ABSTRACT

PERNELL, CHRISTOPHER WARREN. Comparison of the Functional Properties of Egg White Proteins and Whey Protein Isolate in Aerated Food Systems. (Under the direction of Dr. E. Allen Foegeding)

This study investigated the static and dynamic physical properties of protein foams and cake batters made from egg white protein and whey protein isolate. A method of rheological evaluation (vane method) initially employed in the study of soils was used to evaluate protein foam rheology. The method was shown to be a reliable method for determining large-scale rheological properties of protein foams. Egg white protein produced foams with higher yield stress at lower concentrations and shorter whip times than did whey protein isolate. Short lifetime decreases in yield stress were observed in foams of higher protein concentration of both types, which were both concentration and whip time dependent. This is considered to be the result of a restructuring of the system as opposed to collapse of the foam. Cakes made from foams of both types showed different performance properties as well. Cakes produced from egg white protein exhibited a minimum concentration of protein (between 5 and 10% w/w of foam) necessary to form a cake of satisfactory volume. Whey protein isolate was unable to form a satisfactory cake regardless of the concentration studied. Phase contrast microscopy, fluorescence microscopy and differential scanning calorimetry studies suggested that the difference in behaviors might be due to a high degree of phase separation between egg white protein and soluble starch that was not seen in whey protein containing systems.
This phase separation appears to allow significant matrix development in egg white protein containing cakes that can support the volume of the final product.
COMPARISON OF THE FUNCTIONAL PROPERTIES OF
EGG WHITE PROTEIN AND WHEY PROTEIN ISOLATE
IN AERATED FOOD SYSTEMS

by

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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

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APPROVED BY:

Chairman of Advisory Committee
Christopher Warren Pernell was born, raised, educated, and will probably die in Raleigh North Carolina. What else is there to say.
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# TABLE OF CONTENTS

List of Tables .............................................................................................................vii

List of Figures ..............................................................................................................viii

**Introduction** ........................................................................................................... 1

References ...................................................................................................................5

**Manuscript 1.** Measurement of the Yield Stress of Protein Foams by Vane Rheometry

Abstract .......................................................................................................................8

Introduction ..................................................................................................................9

Materials and Methods ...............................................................................................12

Results and Discussion ...............................................................................................16

Conclusion ....................................................................................................................20

References ...................................................................................................................21

**Manuscript 2.** Description of the Mechanical Behavior of Protein Foams

Abstract .......................................................................................................................29

Introduction ..................................................................................................................30

Materials and Methods ...............................................................................................33

Results and Discussion ...............................................................................................38

References ...................................................................................................................48
**Manuscript 3.** Comparison of Bulk Physical Properties of Angel Food Cakes Containing Egg White Protein and Whey Protein Isolate

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>61</td>
</tr>
<tr>
<td>Introduction</td>
<td>62</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>64</td>
</tr>
<tr>
<td>Results</td>
<td>69</td>
</tr>
<tr>
<td>Discussion</td>
<td>74</td>
</tr>
<tr>
<td>Conclusion</td>
<td>85</td>
</tr>
<tr>
<td>References</td>
<td>86</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Manuscript 2;

Table 1. Surface tension and viscosity of foaming solutions ..........................50
Table 2. Overrun of foams determined by mass measurement.........................51

Manuscript 3.

Table 1. Enthalpy ($\Delta H$) onset ($T_o$) and peak temperature ($T_p$) values of the major peak observed in DSC analysis of formulations at various stages of cake batter production.................................................................88
Table 2. Composition of formulations on a weight percentage basis..................89
LIST OF FIGURES

Manuscript 1;

Figure 1  Schematic of vane attachment…………………………………………………23
Figure 2.  Whipping configuration and measurement positions for each mixer
Model…………………………………………………………………………………24
Figure 3.  Yield stress vs. whipping time profiles of egg white protein and whey
protein isolate foams……………………………………………………………25
Figure 4.  Comparison of point and slope methods of vane rheometry for protein
Foams……………………………………………………………………………….26
Figure 5.  Comparison of force gradient effects on vanes of different lengths………..27

Manuscript 2;

Figure 1.  Yield stress vs. whipping time profiles of EWP and WPI foams………..52
Figure 2.  Time dependence of yield stress of foams varying in whip time………..53
Figure 3.  Air phase volume of foams determined by mass measurement………..54
Figure 4.  Mean detectable bubble size vs. time………………………………………55
Figure 5.  Yield stress vs. air phase volume by mass measurement…………………..56
Figure 6.  Mean detectable bubble size……………………………………………..57
Figure 7.  Air phase volume as determined by image analysis………………………..58
Figure 8.  CSLM images of EWP and WPI foams……………………………………59

Manuscript 3;

Figure 1.  Final cake volumes………………………………………………………..90
Figure 2.  Expansion of cakes during baking…………………………………………91
Figure 3.  Changes in phase angle upon cooking……………………………………..92
Figure 4. Expansion and rheological changes occurring in WPI containing cakes during baking .................................................................93

Figure 5. Expansion and rheological changes occurring in EWP containing cakes during baking ..............................................................94

Figure 6. Phase contrast micrographs of cake batter systems following simulated Baking ..........................................................95

Figure 7. Bright field and fluorescence micrographs of cooked EWP batters .......96

Figure 8. Bright field and fluorescence micrographs of cooked WPI batters ......97

Figure 9. Phase contrast images of non-batter cooked samples ..........................98
“One can only see what one observes, and one observes only things which are already in the mind”
-Alphonse Bertillon

“Make for thyself a definition or description of the thing which is presented to thee, so as to see distinctly what kind of a thing it is in its substance… For nothing is so productive of elevation of mind as to be able to examine methodically and truly every object which is presented to thee in life”
-Marcus Aurelius
INTRODUCTION

Proteins are employed extensively in processed foods to improve the nutritional and textural properties of the food product. These proteins come from many sources and are available in many forms. They exhibit widely varying functional properties which include solubility, water holding, gelling, emulsifying capacity and foaming (Kinsella, 1981). Food technologists can utilize these materials to their advantage in developing new products or improving existing ones. They would also like to be able to substitute one protein for another or combine proteins to obtain specific functional behaviors and reduce costs. Protein suppliers on the other hand would like to be able to manipulate and control the properties exhibited by their products in order to gain entrance into markets dominated by other protein sources. One such example is the interest in utilizing whey proteins to substitute for more costly egg white proteins in aerated foods.

As early as 1930 researchers were investigating the use of whey proteins as egg white replacements (Peter and Bell, 1930). This is understandable because very few other protein sources show functional properties, such as foamability, heat coagulability and high sulfhydryl / disulfide content, which are similar to those of egg white (To et al., 1985). Since then a great deal of research has been conducted in an effort to understand why egg white proteins function in the way they do and how whey proteins can be modified or manipulated to function similarly, particularly with respect to foaming properties. MacDonnell et al (1955) investigated the various fractions of protein species in egg white relative to whole egg white in order to establish the contributions of each to the foaming properties of egg white. Li-Chan and Nakai (1989) described the foaming properties of each of the egg white fractions from a biochemical point of view. In
general, however, whole egg white is used as a control for comparison of the foaming properties of whey proteins.

Many modifications of whey proteins have been investigated with respect to their foaming behavior. Chemical modifications have been conducted in order to investigate the effects of changing the hydrophobic/hydrophilic character of the proteins (Brinegar and Kinsella, 1981; Phillips and Kinsella, 1990). Similar effects induced by heat denaturation have also been investigated (Richert et al., 1974; de Vilbiss et al., 1974; Zhu and Damodaran, 1994; Townsend and Nakai, 1983). Enzyme induced hydrolytic effects on the foaming properties of whey proteins have been studied (Althouse et al, 1995; Kuehler and Stine, 1974), as have the effects of salts on the foaming properties of whey proteins (Cotterill et al., 1992; Zhu and Damodaran, 1994; Phillips et al., 1991). Much has been learned from these studies but only relative to the function of the proteins in forming and stabilizing foams at room temperature. This is an important distinction because rarely is a protein foam a final product in itself; it must usually undergo further processing. These studies have not yielded information that explains the behavior of the foam during further processing, i.e. the ability of the foam to produce a satisfactory cake. This could reflect an incorrect approach to the problem.

The ultimate goal in these studies is to be able to predict the behavior of the protein, within the various stages of processing, based on the structure and known chemical properties of the proteins (de Wit, 1984). In order to fulfill these wishes, it is necessary to first know exactly how the preferred protein functions in the food of interest and then to know what causes the protein to function in this manner. Only then can other proteins be modified or manipulated to perform in a similar manner. The approach in studying whey protein foaming thus far has been primarily that of “cook and look”. That
is, a modification to the protein is made based on the hypothesis that it will be positively favored by some fundamental factor in the physics of foam formation and/or stability. The hope is that if foaming capacity or stability is improved, so too may be the performance of the foam upon further processing. The assumption is that the forces that govern formation and stability in making the foam also govern the formation and stability during further processing. This has not been found to be the case. Perhaps a better approach to the problem may be to investigate the behavior of the foam as an ingredient in itself as opposed to immediately studying the protein’s behavior within the foam. In this way the important characteristics of the foam, with respect to its behavior in the system, can be targeted as a goal for more specific modifications of the protein. Rheological analyses of the foam’s behavior might uncover these characteristics.

In the formation of a cake, a liquid foam (batter) is transformed into a solid foam. Rheological measurement of this transition and the events that lead up to it should provide a wealth of information as to the chemical and physical phenomena that occur during baking. The rheology of liquid foams is a difficult issue owing to the complexity of their structure. The bulk of the work that has been done on the subject has involved theoretical prediction of the flow behavior based on geometrical models of the structure in two or three dimensions (Jeronimidis, 1988; Kraynik and Hansen, 1987). Physical measurement of the rheological properties of foams has been limited as well, due to difficulties in preventing slip at the boundaries. One apparatus has been proposed, however, which would eliminate this obstacle. Prud’homme and Khan describe the use of a vane device as a potential tool for measuring the yield stress of foams, (Prud’homme and Khan, 1996). This geometry has been shown to be effective in measuring the yield stresses of a number of food emulsions and concentrated suspensions (Missaire et al.,
It is the objective of this study to describe the physical behavior of egg white protein foams, whey protein foams and cakes made from them, in an effort to determine the function of the foam in producing a satisfactory cake.
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Measurement of the Yield Stress of Protein Foams by Vane Rheometry

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ABSTRACT

The yield stresses of protein foams made from varying protein type (spray dried egg white and whey protein isolate), protein concentration, whip time and mixer model were evaluated using vane rheometry. Two methods of analysis (point and slope methods) were investigated. Yield stress values determined by slope and point methods were similar for egg white protein foams but did not agree in the analysis of whey protein foams. Point method values were very reproducible in all foams tested. Egg white protein solutions formed stiffer foams more rapidly and at lower protein concentrations than foams made from whey protein isolate. Vane rheometry was shown to be a reliable method of determining yield stress in protein foams.

Keywords: foams, yield stress, vane method
INTRODUCTION

Foams in both fluid and solid form are present in a large number of foods. Products such as ice cream, whipped topping, nougats, meringues, breads, cakes, cookies and various batters for baked goods are either foams in finished form or incorporate foams at some stage of production. In most cases foams are produced as a preliminary stage in the making of a food product and subsequently subjected to further processing. This means that the foam as a bulk material can be thought of as an ingredient of sorts in the production of the food item. As with any ingredient, knowledge of the chemical and/or physical properties of the material, and how these properties are affected by further processing, is vital to efforts to employ them or modify them for use in a product formulation.

Many methods of evaluating food foams are available in the literature (Halling, 1981). Among these, measurements of overrun and stability are by far the most common. These methods seek to model the foaming behavior of a given protein formulation for predictive and comparative purposes. It is also hoped that these methods may help to elucidate the molecular mechanisms and interactions that are important in the formation and stabilization of a foam. The complex nature of the constructive and destructive mechanisms related to foam formation and stability suggest, however, that stability and overrun may have little value as anything other than tools for comparison of foams (Halling, 1981). In general, these evaluations are performed on foams under isothermal conditions where the foam is the final product. As such, the results do not necessarily apply to foams that are further processed, i.e. made into batter and baked to produce cakes. Foams made from different kinds of proteins, different protein concentrations and
various other compositional formulations have been compared by these methods (Arunepanlop et al, 1996; Cotterill, et al, 1992; Phillips and Kinsella, 1990; Khan et al, 1979).

