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Executive Summary

"Acid and Alkali Unfolding and Refolding Strategies to Improve the Foaming Properties of Egg White Proteins"

Hordur G. Kristinsson
Ast. Professor
Department of Food Science and Human Nutrition
University of Florida
Gainesville, FL

A) Project overview

Eggs and egg whites (albumen) are an important commodity in the US and are used in a variety of food products due to the versatile nature of the various proteins found in the albumen. Variable foaming performance of egg albumen has always posed a problem in the food industry. Several different methods have been proposed in the past to improve foaming properties of egg whites, most focusing on heat denaturing the proteins. However, if heat treatment is too extensive, protein concentration too high or the protein is at a pH and ionic strength that favors aggregation, coagulation may occur and foaming properties are adversely affected. One way to increase the foaming functionality of egg proteins would be to induce a conformational change without heat that leads to a protein of increased hydrophobic and flexible nature, i.e. partially denature the protein. Employing different "extreme" pH treatments is an alternative means to heat, to induce a positive conformational change in the egg albumen proteins to increase their foaming properties. To the best of the investigators knowledge it had not been previously studied how controlled acid and alkali denaturation followed by pH readjustment to renaturing conditions affected the foaming properties or conformation of the albumen proteins, collectively or individually. This was the overall goal of the project described in this final report. Previous studies by the investigator on fish and land animal muscle proteins had demonstrated that certain extreme pH treatments where proteins are subjected to a very low or high pH and then readjusted to different pH values in the native pH range of the proteins, led to dramatic improvements in the functional properties of these proteins. We wanted to investigate how a large variety of pH treatments would (a) affect the foaming properties of egg albumen proteins, (b) structural and conformational properties of egg albumen proteins, and (c) the function of selected pH treated proteins in actual food products.

The unfolding of egg albumin proteins and ovalbumin was studied as a function of pH, to help us with the selection of unfolding and refolding pH values. Egg albumin solutions were subjected to a large number of unfolding pH values (pH 1.5-3.5 and pH 10.5-12.5) and refolding pH values (pH 4.5-8.5). The effect of different salt concentration (~0-300 mM) and different salt ions were evaluated. We also studied the effect of unfolding and refolding time on the foaming properties of the proteins. Controls were egg albumens adjusted directly to the pH of interest in the pH range 4.5-8.5. Functional tests in these studies were (a) foam overrun, (b) foam stability, (c) liquid drainage, and (d) foam rheology. Structural and conformational test conducted on the

proteins were (a) tryptophan fluorescence, (b) surface hydrophobicity, (c) reactive sulfhydryl groups, and (d) SDS-PAGE (reducing and non-reducing). Selected pH treated egg albumens were also compared to untreated egg albumens as ingredients in angel food cake and meringue. Both products were subjected to sensory evaluation, and the angel food cake rheological properties were evaluated before, during and after heating.

Both protein systems followed a similar unfolding curve, as proteins denatured below pH 4-4.5 and above pH 10-10.5. The results demonstrated that foaming capacity of egg albumen and the stability of the foam, or both, could be improved by an unfolding and refolding regime by choosing proper unfolding and refolding pH values. The foaming capacities of egg albumen were greatly improved when the refolding was at pH 6.5, 7.5 or 8.5, while the foaming capacities could be either slightly increased or in a few cases decreased when the refolding was at pH 4.5 or 5.5 compared to the controls. The foam stability was in almost all cases improved by the unfolding and refolding treatments except for a few cases of unfolding at pH 1.5 or 10.5. The foam stability and liquid drainage were improved most when the unfolding was at pH 12.5. Analysis of total and surface sulfhydryl groups, surface hydrophobicity and SDS-PAGE provided strong evidence that the partial unfolding of egg albumen proteins as well as the interactions among egg albumen proteins through disulfide and/or hydrophobic groups dictated the improvements in foaming properties. The increase in surface hydrophobicity showed better correlation with the improvement of foaming properties than the change of surface sulfhydryl content did.

Rheological tests revealed that foams at pH 4.5 and 8.5 made from egg albumin proteins after pH-induced unfolding and refolding treatments behaved as highly elastic materials. Static and dynamic yield stress was investigated using small, steady shear experiments. Yield stress was measured also in oscillation mode and high correlation was found between dynamic yield stress and critical stress amplitude. All pH treatments led to firmer foams than untreated foams at pH 8.5. At pH 8.5 the control foam had a very weak structure while pH-treated foams at pH 8.5 had values that were even higher than the values for the control at pH 4.5 (which is an ideal foaming pH for egg albumin). It was shown that an increase in yield stress of foams after drainage is related to foam stability and liquid drainage. It was demonstrated that unfolding egg albumin at low or high pH followed by refolding leads to a substantial increase in foam firmness and gives the foam different properties than foams from untreated egg albumin. Our work also demonstrates that yield stress of foams measured by small steady shear rate using a special cross-hatched parallel plate geometry which prevents slippage is an excellent method to determine their firmness.

Rheological tests on angel food cake made from foams with pH treated eggs, demonstrated that there was not necessarily a link between the initial cake batter firmness and the final firmness. It was however demonstrated that some of the pH treated egg albumens gave a stronger cake, and exhibited a different rheological behavior than cakes made from untreated egg albumens. Sensory work on angel food cakes revealed that some of the pH treatments did improve the cake volume and gave a soft, fluffy, yet firm cake which was preferred by tasters. Smaller differences were found in meringue made from pH treated eggs and untreated eggs, except that the meringue from pH treated eggs had a less brittle crust.

Several egg and egg ingredient companies have shown significant interest in the technology we have developed this year, and have contacted us to schedule industrial scale trials. We have been reluctant to do this, as we are still working on optimizing the use of the pH treated eggs in actual food products, as well as continuing to evaluate pasteurization of liquid eggs treated at high and low pH. We anticipate being ready for discussions and trials by this spring.

B) Publications and presentations connected to this grant

Peer reviewed publications

1. Liang, Y. L. and Kristinsson, H. G. 2005. Influence of pH-induced unfolding and refolding of egg white proteins on their foaming properties and conformation. *J. Food Sci.* 70, C222-230.
2. Mleko, S., Kristinsson, H.G. and Liang, Y. L. 2005. Rheological Properties of Foams from pH Unfolded and Refolded Egg Albumen Proteins. *Lebensm. Wiss. Technol*. (*in review*).
3. Mleko, S. and Kristinsson, H. G. 2005. Rheological Properties of Angel Food Cake with pH Unfolded and Refolded Egg Albumen. *In final stages of preparation. Planned for submission to J. Food Sci. in December 2005*.
4. Liang, Y. L. and Kristinsson, H. G. 2005. Influence of Ca^{2+} on the foaming properties of egg albumen subjected to low and high pH unfolding treatments. *In final stages of preparation. Planned for submission in December 2005*.

Presentations

1. Liang, Y. and Kristinsson, H. G. 2005. Foaming properties of egg albumen after a pH-induced unfolding and refolding regime in the presence of Ca^{2+}. IFT Annual Meeting, July 15-20, New Orleans, LA. Abstract 71B-22

2. Liang, Y., Kristinsson, H. G. and Mleko, S. 2005. Rheological properties of egg albumen after a pH-induced unfolding and refolding regime. IFT Annual Meeting, July 15-20, New Orleans, LA. Abstract 71B-23

3. Liang, Y. L and Kristinsson, H. G. 2004. Influence of pH-induced unfolding and refolding of egg white proteins on their foaming properties and conformation. IFT Annual Meeting, Las Vegas, NV, July 12-16, 2004, Abstract 17E-13.

