. This could be partially due to the extra five minutes and additional hand mixing needed to produce the cake batter. However, WPI and WPI/EWP combinations showed bubble sizes larger than EWP cake batters from the first image. This indicated that some destabilization process was at work even before heating the cake batters. Disproportionation was observed in time lapse movies of foams (see previous chapter), and thus is likely also at work in cake batters. Also, some coalescence was observed when preparing and focusing the microscope for imaging. The addition of cake flour and powdered sugar, plus the action of folding the dry ingredients into the protein-sucrose foam, increases the instability of WPI foams, but appears to have no negative effects on EWP foams.

WPI foams showed large bubbles present at 25°C which grew to extremely large bubbles with an increase in temperature. Batters composed of 25% WPI and 75% EWP followed a very similar pattern, starting off with large bubbles growing larger, indicating that the interface was dominated by WPI even at only 25% substitution. However, EWP batters showed smaller bubbles from the first measurement and had enough of a network stabilizing the bubbles that they grew at a much slower rate. Even at temperatures higher than 55°C, EWP batters showed a stable network, shown in Figure 11. Bubbles continued expanding with heating, but did not coalesce into extremely large bubbles as seen in the WPI and WPI/EWP combination cake batters.

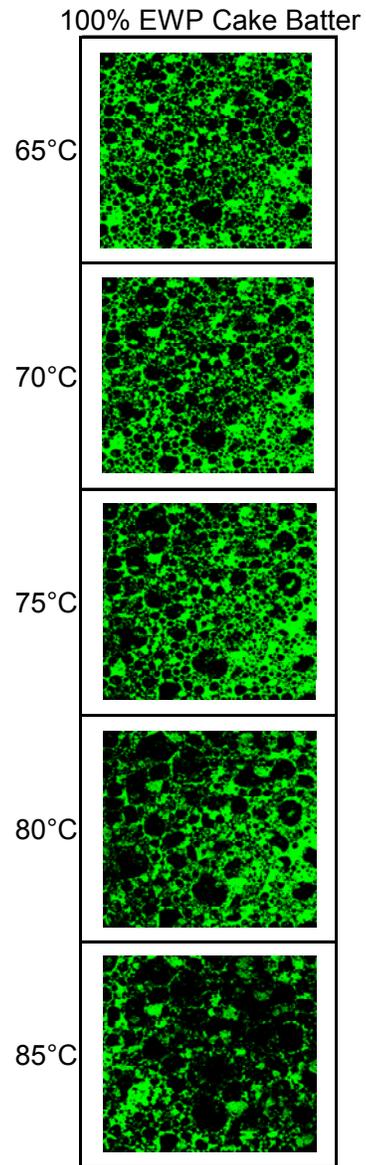


Figure 11: Confocal images of 100% EWP angel food cake batter with heating (continuation of images of EWP cake batter seen in Figure 10). Field size is 3.6 mm x 3.6 mm.

Pernell and others (2002b) investigated heating angel food cakes made with EWP and WPI. When baked in an oven, the cakes showed similar cake height changes until reaching an internal temperature of about 75°C. After this point, 10% WPI cakes declined in volume while 10% EWP cakes continued to expand and maintained the maximum volume (reached around 95°C) (Pernell et al, 2002b). In Figure 10, differences in the microstructure of the cake batters could be seen at much lower temperatures. Also, Pernell and others found DSC peak temperatures on cake batters to be 94°C for 10% EWP and 91°C for 10% WPI cake batters. This mainly corresponds with starch gelatinization, as the peak temperature for cake batter without protein was 89°C (Pernell et al, 2002b). However, Figure 11 shows whipped cake batters set up structures at much lower temperatures. EWP batters set up a structure maintained throughout the heating process, while WPI and WPI/EWP combination batters failed to form a network, resulting in bubble destabilization and huge bubbles by 55°C. The bubble network was destabilized long before starch gelatinization could assist in setting up a solid structure.

Visual observation of the microstructure of cake batters corresponds to rheological data on cooking batters also investigated by Pernell and others (2002b). Phase angle data on cakes made with EWP and WPI were collected during a heating process mimicking baking. Both batters had a phase angle measurements between 0 and 45, which signified the batters were acting like elastic solids. At the start of the temperature ramp, the batters showed comparable phase angles, but diverged by 60°C. The phase angle of the WPI batter increased until it reached

about 90°C, which suggested the batter became more viscous. In contrast, the EWP batter showed a continuous decrease in phase angle, implying that structure became more elastic with heating (Pernell et al, 2002b). This corresponds with observations on the microstructure of cake batters seen in Figures 10 and 11. By 55°C, the WPI and WPI/EWP combination batters showed large bubbles, indicating a breakdown of the original foam structure. However, EWP exhibited small bubbles characteristic of an elastic network structure and maintained this structure through heating to 85°C.