It has been shown that by altering the composition of foaming solutions and/or foaming conditions, foams of similar overrun or stability can be produced (Cotterill et al, 1992; Johnson and Zabik, 1981). However, angel food cakes made primarily from whey protein foams have not yet been reported which are equivalent to egg white protein based cakes. This suggests that foam overrun and isothermal stability are not the variables of interest in modeling the performance of protein foams in cake production.

A cursory examination of any household cookbook reveals a common end point for the production of a foam to be used in virtually any recipe. This end point is referred to as the “peak stage” of the foam. The peak stage is described as the ability to form a peak when a dollop of the foam is drawn from the bulk material. The level of peak stage that the foam has reached is determined by how well the peak in the foam resists flow under the influence of gravity (i.e. soft peak, medium peak, etc…). Thus the peak stage is a subjective, semi-quantitative rheological evaluation of the yield stress of the foam. Many recipes include a warning that over or under whipping of the foam (meaning incorrect peak stage) will result in poor performance of the batter upon baking. It seems logical then, to investigate the rheological characteristics of the foam in relation to its performance in producing the final product.

The rheology of foams is a difficult issue owing to the complexity of their structure. The bulk of the work that has been done on the subject has involved theoretical prediction of the flow behavior based on geometrical models of the structure in two or three dimensions (Jeronimidis, 1988; Kraynik and Hansen, 1987). According to these
models, multiple factors influence the rheology of foams including air phase volume, solution viscosity, interfacial viscosity, bubble size distribution and bubble shape (Weaire, 1989; Princen, 1989). Some of these properties are quite difficult to measure in practice and therefore limit the effectiveness of the models that rely upon them.

Physical measurement of the rheological properties of foams has been limited as well. Traditional rheometer geometries, such as parallel plate and cup and bob systems, tend to experience wall slip and severe sample destruction during sample loading. Prud’homme and Khan adapted a parallel plate rheometer using sandpaper glued to the plates to obtain reliable data on the rheology of polymer foams (Prud’homme and Khan, 1996). They were able to show conclusively that foams do possess a quantifiable yield stress, which is in agreement with other experimental and theoretical works concerning aqueous foams (Weaire, 1989; Princen, 1989). Though the use of parallel plates offers a viable means of measurement of foam rheology, there are limitations. For example, collapse of the foam reduces the height of the foam column, thereby disengaging the foam from the upper plate of the rheometer. A very transient foam would be difficult to measure using this technique. Compression of the foam is also of concern in this technique. Compression causes a distortion of the microstructure, which changes the rheological properties of the material.

Prud’homme and Khan describe the use of a vane device as a potential tool for measuring the yield stress of foams (Prud’homme and Khan, 1996). According to Zhang et al (1998), the method was first described in an application in soil mechanics where it was used to measure soil cohesion. Dzuy and Boger (1983) later used a vane rheometer to measure yield stresses in concentrated suspensions of red clay. In this work they showed that the yield stress of clay suspensions could be calculated as a function of the
maximum torque experienced by the rheometer during testing. This method has been adopted by researchers in food science to measure the yield stresses of various concentrated suspensions such as ketchup, apple sauce and baby food (Missaire et al., 1990; Yoo and Rao, 1995, Yoo et al., 1995) as well as more complex food structures such as ice cream (Briggs et al, 1996). The general consensus is that the vane method is a reliable and easy method to use in evaluating the rheological properties of concentrated suspensions. Foams can be thought of as concentrated suspensions of colloidal and supercolloidal proportions with a liquid continuous phase and air cells as the suspended phase. Therefore the method should be applicable to the measurement of foam properties.

This study was conducted to test the viability of the vane method with respect to characterizing protein foams of varying composition and processing treatment. In subsequent investigations we will use this method to test the hypothesis that the yield stress of a protein foam is correlated to the functional properties of the foam in complex, further processed systems.

MATERIALS AND METHODS

**Egg white and whey protein isolate.** Commercial samples of whey protein isolate (WPI) (BiPro, Davisco Foods International, Inc., LeSueur MN.) and spray dried egg white protein (EWP) (P-18-J, Henningsen Foods, White Plains, N.Y.) were obtained and stored at room temperature until used. Protein content of WPI and EWP samples was determined by the Macro-Kjeldahl method (AOAC, 1984) using N factors of 6.38 and
6.25 respectively. Extra fine granulated sugar (Dixie Crystals) and was purchased from a local grocery store and stored at room temperature.

**Foam Preparation.** Foams were prepared from stock protein solutions using each of two standard kitchen mixer models (Sunbeam Mixmaster with 1.5 qt. bowl, Sunbeam Appliance Co., Oakbrook, IL. and Kitchen Aid model K45 with 4.5 qt. bowl and wire whip attachment) attached to a Staco voltage regulator (Staco Inc., Dayton OH.) set at 95%.

**Yield Stress.** Foam yield stress was measured with a Brookfield model DV-I 25xLVTDV digital viscometer (Brookfield Engineering Laboratories Inc., Stoughton MA.) equipped with a custom-made vane attachment. Four - bladed stainless steel vanes (Accu-tool, Raleigh, N.C.) were machined from bar stock and adapted to fit the Brookfield viscometer. All vanes had diameters of 10 mm and varied in length from 20 mm to 32 mm (Fig. 1). Height to diameter ratios of the vanes used were 2.0, 2.4, 2.8 and 3.2.

**Procedure.** The formulation used for foam preparation was modified from Cotterill et al. (1992). Stock solutions were prepared on a w/w protein basis with 90 g deionized water and 16.2 g sugar per treatment multiplied by the number of treatments per experiment.

Protein powders were hydrated by adding the powder to approximately 80% of the deionized water at room temperature with gentle stirring for approximately one hour or until all protein was solubilized. Care was taken to minimize foaming during stirring. Once the protein was dissolved, the remaining water and sugar was added and stirring continued for 30 minutes to dissolve the sugar and any protein that remained on the sides
of the container. The solution was held at 4\(^\circ\)C over night to allow for maximum hydration of the protein. Solutions were brought to room temperature (23 \(\pm\) 2\(^\circ\)C) the following morning by gentle stirring. All foams were prepared from ambient temperature solutions.

Foams were prepared by mixing 100 ml of stock protein solution in each of the two mixer models for a prescribed period of time at a standard speed. The Sunbeam mixer was set at speed 9 (scale = 1 - 9) and the Kitchen Aid mixer was set at speed 6 (settings = 1,2,4,6,8 and 10).

Upon completion of whipping, the beater(s) were removed from the foam as gently as possible to minimize disruption of the foam. Stiffer foams had a tendency to remain attached to the beaters, in which case the foams were removed from the beater(s) with a rubber spatula as gently as possible and returned to the bowl.

Yield stress measurements were obtained as quickly as possible after removing the foam from the mixer. The bowl containing the foam was placed on a scissors stand beneath the viscometer and gently raised until the top of the vane was flush with the top of the foam column. The viscometer was then set to zero and the motor engaged to begin the measurement. Three measurements were obtained from fixed points in each bowl of foam and three replications of each treatment were performed.

The total elapsed time necessary to measure all three points within a bowl was approximately three minutes. In order to account for any potential changes in the yield stress of the foam over the measurement period, the order of measurement at the fixed positions was systematically changed with each replication.

The measurement positions within a bowl were arbitrarily fixed relative to the position of the beater(s) upon completion of whipping. The measurement positions were
laid out in a square pattern with the final beater position being in the lower right hand corner and the three measurement positions occupying the remaining corners and read counter-clockwise from the top right corner (Fig. 2).

Two techniques were used to convert torque readings obtained by vane measurements into yield stress values (Dzuy and Boger, 1983;1985). The first, referred to as the slope method, accounts for the torque imparted to the ends of the vanes by the material. In this method, multiple measurements are taken on a test material with a series of vanes of the same diameter but differing in length. A plot of the maximum torque recorded by the viscometer vs. vane height is constructed. The slope of this line is then used to calculate the yield stress according to:

\[
\sigma_0 = \frac{2a}{\pi d^2}
\]  

where \( \sigma_0 \) is the yield stress, \( a = \) the slope and \( d = \) the vane diameter. The second method, called the point method, assumes that end effects are negligible and thus requires measurement with only a single vane. In this method, the maximum torque response is recorded for each measurement to be used in calculating the yield stress according to the formula (Steffe, 1992):

\[
\sigma = \frac{M_0}{h - \frac{1}{6} \left( \frac{\pi d^3}{2} \right)}
\]
where h and d are the height and diameter of the vane, respectively, and \( M_0 \) is the maximum torque recorded by the viscometer (Fig. 1).

**Statistical analysis.** Data were analyzed by the ANOVA procedure of SAS (SAS Institute Inc., 1988).

**RESULTS AND DISCUSSION**

The vane method employs a multi-bladed vane attachment, which is connected to a rheometer and immersed in the test material (Figs. 1&2). When the rheometer is engaged, stresses or strains (depending upon the type of rheometer used) are applied to the material and the resulting response is measured in the form of torque or displacement. These values can then be converted to stress by accounting for the geometry of the vane. A key assumption in this method is that the material yields along a cylindrical boundary defined by the diameter of the vane. The vane geometry is representative of the Searle (rotating bob in a stationary cup) geometry in that the vane, when rotating, carves out a cylinder in the test material that is analogous to the shear surface of the bob in the Searle geometry. The material within the cylindrical boundary determined by the diameter of the vane moves as a solid body. The blades of the vane represent equally spaced radii of the bob. It has been shown that only four blades are necessary to accurately assess the rheological properties of a material (Zhang et al., 1998, Dzuy and Boger, 1983). One advantage of using this technique is that the yield surface of the material is well defined by the cylindrical surface carved out of the material by the vane during measurement and is located in the material itself as opposed to lying along the surface of the bob. It is because of this “imaginary” yield surface that the vane geometry can avoid the wall slip
problem experienced with other geometries. In addition, the blades of the vane are quite thin and have very little cross sectional area, so the vane can be lowered into the sample with minimal damage to the structure of the test material. This is quite important with respect to foams because the structure is very delicate.

It has been reported that, when forming foams by whipping, the foaming response of the dispersion is dependent on the type of mixer used (Halling, 1981; Kinsella, 1984). Though these conclusions were based on analysis of foam overrun and stability studies, it was found that this is also true for foam yield stress. This study employed two mixers that varied in beater number, beater geometry and probably shear speed, though no calibration of rotational speeds was conducted. The same general trends were seen in the yield stress profiles of egg white protein (EWP) foams using both mixers; however, significant differences were noted between the values obtained for each (Fig. 3a). In general, EWP foams made with the Kitchen Aid mixer tended to have slightly lower yield stress than those made with the Mixmaster (Fig. 3b). No differences were seen in the yield stress of whey protein isolate (WPI) foams made with either mixer from 10% protein solutions, but there was a significant difference in the yield stress of 15% and 20% protein WPI foams (P < 0.05). In this case, the Kitchen Aid mixer produced foams that were higher in yield stress than those made with the Mixmaster. Overall the Mixmaster mixer produced more reproducible foams and was therefore selected as the mixer to use for all subsequent experiments.

Yield stress values obtained by the slope and point methods of analysis in this study were in very good agreement with respect to EWP foams, regardless of protein concentration. Significant differences were seen however, between slope and point method evaluations of WPI foams at all concentrations (P < 0.01). This difference was
not due to compounded error within the slope calculation in that the linearity of the
torque values generated in the slope method for all foams was quite good ($r^2 \geq 0.96$).

WPI foams displayed irregular trends over whipping time when evaluated by the
slope method as compared to the linear increase in yield stress shown by the point
method (Fig. 4). The lack of agreement between slope and point methods for WPI foams
suggests that there is anisotropy in the structure of the foams at any given whip time.
Furthermore, the evolution of the structure with whip time is not regular. This could be a
result of under or over whipping the foam to a point where either a stable structure has
not yet been reached or the stable foam has been broken down by excessive whipping.
The fact that the point and slope method curves are in such good agreement in the EWP
foams is an indication that these are stable, isotropic foams and that end effects are
negligible in the calculation of yield stress from vane data.