4. Kristinsson, H. G. and Ingadottir, B. Acid and alkali unfolding and refolding strategies improve the foaming properties of egg albumen. IFT Annual Meeting, Chicago, IL, Abstract 42-4, 2003.

PART I

Investigations into the effect of acid and alkali unfolding and refolding strategies on the foaming, rheological, structural and conformational properties of egg albumen

INTRODUCTION

Products of egg albumen are important food commodities as they find uses in a vast number of different food formulations. One of the most important uses of egg albumen is to form a foam when its solution is exposed to gas supersaturation or mechanical forces (Walstra 1996; Nakamura and Doi 2000). The foaming properties of egg albumen are affected by many factors such as protein concentration, pH, ionic strength, yolk contamination, heat damage, etc. Obtaining egg albumen with better foaming properties by properly understanding and managing the factors influencing foaming is desired for the commercial production of egg albumen commodities.

Foams are colloidal systems in which tiny air bubbles are dispersed in an aqueous continuous phase (Damodaran 1997). Amphiphilic molecules are needed for creating and stabilizing the air bubbles in the liquid phase. Many proteins can serve as effective foaming agents and stabilizers. For the egg albumen proteins which are mostly globular proteins, an increase of their surface hydrophobicity and flexibility by partially unfolding the proteins is expected to make them better surfactants (i.e. foaming agents) and improve their foaming properties. Structural modification of egg albumen proteins could be obtained by partial unfolding of the proteins. Moderate heat treatment on egg albumen has been reported to improve their foaming properties by increasing its surface hydrophobicity and causing changes in protein conformation (Kilara and Harwalkar 1996; Hagolle and others 2000). However, heat treatment is expensive and might cause protein aggregation which would adversely affect foaming (Kilara and Harwalkar 1996). Altering the pH of a protein medium is another well known method to unfold proteins. pH-induced unfolding and its effect on foaming has been studied with egg proteins. However, the proteins were adjusted to and foamed at extremes of pH (pH 1-3 or pH 11-13) which are not practical pH values for most food systems. Recently, it has been demonstrated that proteins can be kept partially unfolded with modified functionalities if they are unfolded first at extreme pH values and then partially refolded by readjusting the pH back to the "non-denaturing" pH range of the proteins (Kristinsson 2002; Kristinsson and Hultin 2003a, 2003b, 2004). Thus, by employing different pH unfolding and refolding regimes, it may be possible to improve/modify the foaming properties of egg albumen by creating partially unfolded structures with increased surface hydrophobicity, flexibility and reactivity. Also, calcium (Ca^{2+}), a cross-linking agent, is known for its interaction with large molecules and changing their physical properties. The presence of Ca^{2+} may add additional improvements on foaming properties of egg albumin treated by the pH-induced unfolding and refolding regime. Egg albumen with different foaming properties might therefore be tailored by controlling the unfolding and refolding pH values used and used for different food applications.

Qualities of foams are typically measured by foam volume (overrun), foam stability and liquid drainage. These tests are simply done by producing a foam, recording the increase in volume (overrun), monitoring the separation of the foam into a foam and liquid phase (stability) and

recording the amount of liquid drained from the foam (liquid drainage). An increase in foam volume and stability and reduction in drainage are considered positive for food foams. Any change in foam volume and stability, as related to changes in the proteins are expected to greatly influence the rheological properties of foams. The properties of most importance for an egg based foam are (a) yield stress, which is defined as the stress below which no flow is observed in the foam upon experiencing a certain shear stress, and (b) storage modulus (G'), which tells how stiff/rigid or strong the foam is. The yield stress can be furthermore broken down into static yield stress and dynamic yield stress, which give different information on the foam properties. "Fluid foams" like egg albumen foams are viscoelastic materials and its viscous and elastic components can be investigated using dynamic rheology. These properties are however inherently difficult to measure, especially for relatively weak foams such as egg albumen foams. First of all, foams are instable because of liquid drainage caused by gravity and Ostwald ripening, i.e. creation of larger bubbles from smaller ones (Gardiner et al. 1998). Additionally, the generation of a liquid film slip layer at the wall during the measurement will affect the accuracy of measurement. Some techniques have been applied to minimize wall slip. Khan et al. (1988) proposed parallel plate geometry with sandpaper disks attached to the surface of the parallel plates. Using this method, liquid resides in the depressions between the particles of the sandpaper, which can eliminate the effect of the liquid film slip for rheological measurements. Zhong and Wang (2003) supported this methodology and found that wall slip is preventable by attaching sandpapers onto the parallel plate surfaces of the flow cell.
The objectives of this first part of our research were to perform an extensive investigation on the foaming properties (foaming capacity, foam stability and foam rheology) and the structure of egg albumen proteins after a pH-induced unfolding and refolding regime in the presence or absence of calcium ions and to develop an understanding on the relationship between the improved foaming properties of egg albumen and its structural change.

MATERIALS

Egg albumin powder of same lot no. was purchased from Fisher Scientific (Pittsburgh, PA). PRODAN (6-propionyl-2-dimethylaminonaphthalene) was obtained from Molecular Probes, Inc. (Eugene, OR). Ellman's Reagent (5,5-Dithiobis(2-nitro-benzoic acid)) and purified ovalbumin was obtained from Sigma Chemical Co. (St. Louis, MO). Other chemicals were purchased from Fisher Scientific (Pittsburgh, PA) and Sigma Chemical Co. (St. Louis, MO).

METHODS

Protein conformational changes as a function of pH

Protein conformational changes of egg albumin collectively and ovalbumin (the most abundant protein in egg albumin) were studied from ph 1.5 to 12.5, to determine what pH ranges to select for the unfolding and refolding experiments. Changes in protein tryptophan fluorescence were determined using a Perkin Elmer LS-45 luminescence spectrofluorometer. Proteins solution (egg albumin and ovalbumin) were made from pH 1.5-12.5 at 0.01 mg/mL and samples excited at 280 nm and emission at 340 nm followed.

Exposure of hydrophobic residues as a function of pH were be assessed by the proteins ability to bind to a hydrophobic fluorescent dye, 8-anilino-1-napthalene-sulfonic acid (ANS). A solution

of 0.1 mg/mL at pH 1.5-12.5 was serially diluted to give 0.01-0.1 mg/mL solutions and excess ANS added (10 uL of 10 mM ANS in 1 mL protein solution). Samples were excited at 380 nm and emission read between 400 and 550 nm using a Perkin Elmer LS-45 luminescence spectrofluorometer.

The pH-induced unfolding and refolding regime

Egg albumen solutions (100 mL) were prepared at a concentration of 2.5% (w/v) in the absence of calcium ions (in 100 mM NaCl solutions) or in their presence (in solutions of 100 mM NaCl and 10 mM $CaCl_2$). We chose 2.5% based on our previous work (Ingadottir and Kristinsson 2003). At this concentration, good repeatability and comparison between different treatments could be obtained and the foam could be handled with relative ease. The egg albumen solution was adjusted to either low pH values (pH 1.5, 2.5 or 3.5) or high pH values (pH 10.5, 11.5 or 12.5) using 2N HCl or 2N NaOH, and then held 60 min for unfolding. After holding, the solution was readjusted to different refolding pH values (pH 4.5, 5.5, 6.5, 7.5 or 8.5). The solutions (either of 100 mM NaCl or of 100 mM NaCl and 10 mM $CaCl_2$) was then added to each sample bringing their final volume to 110 ml. This brought the protein concentration down to ~2.3%. After 45 min holding for refolding, the samples were whipped with a BIO Homogenizer (M133/1281-0, Biospec Products Inc., Bartlesville, OK) with a foaming disk attachment at speed 2 in 600 mL glass beakers for 1 min. The volume of the foam generated was recorded and used to calculate foaming capacity. The foamed samples were held for 30 min for the study of foam stability and liquid drainage. The scheme of the regime is shown in Figure 1.