The change in EWP foams around 55°C corresponds with data (currently unpublished) investigating the effect of using pre-heated solutions to prepare angel food cakes. Cake volume data showed no major changes in WPI angel food cakes made with protein solutions heated at temperatures ranging from 20°C to 70°C. However, EWP cakes showed a high volume with solution heat treatments between 20°C and 50°C, but then had a large decrease in volume between 50°C and 60°C. EWP foams observed in this research showed the formation of a network around 55°C. This leads to the hypothesis that a change occurs in EWP solution in this temperature range, resulting in interactions between proteins to form a network. When this solution is later whipped to form angel food cakes, the proteins are not ideally suited for stabilizing foams.

## Conclusion

The yield stress model suggested by Princen and Kiss (1989) fit bubble count per unit area, surface tension, and yield stress data collected on foams made with EWP, WPI, and combinations of the two. WPI and WPI/EWP combination foams showed a linear relationship, while EWP foams deviated from the pattern, exhibiting a higher yield stress. Microstructural examination showed that EWP foams with sucrose formed a network at a lower temperature ( $\sim 55^{\circ}\text{C}$ ) than WPI and WPI/EWP combination foams. Also, EWP cake batters observed using confocal microscopy formed a stable network during heating, while WPI and WPI/EWP combination cake batters showed similar destabilization, resulting in huge air bubbles. These results suggest that foams produced from a combination of WPI and EWP more closely resemble WPI foams, indicating that WPI may dominate the interface in foams where both proteins are present.

## REFERENCES

- Arunepanlop B, Morr CV, Karleskind D, Laye I. 1996. Partial replacement of egg white proteins with whey proteins in angel food cakes. *J Food Sci* 61: 1085-1093.
- Aryana KJ, Haque ZZ, Gerard PD. 2002. Influence of whey protein concentrate on the functionality of egg white and bovine serum albumin. *Int J Food Sci and Tech* 37: 643-652.
- Damodaran S. 2005. Protein stabilization of emulsions and foams. *J Food Sci* 70: R54-R66.
- Dickinson E, Ettelaie R, Murray BS, Du Z. 2002. Kinetics of disproportionation of air bubbles beneath a planar air-water interface stabilized by food proteins. *J Colloid and Interface Sci* 252: 202-213.
- Du L, Prokop A, Tanner RD. 2003. Variation of bubble size distribution in a protein foam fractionation column measured using a capillary probe with photoelectric sensors. *J Colloid and Interface Sci* 259: 180-185.
- Ferrando M, Spiess WEL. 2000. Review: Confocal scanning laser microscopy. A powerful tool in food science. *Food Sci and Tech Int* August 2000: 267-284.
- Hicasmaz Z, Yazgan Y, Bozoglu F, Katnas Z. 2003. Effect of polydextrose-substitution on the cell structure of the high-ratio cake system. *Lebensm-Wiss U-Technol* 36: 441-450.
- Labbafi M, Thakur RK, Vial C, Djelveh G. 2007. Development of an on-line optical method for assessment of the bubble size and morphology in aerated food products. *Food Chem* 102: 454-465.
- Lau CK, Dickinson E. 2005. Instability and structural change in an aerated system containing egg albumen and invert sugar. *Food Hydrocolloids* 19: 111-121.
- Murray B. 2007. Stabilization of bubbles and foams. *Current Opinion in Colloid and Interface Sci* 12: 232-241.
- Pernell CW, Foegeding EA, Daubert CR. 2000. Measurement of the yield stress of protein foams by vane rheometry. *J Food Sci* 65: 110-114.
- Pernell CW, Foegeding EA, Luck PJ, Davis JP. 2002a. Properties of whey and egg white protein foams. *Colloids and Surfaces A: Physiochem and Eng Aspects* 204: 9-21.

- Pernell CW, Luck PJ, Foegeding EA, Daubert CR. 2002b. Heat-induced changes in angel food cakes containing egg-white protein or whey protein isolate. *J Food Sci* 67: 2945-2951.
- Peter PN, Bell RW. 1930. Normal and modified foaming properties of whey-protein and egg-albumin solutions. *Industrial and Eng Chem* 22 (10): 1124-1128.
- Princen HM, Kiss AD. 1989. Rheology of foams and highly concentrated emulsions IV: An experimental study of the shear viscosity and yield stress of concentrated emulsions. *J Colloid and Interface Sci* 128(1): 176-187.
- Prins A. 1988. Principles of foam stability. In: Dickinson E, Stainsby G, editors. *Advances in Food Emulsions and Foams*. New York: Elsevier Applied Science. p 91-122.
- Pugh RJ. 1996. Foaming, foam films, antifoaming and defoaming. *Adv in Colloid and Interface Sci* 64: 67-142.
- Richardson G, Langton M, Faldt P, Hermansson AM. 2002. Microstructure of  $\alpha$ -crystalline emulsifiers and their influence on air incorporation in cake batter. *Cereal Chem* 79(4): 546-552.
- Richert SH. 1979. Physical-chemical properties of whey protein foams. *J Agric Food Chem*. 27: 665-668.
- Yang X, Berry T, Foegeding EA. 2007. IFT AMFE Technical Program Abstracts. 293.