Point method evaluations were quite reproducible for EWP foams (c.v. range =
3.5 – 6.7%). Yield stress values of WPI foams determined by the point method were also
quite reproducible; however, accounting for the elapsed time of measurement was
required in order to achieve precision. It was observed that over the time necessary to
take three measurements in a bowl of foam (approximately 3 minutes), the yield stress
decreased. The decrease was determined by fixing measurement positions in the bowl of
foam and altering the order in which each position was measured over three replications.
Analysis of the results across the entire measurement time showed variations of as much
as 25%; however, when the results of a given treatment were compared on the basis of
order of measurement, a high degree of reproducibility was seen (c.v. range = 1.1 –
7.5%). This time dependent behavior will be investigated in future work, however the
reproducibility of the method when accounting for time is noted here in support of the
viability of the method for foam yield stress measurement. Measurement position was found to have no effect in any of the foams studied.

The slope and point methods of yield stress determination have been shown to give roughly equivalent results for concentrated suspensions, indicating that end effects are not a significant contributor to the total torque response in these materials (Dzuy and Boger, 1985). For isotropic materials this is not surprising, since the area of the circle swept out by the vane ends is small compared to the area of the cylindrical wall swept out by the sides of the vane. Anisotropy in the material may be expected to produce different results between point and slope methods. For example, a structural gradient in the vertical direction corresponding to a gradient in the local (x-y plane) yield stress would be expected to skew the results obtained by the slope method. Thus, the agreement between point and slope method values may be an indication of isotropy or anisotropy. Theoretically, if end effects are negligible, slope and point method evaluations should produce identical yield stress values in a stable, isotropic system. A gradient or non-homogeneity in the variable(s) which influence yield stress (structure, concentration, etc.) will determine whether point method values will be larger or smaller than the slope method values depending upon: (1) the direction of the gradient and / or (2) if the point method values are obtained using the longest vane in the set or a shorter one. For example, if the gradient decreases from the top of the foam column (Fig. 5 a), the torque per unit length of vane decreases with increasing vane length. This results in a relatively more gradual slope than when the gradient is reversed, as in Figure 5 b. In this case, the torque per unit length increases with increasing vane length. Since the calculated yield stress is proportional to the slope, the yield stress value will increase or decrease accordingly, relative to the point method values.
It should be noted that a compounding of error is expected to occur in calculating yield stress via the slope method due to the error present in each of the point method measurements from which the slope is obtained. Nonetheless, a high degree of reproducibility was seen in both the point and slope methods for all foams when the time dependent behavior of the WPI foams was taken into account.

CONCLUSION

Based on the high degree of reproducibility and the strong agreement between point and slope method evaluations seen in EWP foams, we conclude that the vane method is a fast, easy and reliable method for characterizing protein foams. This method could find application as a research tool as well as a quality control procedure in the baking and confectionery industries. Employment of the point and slope methods in tandem could prove to be a useful technique in evaluating anisotropic materials in three dimensions. Future studies will utilize the vane method in an attempt to ascertain the contributions of various physical and microstructural characteristics of protein foams to their rheological behaviors.
REFERENCES


Figure 1. Schematic of vane attachment.
Figure 2. Whipping configuration and measurement positions for each mixer model.
Figure 3. Yield stress vs. whipping time profiles of EWP and WPI foams: (A) 2% EWP (●), 5% EWP (■) and 10% EWP (▲) foams and 10% WPI (○,X), 15% WPI (□) and 20% WPI (Δ). Solid lines = Mixmaster mixer; dotted lines = Kitchen Aid mixer. (B) Mixmaster only. Values are means of three replications. Error bars = standard deviation.
Figure 4. Comparison of point (O) and slope (X) methods for all foams. A, B and C show 2%, 5% and 10% EWP foams, respectively. D, E and F show 10%, 15% and 20% WPI foams respectively.
Figure 5. Comparison of force gradient effects on vanes of different lengths. (A) Decreasing force gradient with vane length. (B) Increasing force gradient with vane length. Boldness of arrows indicates magnitude of force. Dashed line denotes differences in vane length.
Description of the Mechanical Behavior of Protein Foams

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ABSTRACT

Whipped foams made of varying concentrations of egg white protein and whey protein isolate were compared by vane rheometry and confocal scanning laser microscopy. Egg white proteins formed stiffer foams with lower protein concentrations and at less whipping time than did whey protein isolate foams. The yield stress was found to decline with time for 10% (w/w) egg white protein foams and for all whey protein isolate foams (10 – 20% w/w) over the time frame investigated (approximately 3 minutes). The decline in yield stress was dependent on protein concentration and whip time. Destabilizing events such as coalescence or disproportionation were not observed in microscopic evaluations or sequential density measurements, indicating that stability in the traditional sense (drainage, collapse) was not responsible for the decline in yield stress.
INTRODUCTION

Foams are represented in the food industry in the form of breads, cakes, cookies, meringues, nougats, ice cream and various baked goods. In some cases the foam is a product in itself, such as whipped topping. In other cases the foam is produced as a step in the process of manufacturing a food product and must undergo further processing before the product is complete. Food technologists would like to know the important variables in foam production, stabilization and behavior upon further processing in order to predict and control the properties of products made from or incorporating foams. To date, these properties are, at best, only moderately understood. In particular, the mechanical properties of foams and the factors responsible for them are poorly understood.

Foams are typically classified in two ways. Solid foams are elastic or plastic materials that consist of a discontinuous or continuous air phase dispersed in a continuous solid phase. In terms of foods, these are typically cooked products such as breads or cakes. The structure is stable and long-lived. The solid phase may be comprised of a single ingredient or several individual components and the mechanical (rheological) behavior is dependent primarily on the physical properties of the solid phase and the bulk density of the material (Smith, 1988). Liquid foams are much more difficult to characterize. In general, they consist of a discontinuous air phase that is dispersed within a continuum of liquid. They are metastable, with lifetimes ranging from a few seconds to several days, and they require surface active agents to maintain stability by reduction of surface tension (Dickinson, 1992; Jeronimidis, 1988). At low air phase volume they are
viscous fluids, but at higher air phase volumes they are viscoelastic materials which exhibit a yield stress (Princen, 1988). Liquid food foams are particularly difficult to characterize in terms of mechanical behaviors because the various means of manufacturing and the nature of the surface active species (proteins, fatty acids, ionic and non-ionic surfactants) that are employed in creating them result in widely varying structures with widely varying surface properties. In addition, the structural arrangement of foams, with a fluid continuous phase but potentially highly concentrated dispersed phase, makes traditional methods of measurement problematic.

Protein foams are a special subset of food foams that have intrigued investigators for many years. Bikerman, for example, proposed a unit of foaminess ($\Sigma$) in 1938 that relates the volume of protein foam formed in a given time to the volume of air used to generate the foam. Primarily it has been the ability of a given protein species or specific combinations of proteins to form and/or stabilize a foam that has been investigated. Standardized methods of foam production have been developed to enable comparison of the foaming and foam stabilizing properties of different protein sources on an equal energy input basis (Phillips et al., 1990). These methods have provided little information as to the mechanisms involved in foam formation and stabilization, however, due to the failure to accurately describe the structural and surface properties of the foam (Halling, 1981). These are problems that hinder the modeling of the rheological properties of foams as well.

A number of researchers have developed theoretical models of foam rheology based on dimensional analysis and force balances of spatially periodic, two-dimensional unit cells (Princen, 1988; Khan and Armstrong, 1986; Kraynik and Hansen, 1986). Others have experimented with highly concentrated emulsions as models for the
rheological behavior of foams (Princen and Kiss, 1986; Yoshimura et al., 1987). Based on these models, they have determined that the main properties that govern foam rheology are surface tension, mean bubble size and air phase volume. These models are certainly helpful, but they are limited. Theoretical models based on two-dimensional analyses do not translate perfectly into three dimensions and polydispersity of air cell sizes is problematic. Emulsion based models can be misleading because surface tensions (air / water interface) of protein dispersions are typically several times greater than interfacial tensions (water / lipid interface) and compressibility of air cells is ca. $10^5$ times that of emulsion droplets (Dickinson, 1992).

Coulombic effects on foam rheology add another dimension of complexity to the issue. Studies of the effects of surface potential on the rheology of hard sphere systems with uniform surface loading of ionic surfactant have shown that flow properties are strongly dependent on particle phase volume, electrolyte concentration and surface area (Woods and Krieger, 1970; Benzing and Russell, 1981). These systems have exhibited quite different properties from those of similar hard sphere systems which are often used to model food emulsions and foams. However these studies have not been carried out for systems with dispersed phase concentrations above ca. 60%; well below that of most food foams. Proteins are zwitterions, each with its own isoelectric point, above and below which the molecule carries a net charge. Food protein sources are nearly always cocktails of proteins as opposed to being single protein species. Thus electrostatic forces are always at play in protein containing systems. The extent to which these forces effect the rheology of protein foams should then be dependent on multiple factors including surface area of the foam, salt content and preferential adsorption of one species over another resulting in differences in surface charge density. Total characterization of all
these parameters is extremely difficult to achieve even in stable systems, let alone transient materials such as foams.

These experiments seek to describe the rheological behavior of protein foams using a method that has been shown to be reliable for this purpose (vane method, Chapter 1). Differences in the rheology of foams made from different protein sources of varying concentration and total energy input will be discussed.

**MATERIALS AND METHODS**

**Egg white and whey protein isolate.** Commercial samples of whey protein isolate (WPI) (BiPro, Davisco Foods International, Inc., LeSueur MN) and spray dried egg white protein (EWP) (P-18-J, Henningsen Foods, White Plains, NY) were obtained and stored at room temperature until used. Protein content of WPI and EWP samples was determined by the Macro-Kjeldahl method (AOAC, 1984) using N factors of 6.38 and 6.25 respectively. Extra fine granulated sugar (Dixie Crystals) was purchased from a local grocery store and stored at room temperature. Sodium fluorescein was purchased from Sigma Chemical Co. (St. Louis, MO).

**Foam Preparation.** Foams were prepared from stock protein solutions using a standard kitchen mixer model (Sunbeam Mixmaster with 1.5 qt. bowl, Sunbeam Appliance Co., Oakbrook, IL) attached to a Staco voltage regulator (Staco Inc., Dayton OH) set at 95% of supplied voltage.

**Yield Stress.** Foam yield stress was measured with a Brookfield model DV-I 25xLVTDV digital viscometer (Brookfield Engineering Laboratories Inc., Stoughton
MA) equipped with a custom-made vane attachment. Four - bladed stainless steel vanes (Accu-tool, Raleigh, NC) were machined from bar stock and adapted to fit the Brookfield viscometer. All vanes had diameters of 10 mm and varied in length from 20 mm to 32 mm. Height to diameter ratios of the vanes used were 2.0, 2.4, 2.8 and 3.2.

**Confocal Microscopy.** Foams were imaged using a Leica DMRBE confocal scanning laser microscope (Heidelberg, Germany) equipped with a Nikon CFN plan fluor 10x objective (Nikon, Inc., New York, NY). The light source was an argon ion laser (Leica Lasertechnik GmbH, Germany) and only the 488 nm emission line was used. The signal from the sample was collected using an FITC filter and eight scans were averaged to produce an image. All images were recorded using the glowoverunder LUT feature provided in the Leica TCS/NT software. This feature assigns false color to over and under saturated pixels (grey scale values of 255 and 0 respectively) to allow visualization of the maximum range of grey scale values without loss of information. Images were analyzed using Adobe Photoshop (Adobe Systems, Inc., Grove City, OH) and the Image Processing Tool Kit (Reindeer Games, Inc. Asheville, NC).

**Procedure.** The formulation used for foam preparation was modified from Cotterill et al. (1992). Stock solutions were prepared on a w/w protein basis with 90 g deionized water and 16.2 g sugar per treatment multiplied by the number of treatments per experiment.