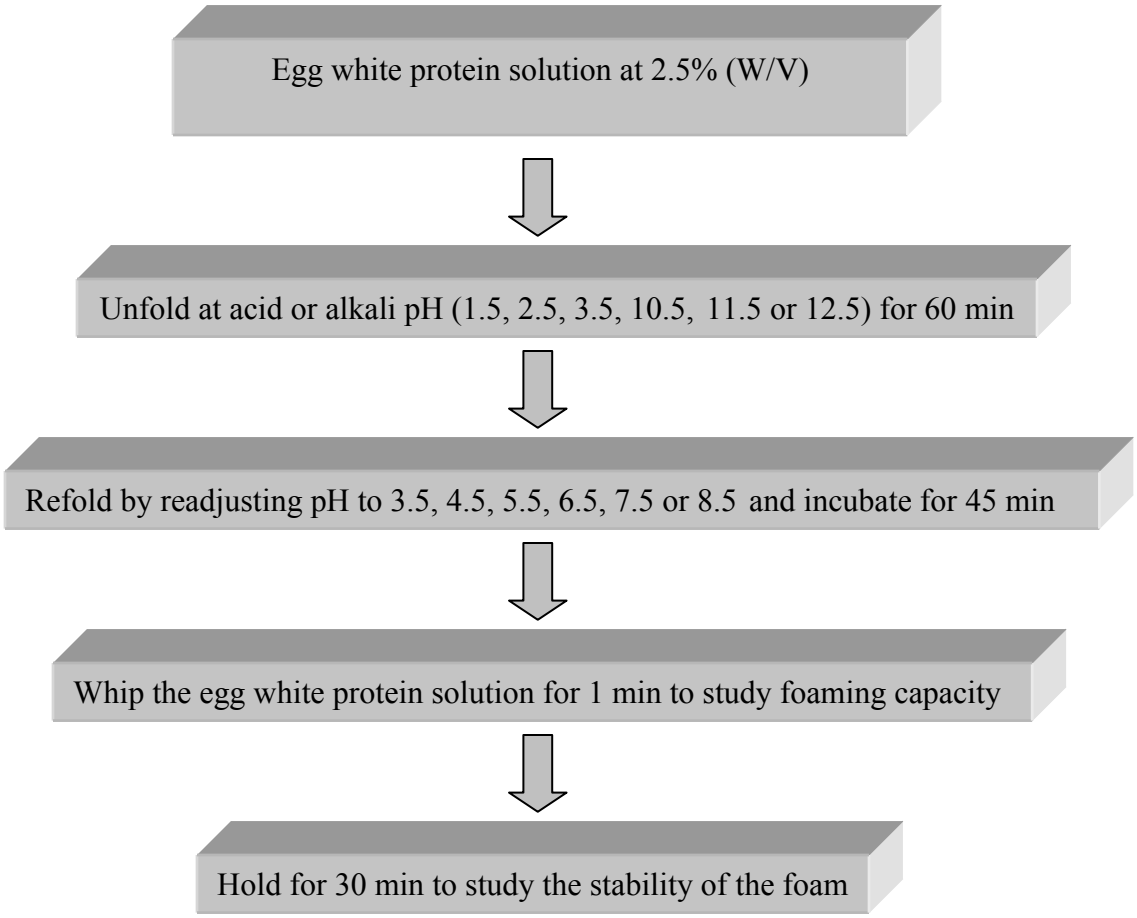


Figure 1. Scheme of the pH-induced unfolding and refolding of egg white proteins and the study of foaming capacity and foam stability.

The 60 min unfolding time and the 45 min refolding time were selected based on previous studies we have done on egg albumen (Ingadottir and Kristinsson 2003). These studies demonstrated, using trypophan fluorescence and protein hydrophobicity (ANS binding), that 60 min was more than sufficient to reach a stable "unfolded" structure and that 45 min was also more than sufficient to reach a stable "refolded" structure. It is however worth mentioning that the terms "unfolded" and "refolded" do not necessarily reflect fully unfolded and refolded structures, since different levels of unfolding and refolding can be achieved, depending on treatment.

In addition to the 60 min unfolding time and 45 min refolding time, a short (~0 min) unfolding or refolding time was also studied using egg albumin solution in absence of calcium ions. For the ~0 min unfolding time treatment, the egg albumen solutions were adjusted to pH 1.5, 2.5, 11.5 or 12.5 and then immediately (~0 min) readjusted to either pH 4.5 or 8.5. After holding at pH 4.5 or 8.5 for 45 min, the egg albumen solutions were whipped and studied for their foaming properties. The ~0 min unfolding here means immediate readjustment of pH to 4.5 or 8.5 after the pH of egg albumen solutions were adjusted to 1.5, 2.5, 11.5 or 12.5. The actual time taken to adjust the pH from 1.5, 2.5, 11.5 or 12.5 to 4.5 or 8.5 was about 2 min. The ~0 min refolding time treatment was done in exactly the same fashion as the ~0 min unfolding time treatment except for using 60 min as the unfolding time and ~0 min for refolding (i.e. foams were formed immediately after the pH of egg albumen solutions was adjusted to 4.5 or 8.5).

Evaluation of Foaming Capacity and Foam Stability

a) Foaming capacity

Foaming capacity was studied by whipping egg albumen solution as described above and calculating relative overrun (Hammershoj and Qvist 2001):

$$\text{Relative overrun} = V_0/V_i$$

where:

V_i: initial liquid volume
V_0: foam volume at 0 min

b) Foam stability

The foam stability was studied by holding the foam for 30 min and comparing the volume of foam after 30 min with the initial foam volume (0 min) (Hammershoj and Qvist 2001):

$$\text{Foam stability} = V_{30}/V_0$$

where:

V_{30}: foam volume at 30 min
V_0: foam volume at 0 min

c) Liquid drainage

Liquid drainage was calculated from the liquid drained from the foam in a 30 min period (Hammershoj and Qvist 2001):

$$\text{Liquid drainage} = 1-(V_i-V_{L30})/(V_i-V_{L0})$$

where:

V_i: initial liquid volume
V_{L30}: volume of liquid at 30 min
V_{L0}: volume of liquid at 0 min

Measurement of protein concentration
The protein concentration of egg albumen sample was determined by the Biuret method (Torten and Whitaker 1964) using bovine serum albumen as a standard.

Measurement of the surface and total sulfhydryl contents
The surface and total sulfhydryl contents were measured by the method of Kim and others (2003) with modifications. The egg albumen samples subjected to the measurements were first diluted to 1/20 of the original concentration with phosphate buffer (10 mM phosphate,100 mM NaCl, pH 8.5). To measure the surface sulfhydryl content, an aliquot (80 μL) of Ellman's reagent (10 mM) was added to 1.5 mL of the diluted egg albumen solution. The mixtures were then incubated at room temperature for 1 hr. The absorbance of the mixture was measured at 420 nm using an Agilent 8453 diode array UV-visible spectroscopy system (Agilent Technologies Deutschland GmbH, Waldbronn, Germany). A molar extinction coefficient of 13,600 mol/cm was used to calculate the amount of sulfhydryl groups per gram protein.