Protein powders were hydrated by adding the powder to approximately 80% of the deionized water at room temperature with gentle stirring for approximately one hour or until all protein was solubilized. Care was taken to minimize foaming during stirring. Once the protein was dispersed, the remaining water and sugar were added and stirring continued for 30 minutes to dissolve the sugar and any protein that remained on the sides
of the container. The solution was held at 4°C over night to allow for maximum hydration of the protein. Solutions were brought to room temperature (23 ± 2°C) the following morning by gentle stirring. All foams were prepared from ambient temperature solutions.

Foams were prepared by whipping 100 ml of stock protein solution for a prescribed period of time at speed 9 (scale = 1 - 12).

Upon completion of whipping, the beaters were removed from the foam as gently as possible to minimize disruption of the foam. Stiffer foams had a tendency to remain attached to the beaters, in which case the foams were removed with a rubber spatula as gently as possible and returned to the bowl.

Yield stress measurements were obtained as quickly as possible after completion of mixing (ca. 1 min). The bowl containing the foam was placed on a scissors stand beneath the viscometer and gently raised until the top of the vane was flush with the top of the foam column. The viscometer was then set to zero and the motor engaged to begin the measurement. The rotational speed of the viscometer was set to 0.3 rpm for all experiments. Three measurements were obtained from fixed points in each bowl of foam and three replications of each treatment were performed.

The total elapsed time necessary to measure all three points within a bowl was approximately three minutes. In order to account for any potential changes in the yield stress of the foam over the measurement period, the order of measurement at the fixed positions was systematically changed with each replication.

The measurement positions within a bowl were arbitrarily fixed relative to the position of the beaters upon completion of whipping. The measurement positions were laid out in a square pattern with the final beater position in the lower right hand corner.
and the three measurement positions occupying the remaining corners and read counterclockwise from the top right corner (Chapter 1, Fig. 2).

Two techniques were used to convert torque readings obtained by vane measurements into yield stress values (Dzuy and Boger, 1983;1985). The first, referred to as the slope method, accounts for the torque imparted to the ends of the vanes by the material. In this method, multiple measurements are taken on a test material with a series of vanes of the same diameter but differing in length. A plot of the maximum torque recorded by the viscometer vs. vane height is constructed. The slope of this line is then used to calculate the yield stress according to:

\[ \sigma_0 = \frac{2a}{\pi d^2} \]

where \( \sigma_0 \) is the yield stress, \( a \) = the slope and \( d \) = the vane diameter. The second method, called the point method, assumes that end effects are negligible and thus requires measurement with only a single vane. In this method, the maximum torque response is recorded for each measurement to be used in calculating the yield stress according to the formula (Steffe, 1992):

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

\[ \text{eq. 2} \]
where \( h \) and \( d \) are the height and diameter of the vane respectively and \( M_0 \) is the maximum torque recorded by the viscometer (Fig. 1).

For confocal microscopy, foams were prepared as above with the inclusion of sodium fluorescein at a concentration of 1 mM in the stock protein solution. After whipping, small aliquots of foam were pulled from random locations within the bowl and loaded into a multi-welled microscope slide. The microscope was then focused 50 \( \mu \text{m} \) into the sample and five fields were recorded from random locations within the sample. Fields were identified with respect to order of measure in order to account for the effects of time on the structure. In all samples, the detection pinhole of the microscope was set to its lowest possible setting to allow for maximum confocality. Yield stress values of samples containing fluorescein were compared to non-fluorescein containing samples. Yield stress was not affected by inclusion of sodium fluorescein in the sample.

Overrun of protein foams was determined by density difference between the foam and the protein dispersion from which it was made. Upon cessation of whipping, foam samples were gently transferred to a tared weigh boat (100 ml) in small aliquots to avoid entrapping air pockets. Excess foam was scraped from the top of the weigh boat with a metal spatula to level the upper surface and allow for consistent volume of foam for each measurement. The weight of the foam was then recorded. Multiple measurements were taken for each bowl of foam utilizing most to nearly all of the foam in each bowl and taken from sequentially deeper locations in the foam column. A minimum of four measurements was obtained from each bowl and treatments were analyzed in duplicate. Six measurements were obtained for each protein dispersion in the same size tared weigh boat. Overrun was calculated by the following equation:
\[
\% \text{ Overrun} = \left\{ \frac{[\text{wt 100 ml dispersion} - \text{wt 100 ml foam}]}{\text{wt 100 ml foam}} \right\} \times 100
\]

RESULTS AND DISCUSSION

Foaming behaviors of the proteins in this study can be evaluated in two ways with respect to their yield stress (\(\tau\)) development. The general trend in \(\tau\) development as a function of protein concentration and whip time is compared without accounting for the effects of time on serial measurements within a treatment. These values will be referred to as mean yield stress (\(\tau_m\)). Values of \(\tau\) which account for elapsed time within a treatment will simply be called yield stress. All values of \(\tau\) reported were determined by the point method of evaluation (previous chapter).

Mean yield stress development of foams was quite different between egg white protein (EWP) and whey protein isolate (WPI) (Fig.1). Foams made from WPI required higher concentrations (10% - 20% w/w) and longer whip times to develop a \(\tau_m\) of sufficient magnitude to be reliably measured by the viscometer used (minimum value of 35 Pa based on the viscometer spring constant). In contrast, EWP foams developed quickly in terms of \(\tau_m\) and at lower concentrations (2% - 10% w/w) than WPI foams. Within a protein concentration, EWP foams increased significantly in \(\tau_m\) over the range of 2 – 6 minute whip times before reaching a plateau (5% & 10%) or beginning a slight descent (2%). WPI foams exhibited little or no significant increase in \(\tau_m\) once a foam of suitable strength to be measured was produced. Increased protein concentration led to increased \(\tau_m\) in all EWP foams, as did an increase in WPI from 10% to 15% (w/w); however, further increase of WPI to 20% (w/w) had little effect on \(\tau_m\). This suggests the
possibility of a saturation effect on $\tau_m$ when high levels of WPI are incorporated into a foam.

In order to account for time effects on the yield stress of the foams, three measurements were taken within each bowl of foam with approximately one minute intervals between measurements. Three replications of each treatment were performed. Mean values were compared on the basis of order of measurement and normalized plots ($\tau/\tau_0$) of yield stress vs. measurement order were constructed ($\tau_0$ = the first yield stress measurement in each bowl). Figure 2 displays the results of these analyses. In 2% and 5% (w/w) EWP foams, no difference in $\tau$ was seen with respect to order of measurement. Foams containing 10% w/w EWP exhibited a decline in $\tau$ (2 – 17%) over the course of three measurements that was whip time dependent. All WPI foams showed declines in $\tau$ with time. These were also whip time dependent as well as concentration dependent. The degree of decline in 10% WPI foams reached a similar maximum to that of 10% EWP foams ($\approx$ 18%) after three measurements, but the whip time dependent range (14 – 18%) was smaller. Foams containing 15% and 20% WPI showed similar extents of decline (37%) and were greater than those of 10% WPI. They differed however in rate of decline. The 15% WPI foams showed a linear rate of $\tau$ decline like lower concentration foams, but the 20% WPI foams declined comparatively quickly between the first and second measurements and less quickly between the second and third measurements.

Overrun of all foams was whip time dependent (Fig. 3). All WPI foams and 10% EWP foams increased in overrun with whip time at approximately the same rate within the whip times studied. No decrease was seen in the rate of overrun of these foams, suggesting that there was ample protein in the continuum to support formation of new
interfacial area and thus incorporate more air into the foam. The rate of overrun increase in 2% EWP foams began to slow after approximately 8 minutes of whipping. This may have been due to a depletion of protein from the continuum as whipping continued, therefore reducing the ability of the protein solution to incorporate air into the foam.

Concentration dependence of foam overrun appears to be inversely proportional to surface tension (Table 1). This makes sense in that decreased surface tension allows for more rapid formation of air cells for a given amount of shear (Dickinson, 1992). As a result, dispersions which exhibit a lower surface tension can incorporate more air per unit of time at a given shear rate. An increase in foam overrun from 2% EWP to 5% and 10% EWP reflects the decrease seen in surface tension between 2% and 5% EWP dispersions. Only one data point is available for 5% EWP overrun, but it is similar to the overrun for 10% EWP with the same whip time, just as the surface tensions of the dispersions are similar. No trend representing concentration effects was seen in the overrun profiles of WPI containing foams, nor was there a difference in surface tension of the dispersions.

No time or positional change in overrun of any foam was seen over the time scale in which overrun was measured (7 – 10 minutes). Overrun was measured by density difference between the foam and the protein dispersion from which it was produced. At least four measurements were obtained for each foam, utilizing most to nearly all of the foam in each bowl. Subsequent measurements were drawn from progressively deeper locations in the foam column, but no trends toward more dense foams were encountered with this progression. Thus no detectable drainage (the standard phenomena studied to determine stability) or gradient in air phase volume was observed over this period. However, as mentioned before, there was a significant whip time and concentration dependent decrease in $\tau$ over the first three minutes or so after foams of higher protein
concentration were produced. This suggests that the change in rheological properties reflects either a considerable sensitivity of the method to local foam stability or a restructuring of the material with time that is unrelated to stability.

Based on studies utilizing very stable oil and water emulsions, Princen (1988) proposes that the yield stress of a foam may be given by:

$$\tau_0 = \frac{\sigma}{R_{32}} \phi \cdot Y(\phi)$$

where $\tau_0$ = yield stress, $\sigma$ = surface tension, $R_{32}$ = the Sauter mean bubble radius ($\approx 3$ times the volume per surface area; $3V/S$), $\phi$ = air phase volume and $Y(\phi)$ is an empirical function of the system being investigated. Thus a change in S/V ratio, surface tension, $\phi$ or $Y(\phi)$ should lead to a change in $\tau$. Coalescence would lead to a decrease in the S/V ratio, which could reduce $\tau$. Zhu and Damodaran (1994) report a decrease of approximately 20% in interfacial area of 5% WPI foams at pH = 7.0 in the first 5 minutes after foaming using a sparging technique to produce the foam. However it is doubtful that this occurs to an extent which would be solely responsible for such a loss of $\tau$ within the time frame investigated at such high protein concentrations. In microscopic observations of our foams over periods of more than 5 minutes, no coalescence was seen and no significant increase in mean bubble size was encountered (Fig. 4). According to Phillips (1981), little or no perceptible change in surface tension occurs in EWP or WPI films, within the time scales investigated here. Therefore the decrease in $\tau$ does not appear to be due to a change in surface tension. It can be assumed that $\phi$ does not
decrease either, and in fact, $\phi$ did not exhibit the effects predicted by this equation in these experiments. Figure 5 shows that 10% EWP foams possessed a 3 fold increase in $\tau_m$ over 10% WPI foams at the same overrun ($= 725\%$) with a lower $\sigma$ and equivalent mean bubble diameter (Fig. 6). Princen and Kiss reported no time dependency in the $Y(\phi)$ correction factor proposed in equation 3, thus it is apparent that while this model may well describe the mechanical behavior of concentrated emulsions, it fails to describe some factor(s) or combination of factors which are important to the rheology of foams.

Historically, one of the primary evaluations of foams has been stability as determined by drainage of liquid from the foam. Drainage results in a gradient in air phase volume, which should result in an increase in $\tau$ that ascends within the foam column according to the above equation. Alternatively, it could be stated that drainage results in a decreasing gradient in air phase volume. It has been shown that, within the time frame investigated here, this gradient in drainage is linear (Kim and Kinsella, 1985; Blecker et al., 1997). In the previous chapter, discrepancies between point and slope methods of $\tau$ evaluation were discussed. It was concluded that anisotropy or a gradient in the factor(s) that determine $\tau$ within the foam may cause these discrepancies. In particular it was hypothesized that a gradient in the $\tau$ determining factor(s) would produce a regular deviation from point method measurements. The trends in $\tau$ development for all WPI foams, as determined by point method analyses, were basically linear with respect to whip time (Fig. 1). Therefore, drainage (stability) in slope method analyses should result in a linear trend with a lesser slope than in point method analyses, since the gradient in $\phi$ decreases. This trend was not seen in the data (Chapter 1, Fig. 4). In fact no regular trends were seen in slope method measurements of WPI foams as
compared to the point method values. The lack of a trend in either the slope method evaluations of foam rheology or serial measurements of overrun within a bowl of foam suggest that stability, in terms of drainage and/or collapse, is not responsible for the decreasing yield stress with time in high concentration foams. However, there may be a restructuring of the material that is responsible for the decrease in yield stress with time.

Phase volumes of air as determined by conventional mass measurement were between 85 and 89% (Fig. 3). Air phase volume fractions determined by image analysis from confocal scanning laser microscopy (CSLM) images however were between 57 and 75% (Fig. 7). The discrepancy in these values could be due to two factors. During mass measurement, small air pockets were unavoidably introduced into the weigh boats upon transfer of foam, meaning that these values are over estimations of the true $\phi$. On the other hand, the lack of small bubbles seen in microscopic evaluation suggests that some of the bubbles in the foam may not have been accounted for by CSLM. According to the formula: $R = 0.4\lambda / N.A.$ commonly employed to calculate lateral resolution in confocal microscopy, a minimum bubble diameter of 0.69 $\mu$m, corresponding to an area of 0.37 $\mu$m$^2$ should have been resolvable ($\lambda = 520$ nm, N.A. = 0.3). In fact, the smallest bubble profile encountered was 1.8 $\mu$m$^2$ and in most of the images analyzed the smallest profile encountered was greater than 6.0 $\mu$m$^2$. This is likely due to the fact that smaller bubbles are less likely to be sectioned by the microscope than larger ones. Thus, the mean diameter of bubbles as determined by analysis of these images may or may not be a true mean of bubble sizes. However, small bubbles account for a small amount of the total volume. In addition, bubble profiles in the CSLM images were quite circular, suggesting that maximum packing volume had not been met (Fig. 8). This is an indication that $\phi$ as
determined by CSLM is a more reliable measure than that made by conventional mass measurement.

All foams in this study were produced from pH 7.0 dispersions. At this pH, all WPI proteins and all but one of the EWP proteins exhibit a net negative charge. Lysozyme is net positive at this pH. As protein concentration is increased, the net chemical potential of the system increases due to increased charge density. This potential could be the driving force behind a redistribution of the air cells in the material. In the case of EWP foams, lysozyme may be stabilizing the foam by suppressing the surface charge of the bubble through electrostatic interaction with adsorbed proteins (MacDonnell, 1955).

It is not hard to visualize an order within the three dimensional structure of the foam that comes about as a result of whipping. Smaller bubbles can become entrained in the wake of the beaters to the exclusion of larger ones. Thus, maximum close packing of the bubbles is not achieved instantly. Restructuring of the system following cessation of whipping would allow minimization of the net electrostatic repulsion between air cells. This could account for the decrease in $\tau$ with time in several ways. The net repulsive potential contributes to the resistance to applied stress and therefore to the yield stress. As this potential decreases, so does the resistance. In addition, any movement or flow that occurs within the system as a result of restructuring, is kinetic energy that does not have to be imparted by the rheometer to initiate flow. Khan and Armstrong (1986) and Kraynik and Hansen (1986) found that yield stress is sensitive to the orientation of a unit cell relative to the direction of shear. Accordingly, any order in the structure would result in increased yield stress that would be diminished as the structure approaches randomness.
Like the concentration dependence of the rate of decrease in $\tau$ with time, the whip time dependence may also be the result of restructuring. Adsorption of the protein to the air – water interface favors the reconfiguration of the peptide chain such that non-polar side chains are oriented toward the air phase and polar / charged groups are preferentially oriented toward the water phase. This results in an air cell with a negatively charged surface at this pH. Increasing whip time increased overrun but did not appreciably increase the detectable mean bubble size indicating that there was increased total surface area within the foam (Fig. 6). The decreasing trend in $\phi$ with whip time of 15% WPI foams determined by microscopy, in contrast to the increasing trend in $\phi$ from mass measurement, along with decreasing mean detectable bubble size, suggests that quite a lot of surface area is created in the form of very small bubbles at higher protein concentrations. Greater total charged surface area means greater net potential and thus greater driving force for restructuring.

The Princen model (equation 3) is based on data obtained from measurements of oil in water emulsions that are analogous to hard sphere systems. According to their results, $Y(\phi)$ is an empirically derived, logarithmically increasing function of $\phi$ at $\phi$ greater than the maximum packing volume (0.7405) where $\phi$ is the phase volume of oil. In contrast, the discontinuous phase in whipped protein foams is compressible air and is not analogous to hard sphere systems. Thus the term $Y(\phi)$ in equation 3 remains in question in terms of its contribution to the yield stress of a protein foam.

All other terms in equation 3 have been in contradiction to the results seen in this study. EWP foams exhibit a lower surface tension and equivalent or smaller S/V ratio (as determined by mean detectable bubble diameter) than WPI foams and yet the $\tau_m$ is
greater for EWP foams. Similarly, overruns of as much as 800% in 10% WPI foams exhibited less than 2/3 of the yield stress exhibited by 10% EWP foams with 640% overrun and a lower surface tension. This could be the result of unaccounted for variables, such as Coulombic effects or air cell compressibility, which might have a more pronounced effect on foam mechanical properties than the variables presented in the model. The transient nature of foams (drainage, coalescence, disproportionation, collapse, restructuring) in combination with these unknown factors may further distort the relationship of protein foam mechanical behavior to the Princen model.

Further work is needed to more accurately quantify air cell size distributions and electrostatic contributions to the rheological properties of protein foams. Microscopic analyses at varying levels of magnification concentration could be conducted to more precisely characterize the bubble size distributions of foams. Quantification of the surface and bulk electrical potential of proteins at varying pH compared to surface tension values may yield insight into the ways bubbles might deform and restructure themselves to minimize net system potential. Comparison of the rheological properties of protein foams to those of similar foams produced with neutral surfactants could also yield valuable information.

One note of caution must be mentioned with respect to stereological evaluation and confocal imaging of foams. Due to the difference in refractive index of the air and aqueous phases of the system, three-dimensional reconstructions (z-series) of multiple sections of foams will be distorted from their true shape. The greater the difference in refractive index between the phases, the greater the distortion. Reed et al (1997) investigated the coarsening of soap foams with confocal microscopy, 3-D reconstruction and second-order stereological procedures. Although they concluded that these methods
were valid, they also stated that the methods were in need of further refinement. One of these refinements is an algorithm that accounts for the geometric changes of the foam in the vertical direction due to refractive index difference. Since this study was primarily concerned with relative difference in bubble size as opposed to absolute bubble size, we avoided three-dimensional evaluation of the foams in an effort to minimize the complexity of the study.
REFERENCES


<table>
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<tr>
<th>Protein type</th>
<th>Concentration (wt. %)</th>
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* surface tension and viscosity of foaming solutions ± standard deviations
% Overrun of foams

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* determined by mass measurement ± standard deviations
Figure 1. Yield stress vs. whipping time profiles of: 2% EWP (●), 5% EWP (■) and 10% EWP (▲) foams and 10% WPI (○), 15% WPI (□) and 20% WPI (△). Error bars represent standard deviations of three replications.
Figure 2. Time dependence of yield stress of foams varying in whip time. (A) 2% EWP (B) 5% EWP (C) 10% EWP (D) 10% WPI (E) 15% WPI (F) 20% WPI

Whip times: EWP (◆) 2 min (■) 4 min (●) 6 min (▲) 8 min (✧) 10 min
(□) 12 min (△) 14 min; WPI (◆) 15 min (■) 20 min (▲) 25 min (●) 30 min
Figure 3. Air phase volume of foams determined by mass measurement. (♦) 2% EWP
(■) 5% EWP, (▲) 10% EWP, (◇) 10% WPI, (□) 15% WPI, (△) 20% WPI.
Error bars represent standard deviations of three replications.
Figure 4. Mean detectable bubble size vs. time (●) 5% EWP, 4 minute whip time (■)
15% WPI, 20 minute whip time. Error bars represent standard deviation of at least 3 measurements.
Figure 5. Yield stress vs. air phase volume by mass measurement. (♦) 2% EWP (■)
10% EWP (□) 10% WPI (♂) 15% WPI (○) 20% WPI
Figure 6. Mean detectable bubble size. (■) 5% EWP (□) 10% EWP (●) 10% WPI (○) 15% WPI. Error bars represent standard deviations of at least three measurements.
Figure 7. Air phase volume as determined by image analysis. (■) 5% EWP (□) 10% EWP (●) 10% WPI (○) 15% WPI
Figure 8. CSLM images of 10% EWP foam (top) and 10% WPI foam (bottom)
Comparison of Bulk Physical Properties of Angel Food Cakes Containing Egg White Protein or Whey Protein Isolate

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The baking performance of angel food cakes produced from foams that varied in
whip time, protein type and / or concentration was compared. Cakes were evaluated
based on final volume, dynamic volume change, rheological changes during baking,
thermodynamic transitions during baking and microstructural development during
baking. Whip time in formation of the foam had no effect on final cake volume or
maximum expansion. Maximum expansion was found to be a function of protein
concentration regardless of protein type. Egg white protein exhibited a minimum critical
protein concentration necessary for satisfactory cake production. Whey protein isolate
was unable to prevent cake collapse regardless of concentration. The ability of egg white
protein to form suitable cakes in contrast to whey protein isolate appeared to be the result
of biopolymer incompatibility between egg white protein and soluble starch. This
condition was not present to a significant degree in whey protein formulations.
INTRODUCTION

Concentrated protein is used as an ingredient in a large number of foods to improve or control the textural properties. A large variety of protein ingredients are available to the food manufacturer, each with optimum conditions for use and a range of functional properties. Investigation of the functional properties of proteins and the factors which influence them is necessary for modeling food systems such that processes may be optimized, novel products may be made or cost saving substitutions with other ingredients may be employed in product manufacture (Zasypkin et al., 1997). Angel food cake is an excellent model system for study of protein functionality because it is prepared in a stepwise manner, contains relatively few ingredients, relies on no chemical reactions other than those directly influencing the protein (i.e. no leavening) and is completely dependent on the functional properties of its protein constituents.

A high quality cake is produced when the dynamic events occurring in the system yield a high volume, low-density cake with fine, relatively uniform crumb structure. The observed rise and fall of angel food cakes during cooking gives an indication of the relative importance of constructive and destructive events occurring in the material with time and temperature. During cake rise, constructive events (individual bubble expansion) should dominate over destructive events (coalescence, disproportionation). Once a maximum cake height is reached, the cake can either fall, in which case destructive mechanisms dominate over constructive events, or the volume can be maintained by formation of a matrix which “sets” the cake. The chemical interactions that occur within the system dictate which of these paths will be followed.
Protein participation is vital to the setting behavior but it is not solely responsible as starch is also involved (Hoseney, 1986). It has been postulated that the difference between EWP and other protein sources in forming cakes is related to synchrony in thermal behavior of the EWP and starch (i.e. protein denaturation and starch gelatinization) that isn’t present in other protein / starch combinations (Raeker & Johnson; 1995a). Thermal analyses (differential scanning calorimetry) have been conducted which support this idea (Raeker & Johnson, 1995b). Difficulties arise in interpreting these results in that they are typically carried out in systems that are not representative of true cake batters. Protein concentrations are usually in the range of 0.1% to 3% whereas cake batters typically contain 5% protein or more. In addition, complex systems that include high concentrations of carbohydrate of various forms (i.e. cake batters) are not typically analyzed by thermal means such as DSC. High levels of sugar, for example, have been shown to have dramatic effects on the thermal properties of proteins in solution (Back et al., 1979; Arakawa and Timasheff, 1982). Thus the response of proteins to baking within the cake batter environment is still uncharacterized.