For the measurement of total sulfhydryl content, the diluted egg albumen samples of 0.5 mL were first mixed with 2.5 mL of 8 mM urea, 2% sodium dodecylsulfate, 10 mM EDTA and 0.2 M Tris-HCl at pH 8.5. After adding 50 μL Ellman's reagent, the mixtures were placed in a water bath at 40°C for 15 min. The total sulfhydryl contents of the samples (per gram protein) were then calculated from their absorbance values at 420 nm using a molar extinction coefficient of 13,600 mol/cm.

Measurement of surface hydrophobicity

Surface hydrophobicity was measured by the method of Alizadeh-Pasdar and Li-Chan (2000) using PRODAN as the fluorescent probe. A PRODAN stock solution was prepared at 11.35 μg/mL in methanol. The PRODAN stock solution was kept in a screw-capped vial covered with aluminum foil and sealed with Parafilm (American Can Company, Greenwich, CT). The PRODAN stock solution was stored at -25°C until the day of use. To measure surface hydrophobicity, egg albumen samples of 100 μL, 200 μL, 300 μL, 400 μL and 500 μL were mixed with phosphate buffer (10 mM phosphate, 100 mM NaCl, pH 8.5) giving a final volume of 4 ml. A PRODAN solution of 10 μL was then added to the protein solution. After mixing, the samples were held for 15 min in the dark. The fluorescence emission of the samples was then scanned between 380-560nm (excited at 365nm) using a LS 45 luminescence spectrometer (PerkinElmer, Inc., Wellesley, MA). As egg albumen was a collection of proteins, the fluorescence peak of the samples was a broad peak between 430-460 nm with a maximal fluorescence emission at 435 nm for most of the samples. Thus, the reading at 435 nm was taken as the representing peak reading for the egg albumen proteins as whole. The net fluorescence strength of the sample with PRODAN was calculated by the fluorescence of the sample with PRODAN at 435 nm minus the fluorescence of the sample without PRODAN at 435 nm. The surface hydrophobicity was obtained from the slope of the net fluorescence readings of samples versus the protein concentrations (%, w/v) of the samples.

Protein analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

The SDS-PAGE protein analyses were run on pre-cast linear 4-20% gradient minigels (Bio-Rad Laboratories, Inc., Hercules, CA) using a vertical mini-PROTEAN 3 electrophoresis module (Bio-Rad Laboratories, Inc., Hercules, CA) with a constant voltage of 200 V per gel using a Power Npac 300 power supply (Bio-Rad Laboratories, Inc., Hercules, CA). Aliquots of proteins were added to Laemmli buffer (Bio-Rad Laboratories, Inc., Hercules, CA) giving a concentration of approximately 1 mg/mL. Samples were then heated at 100°C for 5 min. For the SDS-PAGE analysis with a reducing agent, an aliquot of 50 μL 2-mercaptoethanol (Bio-Rad Laboratories, Inc., Hercules, CA) was added to 1mL protein sample in Laemmli buffer before denaturing the proteins.

Measurement of foam rheological properties

Egg albumen solutions were subjected to the pH-induced unfolding and refolding treatment and then foamed as described above. The foams were either directly subjected to rheological testing or allowed to sit for 30 min (led to separated foam) before rheological testing. All rheological measurements were performed at 25^{o}C using a TA Instrument AR 2000 controlled stress rheometer (TA Instruments, New Castle, DE). An acrylic ST X-Hatch (40 mm diameter) plate was used with a cross-hatched surface as the upper plate. To prevent slippage, type 120 pasting sandpaper (3M Technologies, St Paul, MN) was glued to the lower plate. The foam sample was carefully loaded on the sandpaper. The upper plate was then lowered and stopped at a final gap of 2.4 mm to the lower plate. Any excessive sample protruding beyond the upper plate was removed carefully. Samples were allowed to rest for 2 min before analysis.

To determine the linear viscoelastic region of the foams, a strain sweep (frequency 1 Hz) was performed in the range of 0.002-0.05 and changes in storage (G') and loss (G") modulus recorded. Frequency sweep were carried out on the foams in the 0.1-10 Hz range at 0.4% strain and changes in storage (G') and loss (G") modulus recorded. Steady shear experiments were performed on the foams at a 0.05 s^{-1} shear rate for 1200 s and changes in shear stress (Pa) recorded. Dynamic and static yield stresses of the foams were estimated from the steady shear graph. Relative increases in yield stress for the foams were calculated:

$$RIY = Y_s - (Y_f/Y_s)$$

where:

Y_f - yield stress of fresh foam
Y_s - yield stress of the separated foam after drainage

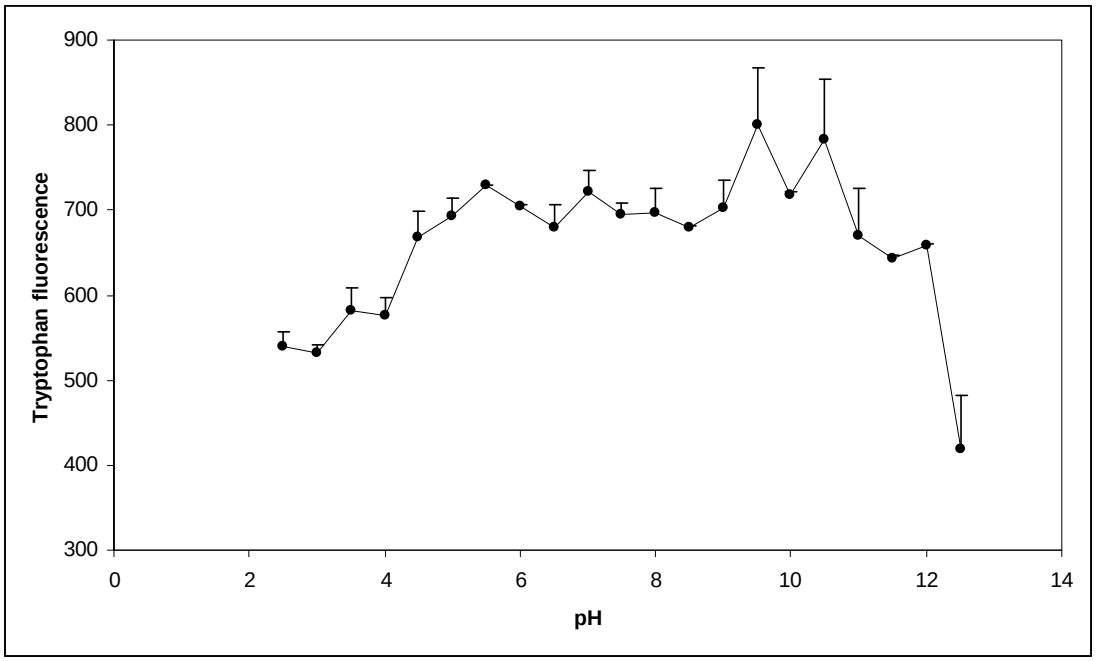
Oscillatory stress sweep was carried out in the range 0.008-100 Pa at 1.0 Hz frequency and changes in storage (G') and loss (G") modulus recorded.

Data handling
All of the experiments and assays were performed in at least duplicate and data are presented as the average in the paper. Analysis of variance (ANOVA) and Tukey's studentized range test were determined using a SAS program.