Baking causes conversion of cake batter from a relatively dense, metastable liquid foam to a light, stable solid foam. The specific events that occur during this conversion are not fully understood. Egg white protein is apparently better suited to this process than other protein sources but it is unclear why this is the case. Many studies have been conducted in an effort to determine the molecular contributions of proteins to cake production and / or foaming properties including chemical treatments of protein from various sources (Cotterill et al., 1992; Phillips et al., 1990; To et al., 1985), fractionation of egg white (MacDonnell et al., 1955; Raeker and Johnson, 1995a; Johnson and Zabik 1981a & b), replacement of egg white by other ingredients (Arunepanlop et al., 1996;
Khan et al., 1979; Raeker and Johnson, 1995b; Gaines and Donelson, 1985), replacement or addition of non-protein components in cake formulations (Pateras et al., 1994) and differences in mechanical processing during batter production (Phillips and Kinsella, 1990; MacDonnell et al., 1955). Individual proteins have been evaluated in terms of their thermal behavior (enthalpy and temperature of denaturation) in an effort to establish time-temperature relationships that may be of importance in cake formation (Back et al., 1979; DeWit and Klarenbeek, 1983; Raeker and Johnson, 1990 a & b). These studies have yielded information on the role of some of the individual components in a batter formulation, however these results may or may not be applicable to actual cake preparation when all ingredients are included at their customary concentrations. What is lacking is information on bulk behavior during cake making which may describe the dynamic changes in the system, allowing for more complete analysis of the role of proteins in making high quality cakes. In this report, the bulk behaviors occurring in angel food cake production are studied by various techniques in an effort to distinguish differences in performance of egg white protein and whey protein isolate.

**MATERIALS AND METHODS**

**Egg white and whey protein isolate.** Commercial samples of whey protein isolate (WPI) (BiPro, Davisco Foods International, Inc., LeSueur MN) and spray dried egg white protein (EWP) (P-18-J, Henningsen Foods, White Plains, NY) were obtained and stored at room temperature until use. Extra fine granulated sugar and 10X powdered
sugar (Dixie Crystals) and bleached cake flour (Swan’s Down) were purchased from a local grocery store and stored at room temperature.

**Foam Preparation.** Foams were prepared from stock protein solutions using a standard household type mixer (Sunbeam Mixmaster with 1.5 qt. bowl, Sunbeam Appliance Co., Oakbrook, IL) attached to a Staco voltage regulator (Staco Inc., Dayton OH) set at 95% of supplied voltage.

The formulation used for foam preparation was modified from Cotterill et al. (1992). Stock protein solutions were prepared on a w/w protein basis with 90 g deionized water and 16.2 g extra fine granulated sugar per treatment. Egg white protein foams were produced in 2, 5 and 10% (w/w) protein dispersions and whey protein isolate foams were produced in 10, 15 and 20% (w/w) protein dispersions. Flavoring and salt were omitted from the formulation to simplify the model.

Protein powders were dispersed by adding the powder to approximately 80% of the deionized water at room temperature with gentle stirring for ca. 1 hr or until all protein was dispersed. Care was taken to minimize foaming during stirring. Once the protein was dispersed, the remaining water and sugar were added and stirring continued for 30 min to dissolve the sugar and any protein that remained on the sides of the container. The solution was then held at 4°C over night to allow for maximum hydration. Solutions were brought to room temperature the following morning by gentle stirring at ambient temperature and adjusted to pH = 7.0 with 1N NaOH or HCl. All foams were prepared from ambient temperature solutions.

Foams were prepared by mixing 100 ml of protein solution at a standard speed (speed 9, range = 1-12) for a prescribed period of time. Upon completion of whipping, the beaters were removed from the foam as gently as possible to minimize disruption of
the foam. Stiffer foams had a tendency to remain attached to the beaters, in which case
the foams were removed from the beaters with a rubber spatula as gently as possible and
returned to the bowl.

Cake Preparation. Cake batters were prepared by first blending 33.0 g cake
flour and 75.8 g 10X powdered sugar and sifting three times. The flour / sugar blend was
then gently folded into the foam in three installments using a total of approximately 30
strokes. The batter was placed in a baker’s pastry bag with a large orifice, round tip for
transfer into the baking vessel (600 ml berzelius beaker). This prevented inclusion of
large air pockets and contact with the sides of the container. The batter (50 g) was
pumped into a beaker and lightly tapped on a countertop to level the upper surface. The
beaker was then placed in a conventional oven pre-heated to 204°C and baked for 15
minutes. Upon completion of baking the beakers were removed from the oven and
inverted on a wire rack to cool at room temperature for 30 minutes. Final cake volumes
were obtained by rapeseed displacement.

Cake Expansion. Changes in cake height as a function of time were recorded
using a cathetometer (5100 series cathetometer, Eberbach Labtools, Ann Arbor, MI).
This device employs a telescope with a single reticle that is attached to a graduated
column by a vernier. Set-up of the instrument required that the graduated column be
vertically true and the telescope be perfectly level. Operation simply involved fixing the
reticle on the upper surface of the batter and raising or lowering the scope to maintain the
reticles position on the surface during baking. Changes in height are read from the
graduated column.

Immediately upon placing a beaker in the oven, the reticle of the cathetometer
was fixed on the upper surface of the batter and the height recorded. Measurements were
taken of surface height at one minute intervals. Normalized profiles of change in cake height with cooking time were constructed for comparison of treatments.

Temperature changes within the batter during baking were recorded at the wall of the beaker and in the center of the batter/cake using 24 gauge, type T thermocouples and Advantech data acquisition software and hardware (American Advantech, Sunnyvale, CA).

**Rheological measurement during baking.** Dynamic rheological data of cake batters during cooking were obtained using a controlled stress rheometer (Stresstech, Reologica Instruments AB, Lund Sweden) with a vane attachment.

Batters were prepared exactly as above and transferred to the cup (diameter = 27.5 mm) of the rheometer via a pastry bag. A four-bladed vane (37mm x 13.5 mm; height : diameter ratio = 2.75) attached to the rheometer was slowly lowered into the batter. The level of batter was then increased to be flush with the top of the vane.

A frequency sweep of 0.1-10.0 Hz and a stress sweep of 0.5 – 1.0 Pa were conducted in oscillatory mode to establish the linear viscoelastic region (LVR) of the material. A frequency of 0.5 Hz at 0.8 Pa was used for all treatments.

A temperature ramp from 34°C to 150°C at 8.5°C per minute was programmed into the rheometer to match the temperature profile recorded by the thermocouple at the wall of the beaker in the cake expansion experiments.

**Microscopy.** Hot stage microscopy was accomplished using a Nikon Optiphot 2 microscope equipped with a Nikon 10x CFW plan phase objective (Nikon Inc., Melville, NY) and Hoffman long working distance condenser with 10x phase annulus (Modulation Optics Inc., Greenvale, NY). The hot stage used was a Linkam THMS 600 controlled by a Linkam TC 92 controller (Linkam Scientific Instruments Ltd., Surrey, England).
Bright field and fluorescence microscopy were performed on a Zeiss Axiophot microscope (Carl Zeiss, GmbH, Jena, Germany) using a xenon arc lamp and DAPI filter cube and equipped with a Hamamatsu Chalnicon black and white camera (Hamamtsu Photonics, K.K., Japan). Image capture was accomplished using Image One software (Universal Imaging, West Chester, PA).

Cake batter formulations were prepared as above with the exception that protein solutions were not whipped into a foam prior to incorporation of powdered sugar and cake flour. A 10 µl aliquot of the liquid batter was sandwiched between two glass cover slips and heated at approximately 80°C per minute while simultaneously being visually observed and recorded on videotape. Phase contrast microscopy was used to observe / record physical changes in the structure of the material during simulated baking. Bright field and fluorescence microscopy were used to analyze the resultant structure of the material after cooking.

**Differential scanning calorimetry.** Differential scanning calorimetry was conducted using a Perkin Elmer DSC 7 equipped with an intracooler II refrigeration unit and dry box (Perkin Elmer Corp., Norwalk, CT). Nitrogen gas at a flow rate of 40 mL/min was used to flush the sample holder and nitrogen gas at 25 psig was used to flush the dry box. The DSC was calibrated with indium (temperature and enthalpy) and dodecane (temperature).

The onset and peak temperatures for heat capacity transitions were recorded for formulations at various stages of completion in the preparation of cake batter. For each treatment of protein type / concentration studied, thermal analysis was conducted on three sub-treatments, i.e. (1) protein and water (2) protein, water and sugar formulation used in
foam preparation and (3) complete cake batter formulation including all sugar and cake flour. A dispersion of sugar and cake flour (no protein) was analyzed as a control.

Samples (≈ 60 µl) were loaded into the manufacturers large volume stainless steel pans (70 µl capacity) and heated from 20°C to 120°C at a rate of 5°C per minute using an empty pan as a reference. Reference pans and empty sample pans were balanced to within 0.2 mg to minimize error due to differences in thermal mass. Duplicate runs were conducted for each sub-treatment and results expressed as the average value of the two.

**RESULTS**

The cakes produced from 10% (w/w of foam) EWP exhibited the greatest final cake volume of all treatments (Fig. 1). This formulation represented satisfactory angel food cakes and was equivalent to the egg white protein concentration found in fresh egg white. Cakes made from lower concentrations of EWP (2% and 5% w/w of foam) and from 10% of foam (w/w) WPI batters exhibited no substantial net volume change in converting batter to cake. The sticking behavior of the two higher concentration WPI containing cakes led to unreliable final cake volume measurements.

Regardless of protein type or concentration, the whip time used to produce the foam in the first stage of batter production had no effect on final cake volume.

The general pattern of cake volume change with cooking time was similar for all treatments up to the maximum expansion point (Fig. 2). The value of the peak height at maximum expansion increased as protein concentration increased and was independent of the whip time to which the foam used in the batter was subjected. Only the highest EWP containing cake (10% w/w of foam) was able to maintain a sufficient volume and symmetry. All other treatments either collapsed to an insufficient final volume or shrank...
to an irregular shape as a result of sticking to the container during baking. The sticking behavior observed in cakes containing a high concentration of WPI occurred upon crust formation at or near the maximum expansion point and prevented the unrestrained collapse of the cake. As a result, the true tendency for these cakes to collapse or set up can only be inferred by the initial rates of collapse following expansion seen in Figure 2 and comparing these rates to the rates of collapse of other treatments which were not inhibited.

The maximum expansion in all treatments occurred at 7 – 9 min of baking, regardless of protein type or concentration.

The most striking difference in the rheological development of EWP and WPI containing cakes was the change in phase angle that occurred during baking. The phase angles of both EWP and WPI containing batters of all concentrations were virtually identical over the temperature range 35 - 60°C (Fig. 3). Both were well within the predominately elastic regime and the curves are basically flat. A divergence occurred at approximately 60°C whereby EWP containing batters tended toward a more elastic structure (i.e. decreasing phase angle) and WPI containing batters moved rapidly toward a more fluid character. At a point that corresponds with maximum expansion and peak heat capacity transitions obtained by thermal analysis, there was an abrupt reversal in phase angle tendencies in WPI containing batters. Eventually the phase angles of all treatments converged to display the expected high degree of elastic behavior.

Similar trends in storage modulus (G’) and loss modulus (G”) development were observed during baking of all cakes (Figs. 4 & 5). During the heating stage that corresponded to expansion of the cakes, both G’ and G” decreased as temperature increased. Near the point of maximum expansion, both of these properties began to
increase. In the case of WPI containing cakes, maximum expansion of the cake occurred immediately prior to or concurrent with the inflection point in the curves of $G'$ and $G''$. EWP containing cakes tended to obtain maximum volume slightly past the inflection point of the curves, into the region of increasing $G'$ and $G''$. Cake formulations which collapsed on baking experienced an increase in $G'$ up to a level between 200 and 400 Pa upon batter to cake conversion, whereas the high concentration EWP containing cake (which maintains a satisfactory volume) displayed a $G'$ surpassing 700 Pa after setting. Likewise, $G''$ increased to approximately 50 Pa in formulations which collapsed and the high concentration EWP cakes showed a 40% increase of $G''$ up to roughly 70 Pa.

Four methods of light microscopy were employed in evaluating the physical changes that occur in various stages of cake batter upon heating.

Polarized light microscopy was used to ascertain the loss of birefringence in the starch present in cake batters. It was determined that birefringence was lost between 90 – 93°C in all formulations including a control which incorporated no protein in the formulation (data not shown). Some birefringence was maintained near the periphery of the samples where rapid drying prior to gelatinization probably occurred, but within the interior of the sample little or no birefringence was seen in any treatment following heating.