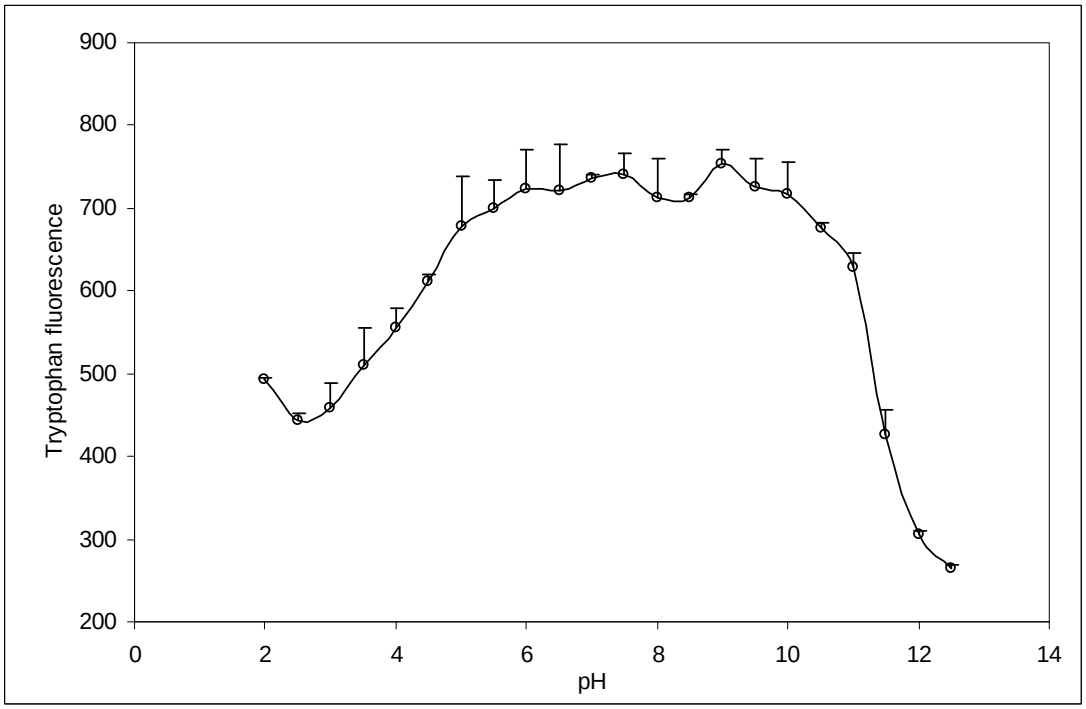
RESULTS

Protein conformational changes as a function of pH

Conformational studies revealed that egg albumin as a whole followed a similar unfolding curve as ovalbumin, when tryptophan fluorescence was used as a probe. Below pH 4-4.5 and above pH 10-10.5 proteins started to unfold, and the unfolding was more extensive for the proteins at extreme high pH. Between pH 4.5 and 10, there was little change in conformation noted for both protein systems tested. This data thus gave us a basis to select the proper unfolding and refolding pH values for the study.



(a)

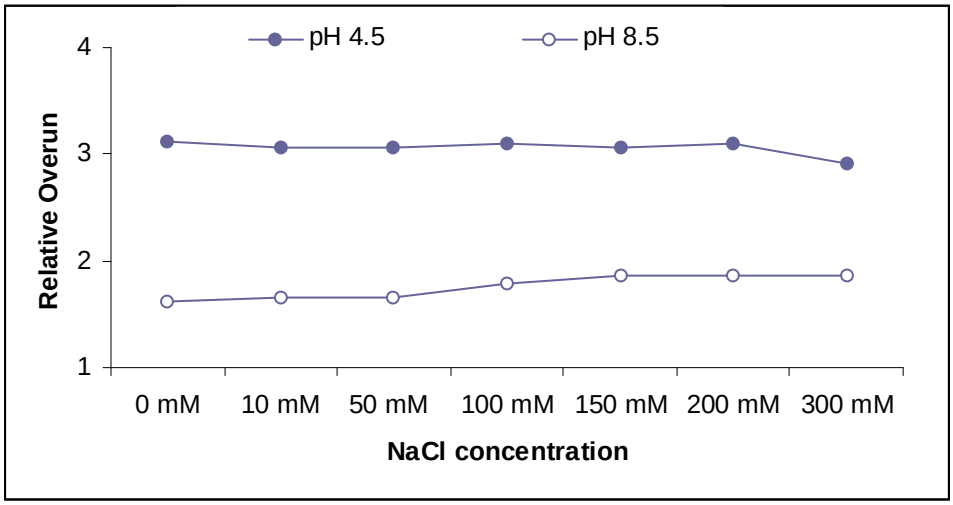


(b)

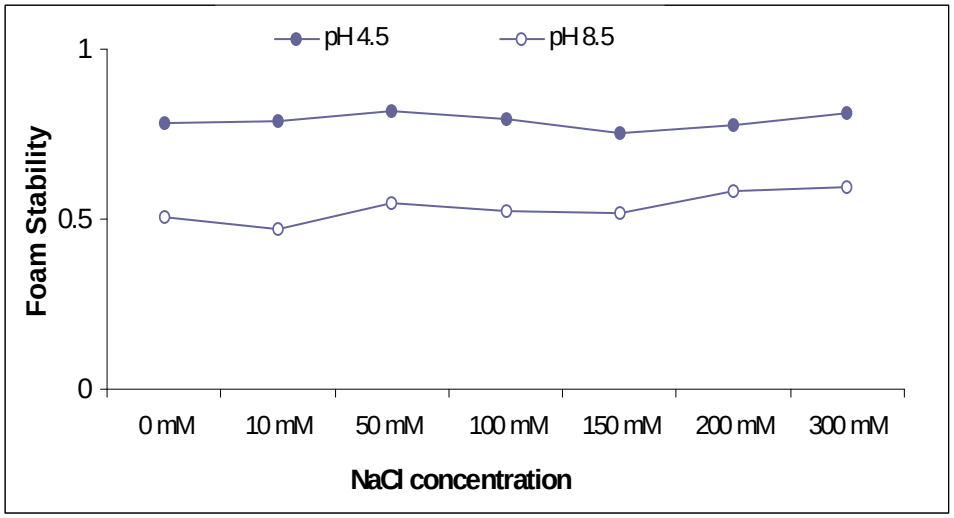
Figure 2. Effect of pH on the tryptophan fluorescence of (a) egg albumin and (b) ovalbumin. A decrease in fluorescence refers to more unfolding/denaturation.

The effect of ionic strength on foaming properties of egg albumen

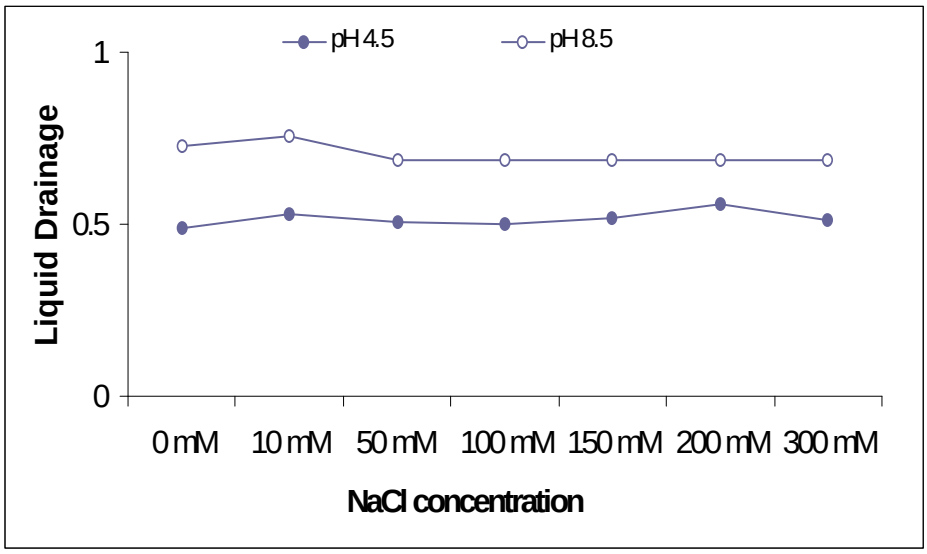
Egg albumin was prepared at 2.5% in 100 ml NaCl solutions with different concentrations to study the effect of ion strength on foam properties. The egg albumin was then adjusted to either pH 4.5 or 8.5. The foaming capacity and foam stability were studied after 45 min incubation and are shown in Fig 3. The results showed that the properties were little affected by the NaCl concentration in the studied range. In the pH-induced unfolding and refolding treatments, addition of acid and base increased the ionic strength slightly. This finding shows that moderate increase in ionic strength during the pH adjustment has no significant effect on the foamability of the unfolded and refolded egg albumen.



(a)



(b)

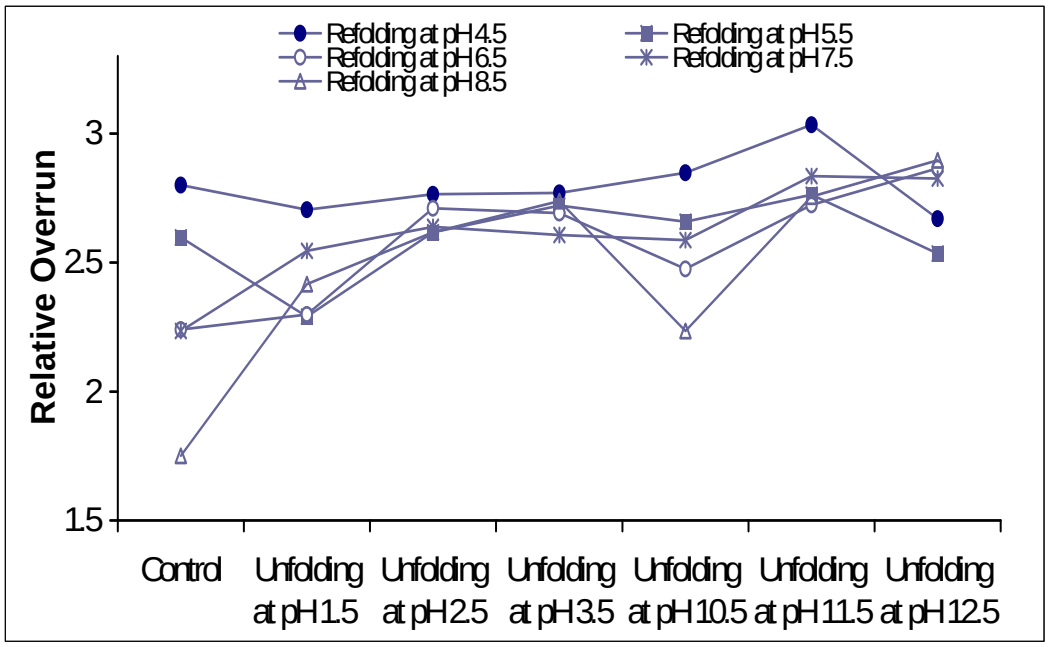


(c)

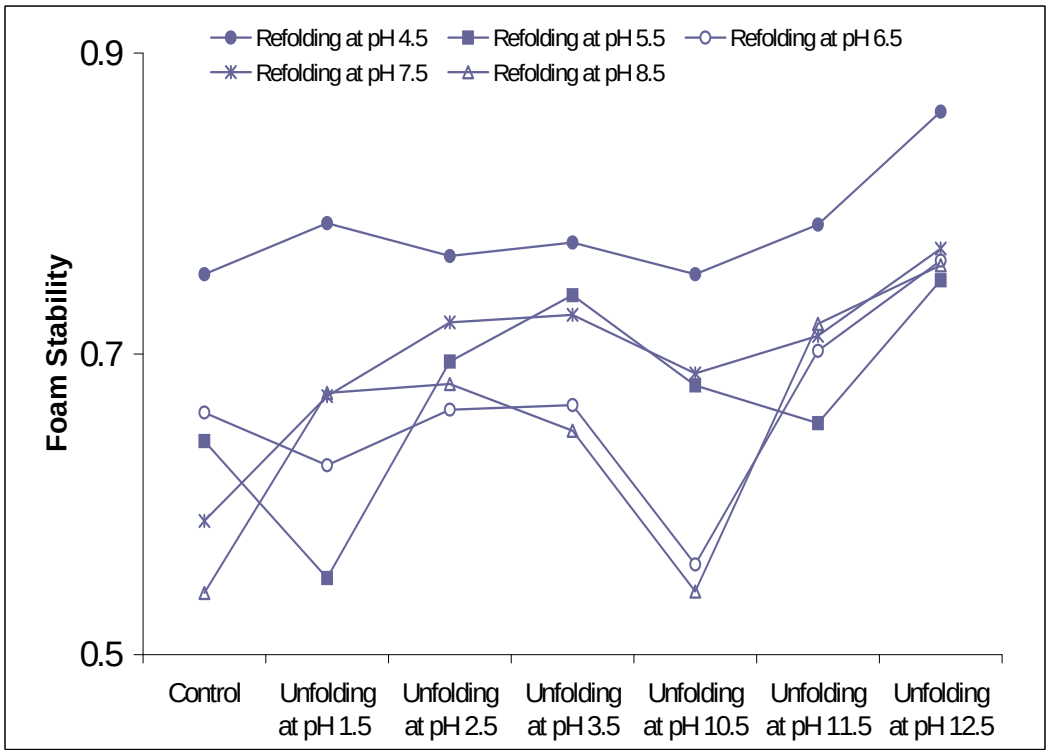
Figure 3. The influence of NaCl concentration on foaming properties of egg white proteins. (a) Foaming capacity; (b) Foam stability; (c) Liquid drainage

The foaming properties of egg albumen after the pH-induced unfolding and refolding regime in the absence and presence of calcium ions.