Structural changes that occurred upon heating various formulations were observed in phase contrast (Fig. 6). WPI foaming solutions (no cake flour or powdered sugar) exhibited no visible structural development throughout the heating process. EWP foaming solutions produced opaque gels of a particulate nature. Gelatinization was clearly evident in all formulations that incorporated cake flour. Upon gelatinization, a
colorless dispersion of starch granules simply faded away as an intricate network of blue fibers developed. This phenomenon was the dominant visible event in all treatments that involved cake flour but there were less prevalent details that differed with protein type and concentration in formulations that contained protein. In samples containing no protein, the blue fibrous network formed within a uniform background of mid-grey tone. Treatments containing WPI exhibited the same blue network but the background was brighter, indicating the presence of protein in the continuum. The intensity of the background increased as WPI concentration increased. The background in EWP containing formulations was noticeably different. This background resembled the bright background in WPI containing samples indicating a proteinaceous nature; however, the protein in these samples clearly formed a separate network with the background in the void spaces exhibiting the same mid-grey tones present in the non-protein containing samples (Figs. 7 & 9). As EWP concentration increased, the intensity of the protein phase increased, as did the degree of separation of the protein network from the gelatinized starch network.

Comparison of the bright field and fluorescence images obtained from all cooked samples supports the observations made in phase contrast evaluation of the samples (Figs. 7 & 8). Bright field images of the EWP and WPI foaming treatments (no cake flour) showed the presence of a particulate structure in the EWP sample and no visible structure in the WPI sample. Areas of brightness in the fluorescence images of the same fields of view are the result of autofluorescence of the protein in the system. No fluorescence was seen in the control (no protein) samples. As can be seen, the EWP foaming treatment exhibits a particulate structure in contrast to the uniform illumination seen in the WPI foaming treatment.
In all samples that contained cake flour, a more intricate structure was observed. Differences between the bright field images of WPI and EWP containing samples were readily apparent. The continuum in the WPI containing samples is quite intricate in structure yet uniform on a relatively large scale, very much like the phase contrast images of the batters that contain no protein. By comparison, the EWP containing samples exhibited a secondary porous network with large void spaces filled by the intricate network described above. Fluorescence images of these samples revealed the network to be proteinaceous, whereas the protein displayed in the fluorescence images of WPI containing samples is shown to be concentrated primarily at the interface of entrapped air bubbles and otherwise distributed somewhat evenly throughout the sample.

Results of thermal analyses of treatments containing EWP agree with the findings of Raeker and Johnson (1995a) and Donovan (1977). The major peak in the thermograms occurred at roughly 81°C and is attributable to denaturation of ovalbumin. Protein dispersions containing sugar (i.e. foaming solutions) resulted in an increase of about 3°C for all peaks. Cake batter samples exhibited only one large peak at approximately 92°C which was very near the peak temperature seen in the control sample of cake flour and sugar without protein.

Samples containing WPI showed similar increases between protein and water samples and foaming solution samples with respect to major peak temperatures though the temperatures were about 7°C lower than in EWP samples (Table 1). In these samples, only one peak was observed near 74°C but it contained a slight shoulder on the low temperature side near 66°C. These values are similar to those reported by De Wit and Klarenbeek (1983) for β-lactoglobulin and α-lactalbumin respectively. As in EWP containing samples, addition of sugar increased peak temperature and this shoulder
approximately 3°C. Protein concentration had no effect on the peak temperatures seen in these two treatments but higher concentration increased the onset temperature by 4–5°C. Also, as in EWP containing samples, these peaks were not present in the cake batter samples or were masked by the lone peak that presumably represents starch gelatinization.

**DISCUSSION**

Cake rise in angel food cakes can only be a result of bubble expansion because they contain no leavening agent. Thus, either the protein films surrounding the individual air cells must expand as volume increases or more protein must be incorporated into the interface via diffusion/adsorption. The fact that, in all cakes, maximum cake expansion increased as protein concentration increased, suggests that the protein remaining in the continuous phase adsorbed at newly created interface allowing expansion to occur. In so doing, protein was depleted from the continuous phase as expansion increased. This has consequences with respect to the volumetric behavior of the cakes.

The results for cakes containing EWP suggest a critical concentration of EWP necessary to produce a stable solid foam (Fig. 1). While 2% and 5% (w/w of foam) EWP formulations were incapable of supporting the expanded volume, the 10% (w/w of foam) EWP formulation produced high cake volumes. Cakes containing WPI on the other hand, though able to expand to a higher degree than EWP formulations, were incapable of producing a matrix that could maintain a suitable cake volume even at double the concentration of EWP. Attempts to substitute other proteins for egg white proteins in angel food cake production have shown that a maximum substitution rate of approximately 30% is feasible before cake quality is too adversely effected (Khan, 1979;
Arunepanlop, 1996). Cake quality in these evaluations was described in terms of several variables, including volume, color, symmetry, crumb structure and overall acceptability as determined by sensory evaluation. Though cakes were deemed acceptable with up to 30% egg white replacement when evaluated on cake volume alone, all substitutions produced significantly less voluminous cakes. It can be hypothesized that substitutions of egg white protein effectively dilute the egg white protein concentration to less than the critical concentration necessary for producing an acceptable cake. The critical concentration must lie somewhere between 5% and 10% EWP in the initial foam and is dependent upon a subjective assignment of minimum acceptable density of a cake. That an apparent critical concentration of EWP exists that cannot be achieved by blending EWP and other protein ingredients suggests mechanistic differences between EWP and other proteins in forming a suitable angel food cake.

Protein type and protein concentration both had significant effects on the performance of the cake batters during baking. The whip time that the foam was subjected to prior to batter formation did not have an effect on either final cake volume (Fig. 1) or dynamic performance (Fig. 2). This is in contrast to the findings of MacDonnell et al. (1955) who reported reduced cake volumes due to over whipping. The whip times studied in this experiment were significantly longer than those used by MacDonnell et al. (1955) but were similar to those used by many other researchers for evaluation of foaming properties of various protein ingredients (Arunepanlop et al., 1996; Phillips et al., 1990). Even the longest whip times reported by MacDonnell et al. (1955) were not sufficient to convert all of the liquid to foam in this experiment using the mixer and speed setting employed here.
Bulk rheological behavior between protein types was similar in many respects. The trends in storage modulus ($G'$) and loss modulus ($G''$) followed the same general pattern in cakes containing both EWP and WPI. As the temperature in the batter increased, both $G'$ and $G''$ decreased (Figs. 4 & 5). Upon setting a minimum was reached in both curves followed by an increase toward more solid characteristics. Hoseney (1986) described setting as the point at which the cake can no longer expand due to great increases in viscosity as a result of starch gelatinization. The time / temperature at which this minimum occurs and its relationship to maximum cake height provide insight into the bulk behaviors that occur in these batters upon baking. Consideration of the factors which effect $G'$ will assist in interpreting the relationship of this property to the bulk behavior observed in producing cakes.

The phase angle ($\delta$) development between cakes containing WPI or EWP is very different (Fig. 3). Phase angle is an indication of the material’s tendency toward more elastic or more fluid behavior. A $\delta$ of less than 45° indicates that elastic forces dominate over fluid forces; above 45° fluid forces are more prevalent. In all treatments the cakes exhibited largely elastic behavior, however the cakes containing WPI showed a strong trend toward more fluid behavior as baking progressed up to the point of setting. The viscous component of the batters is expressed as $G''$. The decrease in $G''$ with temperature was similar for all treatments. This is expected behavior in that most fluids decrease in viscosity as temperature increases. The fact that $G''$ was very similar in both EWP and WPI containing batters, regardless of concentration, suggests that, up to the point of setting, protein has little effect on the viscosity. The shear modulus (G) of foams may be given by the equation:
\[ G = \frac{\sigma}{R_{32}} \phi^{1/3} E(\phi) \]  

eq. 3

where \( G \) is the shear modulus, \( \sigma \) is the surface tension at the air / water interface, \( \phi \) is the air phase volume, \( E(\phi) \) is a constant related to air phase volume and \( R_{32} \) is the Sauter mean radius of the bubble which is approximately \( 3V/S \) or 3 times the volume per surface area (Princen, 1989). \( G' \) is related to \( G \) according to the relationship: \( G' = G \cos \delta \). For purposes of evaluating these results, it is reasonable to substitute \( G' \) for \( G \) in equation 1. According to this relationship, a decrease in \( G' \) should reflect a decrease in surface tension or surface area per unit volume. Assuming spherical bubble shape is maintained during expansion and no destabilizing events occur (coalescence or disproportionation), the rate of \( S/V \) changes according to: \( S = 4.836(V)^{0.6667} \). Thus the \( S/V \) ratio is ever decreasing and \( G' \) would be expected to decrease accordingly. This is observed in the trends in \( G' \) for the batters of both protein types but the effect is much more pronounced in WPI containing cakes. The similarities in \( G'' \) and notable differences in degree of change of \( G' \) with temperature result in the differences seen in phase angle development of these materials. This alludes to the increased occurrence of destabilizing events in the WPI batters as a function of cooking. The effect of coalescence / disproportionation
would be to further slow the rate of surface area growth per unit growth in volume. Such occurrences would lead to a greater decrease in $G'$ as compared to batters which were more stable to coalescence or disproportionation. Further evidence in support of this hypothesis is the subjective observation of the crumb structure of both types of cakes in comparison with microscopical evaluation of the foams from which the batters were produced. Mean bubble size in foams containing WPI was smaller than that of EWP containing foams (Chapter 2). WPI containing cakes however exhibited a coarser crumb than those made from EWP foams. The similarities in $G''$ and notable differences in degree of change of $G'$ with temperature point to the conclusion that the phase angle differences seen in the rheological profiles of the batters prior to setting are the result of EWP containing batter’s ability to greater withstand destabilizing events such as coalescence during baking.

Another difference in the rheological behavior of these systems was the magnitude of elasticity occurring after setting. Cakes containing 10% EWP (w/w of foaming dispersion) showed a rapid increase in $G'$, following setting of the batter, up to a level nearly double that of the other formulations. This indicates the formation of a more elastic matrix within the system. Presumably this matrix provides stability that allows for the maintenance of high volume in the cake upon setting.

Comparison of the rheological and baking performance behaviors of cakes containing low and high concentrations of EWP revealed that the inflection point in the $G'$ curve occurred well before the maximum expansion point in low concentration cakes, whereas in cakes with a high concentration of EWP, this point coincided with maximum expansion (Fig. 4). In addition, the rate of change of the curve was gradual in low
concentration EWP cakes and quite immediate in cakes of high EWP concentration. Both cakes reached a maximum volume at the same time during baking. Maximum expansion of low concentration EWP cakes nearly approached that of high concentration EWP cakes at half the protein concentration (Fig. 2). Thus, if the rates of bubble growth and coalescence were similar between the two materials, it follows that the bulk concentration of protein in the low concentration cakes was reduced to a much greater extent than that of the high concentration cakes. If the concentration goes too low, it can be concluded that the adsorption rate may decrease relative to the rate of surface area increase. This would have the effect of decreasing the net surface excess concentration of adsorbed protein, resulting in an increase in surface tension while still allowing for expansion.

An increase in $G'$ could be attributable to protein gel formation, however the low concentration of protein in the continuum and lack of any apparent endothermic event occurring at this temperature in DSC analysis suggests this may not be the case. According to equation 1, an increase in surface tension results in an increase $G'$. It is in this way that $G'$ can begin to increase prior to maximum expansion of the cake. In contrast, high concentration EWP cakes contain enough bulk protein to maintain a low surface tension via maintenance of a sufficient excess surface concentration, therefore $G'$ in these cakes decreases with expansion as described above until an abrupt change is encountered upon setting. The relatively large amount of protein remaining in the continuous phase at this point is able to form an elastic network, resulting in the rapid increase in $G'$ after setting. The implication is that while low concentration EWP cakes are able to expand to a similar volume as high concentration cakes, the bulk protein
concentration is reduced to a level that cannot form a matrix of sufficient strength to support the cake volume, and thus they collapse. This is in agreement with the findings of MacDonnell et al. (1955) who suggested that it is a suitable concentration of “heat-denaturable bulk” which is necessary to form a matrix capable of supporting cake volume. These authors report that this “bulk” protein is primarily ovalbumin.