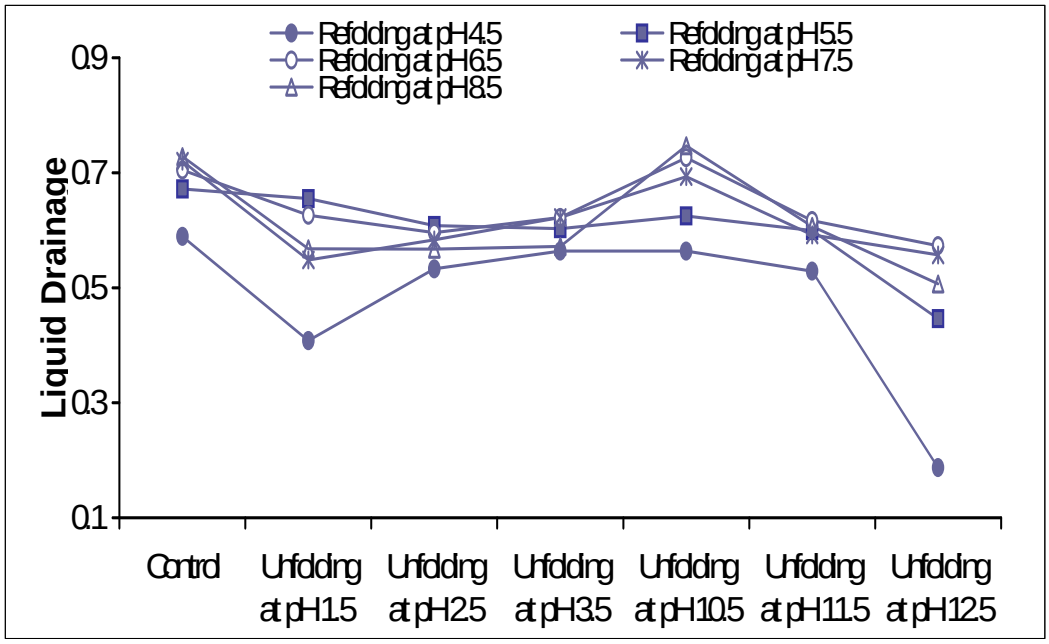
The foaming properties of egg albumen solutions (~2.3% w/v) were studied after the different pH-induced unfolding and refolding regimes. The foaming capacity, foam stability and liquid drainage of the pH-treated egg albumen in the absence of calcium ions are shown in Figure 4. The controls in the figures are samples that were directly adjusted to pH 4.5, 5.5, 6.5, 7.5 or 8.5 from their native pH prior to foaming. The foaming properties of the controls were gradually improved as pH was reduced from pH 8.5 to 4.5. This is in agreement with a previous study by Hammershoj and others (1999) which reported foam overrun in the following order for a highly diluted (0.01%, w/v) egg albumen solution: pH 4.8>>pH 7>pH 9.2>pH 10.7. These authors also reported the highest long term foam stability for the egg albumen at pH 4.8 compared to those at the other pH values. Variable results on egg albumen foamability and foam stability as a function of pH are reported in the literature, which is in part due to the different starting materials, protein concentrations and foaming methods. It is thus difficult to compare data among different publications. Egg albumen is however known to produce the best foams around pH 4-5 which is the range close to the isoelectric points (pI) of many key egg albumen proteins (Linden and Lorient 1999). At the pI, electrostatic repulsion is minimal and hydrophobic interactions are favored, which lead to numerous contacts among the proteins and create stable cohesive foam. A deviation from the isoelectric point would increase charge frequency which works against foam stability since increased repulsion among the proteins would cause the foam to break down (Damodaran 1997).


Refolding at pH 4.5
Refolding at pH 5.5
Refolding at pH 6.5
Refolding at pH 7.5
Refolding at pH 8.5
Relative Overrun
3
2.5
2
1.5
Control
Unfolding at pH 1.5
Unfolding at pH 2.5
Unfolding at pH 3.5
Unfolding at pH 10.5
Unfolding at pH 11.5
Unfolding at pH 12.5

(a)


Refolding at pH 4.5
Refolding at pH 5.5
Refolding at pH 6.5
Refolding at pH 7.5
Refolding at pH 8.5
Foam Stability
0.9
0.7
0.5
Control
Unfolding at pH 1.5
Unfolding at pH 2.5
Unfolding at pH 3.5
Unfolding at pH 10.5
Unfolding at pH 11.5
Unfolding at pH 12.5

(b)



(c)

Figure 4. The foaming properties of egg white proteins after the pH-induced unfolding and refolding regime in absence of calcium (2.5% w/v in 100 mM NaCl). The controls in the figures are samples that directly adjusted to pH 4.5, 5.5, 6.5, 7.5 or 8.5 from their native pH. (a) Relative overrun (b) foam stability (c) liquid drainage

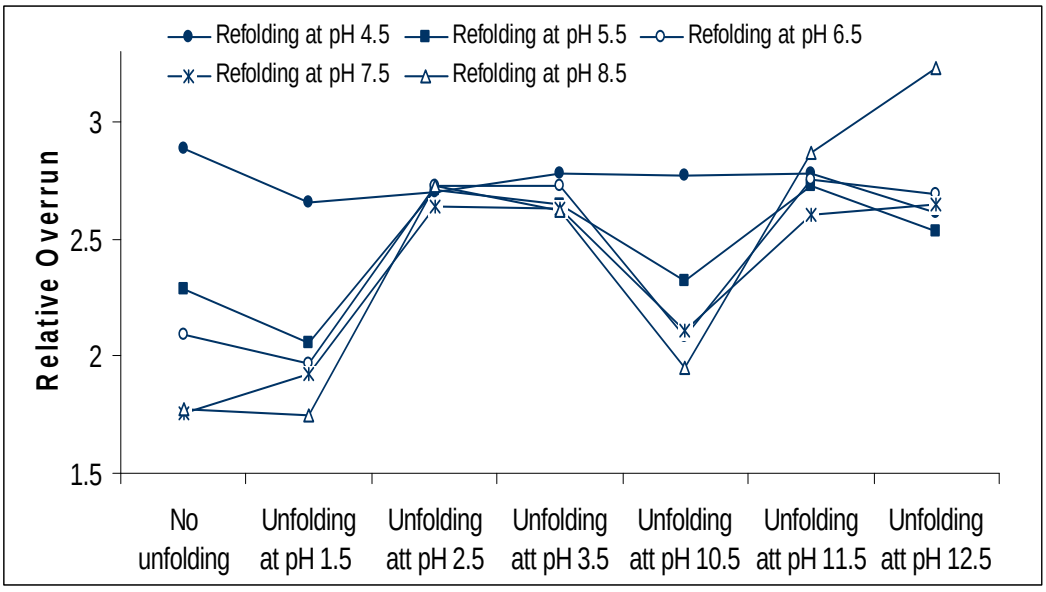
The foaming capacities of egg albumen samples were improved greatly compared to that of the controls when the refolding was at pH 6.5, 7.5 or 8.5. The foaming capacities were either slightly increased or decreased when the refolding was done at pH 4.5 or 5.5. The greatest improvement was seen for the pH 12.5→8.5 treatment with an increase of the foaming capacity from ~1.7 to ~2.9. The foaming capacity was significantly affected by both the unfolding pH values and the refolding pH values ($\rho<0.05$). Overall, the pH treatments refolded to pH 4.5 resulted in significantly higher foaming capacities compared to the treatments refolded to other pH values, among which there was no significant difference ($p<0.05$). No significant difference was found among the pH 2.5, 3.5, 11.5 and 12.5 treatments, among the pH 2.5, 3.5 and 10.5 treatments, between the pH 1.5 and 10.5 treatment, and between the pH 1.5 treatment and the control. Any two treatments not in the same group above were significantly different from each other ($p<0.05$). It was interesting to see that for some pH-treatments the foaming capacities were even better at the high foaming pH than low pH, which is contradictory to the behavior of the controls. These results indicate that the unfolding and refolding regime could greatly improve foaming capacity of egg albumen at pH values where the foaming capacity of "native" egg albumen is relatively poor, i.e. at pH values above 5.5. This could significantly extend the use of egg albumen ingredients in a variety of food products. The foam stability was in almost all cases improved by the unfolding and refolding treatments except for a few cases where unfolding was done at pH 1.5 (pH 1.5→5.5 and pH 1.5→6.5) or 10.5 (pH 10.5→6.5). When the unfolding was done at pH 11.5 and 12.5, the foam stability and liquid drainage were improved at all refolding

pH values. Statistical analysis showed that foam stability and liquid drainage was significantly affected by both the unfolding pH values and the refolding pH values ($\rho<0.05$). The pH treatments refolded to pH 4.5 resulted in significantly ($p<0.05$) higher foam stability compared to the pH treatments refolded to other pH values, among which there was no significant difference. When it came to the effect of the unfolding pH on foam stability, no significant difference was found among the pH 2.5, 3.5, 11.5 and 12.5 treatments, and among the pH 1.5, 2.5, 3.5, 10.5, 11.5 treatments and control. Any two treatments not in the same group were significantly different from each other. With respect to liquid drainage, no significant difference was found between the pH 10.5 treatment and control, and among pH 1.5, 2.5, 3.5 and 11.5 treatments. Any two treatments not in the same group were significantly different from each other.