Thermal analysis revealed only one peak occurring in all cake batter samples. This endothermic peak corresponded roughly with the peak temperature observed in the control (no protein) sample. However, endothermic peaks at lower temperatures, matching those provided in the literature for both protein types, were seen in samples that contained only protein and water. Similar profiles with slightly higher values were observed in foaming solution samples. This suggests a stabilizing effect of the carbohydrate on the protein in the system. Apparently, within a cake batter, protein denaturation and starch gelatinization occur roughly simultaneously. This is important because it allows the elimination of the theory that the difference in functional properties of EWP and WPI in cake making result from differences in synchrony between protein gelation and starch gelatinization. Back et al. (1979) studied the effects of added sugars and polyols on the denaturation temperatures of several fractions of egg white protein. They reported that a 50% (w/w) sucrose solution increased the temperature of denaturation of conalbumin, ovalbumin and lysozyme by 9, 11 and 14°C respectively. The presence of 28% (w/w) sucrose increased the denaturation temperature of ovalbumin by 4°C. Similar effects were observed in the present study, which used similar concentrations of carbohydrates (Table 2). Arakawa and Timasheff (1982) discussed the ability of sugars to stabilize bovine serum albumin and β-lactoglobulin as well as
lysozyme and ovalbumin. Their conclusions support those of Back et al. (1979); that high levels of sugars stabilize protein structure against heat denaturation.

Microscopic evaluation of the development of cakes during and upon completion of heating also supports the idea that a greater amount of elastic matrix is formed in high concentration EWP cakes and suggests a possible mechanistic difference between EWP and WPI in batter to cake conversion. No difference in development of structure was seen between EWP and WPI containing cakes up to the point of starch gelatinization. In fact, no visible differences could be detected between cakes containing protein of any kind and the control treatment that contained no protein. Similarly, no significant differences were seen in thermal analysis of protein containing samples and the non-protein containing control prior to gelatinization. Upon gelatinization however, notable differences occurred. Gelatinization of the starch, which is responsible for “setting” the cake, resulted in the formation of an intricate and uniform network of starch in WPI containing cakes and in the control (no protein) treatment (Fig. 8). The control samples and WPI containing samples differed only in background intensity. The brighter background seen in WPI containing cakes indicated the presence of a uniform protein phase. A secondary network of protein developed in EWP containing cakes that could be readily discerned from the starch network. Within the pores of the EWP network, the background resembled that of the control treatment suggesting little or no protein was present in the pores. Observation of the systems by phase contrast microscopy during baking revealed that in EWP containing cakes, the network of protein began forming at or shortly after the gelatinization of the starch. This suggests that an incompatibility exists between EWP and soluble starch that is not present (at least to a similar degree) between WPI and soluble starch, resulting in a phase separation.
Phase separation is a well-established phenomenon in breadmaking. Numerous researchers have reported the existence of at least three separate phases in bread dough and have remarked on their importance in producing high quality breads (MacRitchie, 1976; Gan et al., 1995; Sahi, 1994; Weegels et al., 1995; Larsson and Eliasson, 1996). A separate gluten phase has been shown to be necessary in producing a loaf with sufficient volume when baked. Angel food cake can be thought of as a bread analog with the gluten proteins replaced by animal proteins. The difference between angel food cakes and bread, with respect to their phase behavior, is that in bread, phase separation takes place during dough formation and is well underway, if not complete, before the dough is ever baked. In contrast, this data shows that the phase separation occurring during baking of EWP containing angel food cakes occurs at high temperatures, in a short time period and only after a change in the physical state of at least one of the components in the batter (i.e. crystalline-to-non-crystalline state of the starch). Cakes containing WPI showed no significant separation of protein from the soluble starch upon gelatinization.

Phase separation studies are typically carried out in ternary systems consisting of two dissimilar biopolymers and water. The biopolymers are almost always specific species rather than bulk materials such as egg white protein, whey protein isolate or starch. This is because slight changes in molecular structure or other properties such as isoelectric point, surface hydrophobicity, etc… can have large effects on the cosolubility or immiscibility of two molecular species in solution (Grinberg et al., 1997). Thus it was not possible to interpret the results obtained in this study at a molecular level due to the diversity of the protein species. In addition, the high temperatures at which these separations occurred (or failed to occur) added another layer of complexity to the thermodynamics involved in describing the molecular behavior seen in these
experiments. However, the general observation that, unlike WPI, EWP tended to phase separate from the bulk upon starch gelatinization, provides information which may be useful to food technologists in product formulation.

Based on the data presented here, we propose the following model to describe the differences in behavior exhibited by EWP and WPI containing cakes. We will consider low concentration EWP cakes (2.6% protein w/w) as a negative control, high concentration EWP cakes (5.3% protein w/w) as our standard and low concentration WPI cakes (5.3% protein w/w) for comparison. This model is divided into 3 stages of interest.

The first stage is the initial heating stage beginning the moment the cakes are placed in the oven and continuing up to approximately 60°C (Figs. 2-5). During this time no differences are seen between any formulations. As temperature increases, air cells in the batter expand causing cake rise (Fig. 2). Viscosity decreases, due to the temperature increase, and $G'$ decreases due to the decreasing S/V ratio that occurs as a result of air cell expansion (Figs. 4 & 5). Phase angles remain constant, indicating that a similar degree of air cell expansion and destabilizing events (disproportionation / coalescence) occur over this temperature range in all formulations (Fig 3).

In the second stage (ca. 60 – 90°C), high concentration EWP cakes expand with relative ease due to a high concentration of protein in the continuum. Low concentration EWP cakes expand to nearly the same degree but the rate of expansion is slightly diminished because of decreasing protein concentration in the continuous phase. This results in increasing surface tension due to decreased rates of protein adsorption, which in turn results in increasing $G'$ as expansion proceeds (Fig. 5). The nearly identical decrease in phase angle for both formulations suggested that bubble integrity was maintained to a similar degree and that individual bubble expansion is more prevalent than coalescence.
(Fig 5). This means that the excess protein in the continuous phase of the high concentration EWP cakes was not functioning to stabilize the cake during this stage except to provide bulk material, which could maintain low surface tension. Low concentration WPI cakes appeared to exhibit a relatively high degree of coalescence as evidenced by the sharp increase in phase angle (Fig. 3) and significant decrease in total expansion relative to a similar concentration of EWP (Fig. 2). The continuous decrease in $G'$ during this stage suggests that, like high concentration EWP cakes with the same amount of protein, sufficient protein content remained in the continuous phase to minimize surface tension (Fig. 4).

Around 90°C, starch gelatinization occurs (Table 1) and begins stage three in the cake development. When this occurred, high concentration EWP cakes developed a protein rich phase within the continuum of gelatinized starch that was clearly visible in phase contrast microscopy (Figs. 6 & 7). This coincides with an abrupt reversal of $G'$ (Figs. 3 & 5). This matrix was apparently of sufficient strength to enable the cake to maintain its volume upon cooling. Similar behavior occurred in low concentration EWP cakes (Figs. 6 & 7), however the decreased concentration of protein in the continuum was unable to form a matrix of sufficient strength to support the expanded cake volume as evidenced by the lesser amount of matrix in these samples. This phase behavior suggests an incompatibility between EWP and gelatinized starch within these systems. WPI containing cakes did not form this separate protein phase to any appreciable extent and therefore were not able to support the expanded cake volume (Figs. 6 & 8). This could be due to a greater affinity of the WPI for the gelatinized starch or to kinetic limitations, i.e. previous gelation of the protein that prevents mobility, thus preventing large scale phase separation. The latter possibility however is questionable, since formation of a
protein matrix prior to starch gelatinization should yield an increase in $G'$ that was not observed in DSC analysis.

**CONCLUSION**

Bulk properties appeared to differ between cakes containing EWP and WPI in two primary respects. First, cakes containing EWP were better able to withstand destructive events during baking, thus maintaining a relatively fine and somewhat uniform crumb structure. More importantly perhaps, EWP containing cakes exhibited a strong tendency towards phase separation and protein network formation that supported the cake volume. A critical concentration of EWP was necessary to form this network to a sufficient level of strength. Cakes containing WPI did not appear to participate in phase separation, at least to the extent that EWP containing cakes did. Modifications to WPI that could induce a similar level of phase separation within the cake batter may yield an ingredient that could replace or partially replace EWP in angel food cake formulations.
REFERENCES


Enthalpy ($\Delta H$), onset temperature ($T_o$), and peak temperature ($T_p$) values of the major peak observed in DSC analysis of formulations at various stages of cake batter production.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Sub-treatment</th>
<th>$\Delta H$ (J/g)$^a$</th>
<th>$\Delta H$ (J/g)$^b$</th>
<th>$T_o$ (°C)</th>
<th>$T_p$ (°C)</th>
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</thead>
<tbody>
<tr>
<td>Low conc. EWP</td>
<td>Aqueous dispersion</td>
<td>8.0 ± 0.8</td>
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<td>74.7 ± 0.0</td>
<td>81.2 ± 0.0</td>
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<td>86.6 ± 0.2</td>
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<td>59.4 ± 1.0</td>
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<td>83.0 ± 0.0</td>
<td>89.1 ± 0.0</td>
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</table>

$^a$ Values given are averages of two replications ± range.

$^b$ Enthalpy calculated on a protein basis

$^c$ Enthalpy calculated on a starch basis
## Composition of formulations on a weight percentage basis

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Production stage</th>
<th>Protein (% w/w)</th>
<th>Sugar (% w/w)</th>
<th>Flour (% w/w)</th>
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<td>Foam solution</td>
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<td>Foam solution</td>
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<td>Cake batter</td>
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<td>Control</td>
<td>Aqueous dispersion</td>
<td>-</td>
<td>42.8</td>
<td>15.3</td>
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</table>
Figure 1. Final cake volumes. Protein concentration expressed as weight percent of initial foam: (✧) 2% EWP (□) 5% EWP (○) 10% EWP (◆) 10% WPI

Values given are means of three replications ± standard deviations.
Figure 2. Expansion of cakes during baking. Protein concentration expressed as % (w/w) of initial foam: (✧) 2% EWP  (□) 5% EWP  (○) 10% EWP  
(♦) 10% WPI  (■) 15% WPI  (●) 20% WPI. Solid line = temperature.
Values given are means of 4 different whip times ± standard deviations.
Dashed lines indicate the measured heights of cakes following sticking of the batter to the container.
Figure 3. Changes in phase angle upon cooking: (●) EWP cakes (■) WPI cakes.

Values given are means of two concentrations (5 & 10% of foam EWP and 10 & 15% of foam WPI) and two whip times (4 & 12 min EWP and 15 & 20 min WPI) within each protein type ± standard deviations.
Figure 4. Expansion and rheological changes occurring in WPI containing cakes during baking: (♦) peak height (●) phase angle (■) $G'$ (▲) $G''$
Figure 5. Expansion and rheological changes occurring in EWP containing cakes during baking: (◆) peak height (○) phase angle (■) G’ (▲) G’’
Figure 6. Phase contrast microscopy of cake batter systems following simulated baking. Protein concentration expressed as weight percent of initial foam. (A) 5% EWP (B) 10% EWP (C) 10% WPI (D) 15% WPI. Arrows denote protein network.
Figure 7. Bright field (a) and fluorescence (b) images of the same field of view in cooked samples. (1) 10 wt. percent EWP foam solution (2) 5% of foam EWP batter (3) 10% of foam EWP batter. Arrows denote protein network.
Figure 8. Bright field (a) and fluorescence (b) images of the same field of view in cooked samples. (1) 10 wt. percent WPI foam solution (2) 10% of foam WPI batter (3) 15% of foam WPI batter.
Figure 9. Phase contrast images of non-batter cooked samples. (A) 10 wt. percent EWP foam solution (B) 10 wt. percent WPI foam solution (C) Batter preparation containing only sugar and cake flour (no protein)