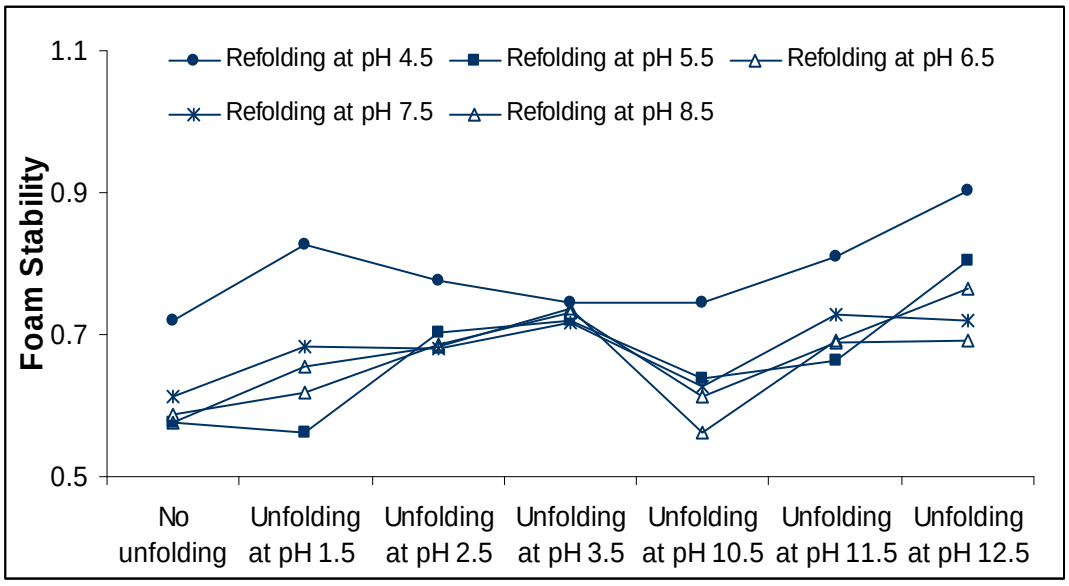
An alkaline unfolding treatment at pH 10.5 was not as effective as these at pH 11.5 and 12.5 in improvement of foaming properties. This could be due to more protein unfolding seen at pH 11.5 and 12.5 compared to pH 10.5 at which pH egg albumen shows minimal unfolding using tryptophane fluorescence as an indicator of structural change (Ingadottir and Kristinsson 2003). Although the unfolding and refolding regime did not greatly improve the foaming capacity of egg albumen when the refolding was done at pH 4.5 and 5.5, the foaming stability and liquid drainage were generally improved in these cases. The most stable foams obtained were the foams generated by egg albumen proteins after unfolding at pH 12.5 and refolding at pH 4.5. Thus, the foaming capacity of egg albumen, the stability of the foam generated, or both, could be improved by the unfolding and refolding regime by choosing proper unfolding pH and refolding pH.

It was interesting to note that the foaming capacity of egg albumen unfolded at pH 1.5 were in general not as good as those of unfolded at pH 2.5 and 3.5. Conformational data with ovalbumin, the main protein in egg albumen, suggests that ovalbumin is more unfolded at pH 2.5 and 3.5 than at pH 1.5 (Ingadottir and Kristinsson 2003). At pH 1.5, ovalbumin appears to refold possibly via Cl^- induced refolding due to high level of HCl at that pH (Goto and Fink 1994; Ingadottir and Kristinsson 2003). The pH 2.5 and 3.5 treated proteins may thus have a more open and misfolded structure (i.e. not refolded back to its native structure) which results in better foaming properties.

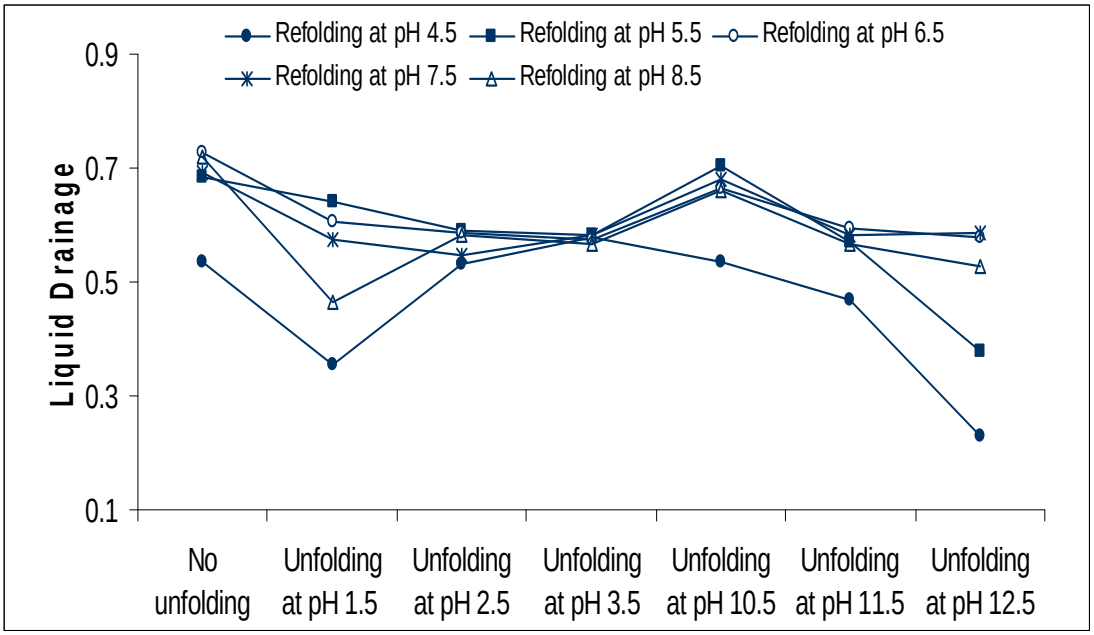
The foaming capacity, foam stability and liquid drainage of the pH-treated egg albumen in the presence of calcium ions are shown in Figure 5. They were improved in similar ways as the albumings with no calcium (Fig. 4). No obvious overall improvement in the foaming properties was observed for these treatments compared to the treatments without calcium. Some of the treatments were however more effective in the presence of calcium. For example the pH 12.5→ 8.5 treatment had a dramatic improvement in foaming properties, more than the sample treatment adjusted to pH 4.5.


Refolding at pH 4.5
Refolding at pH 5.5
Refolding at pH 6.5
Refolding at pH 7.5
Refolding at pH 8.5
Relative Overrun
3
2.5
2
1.5
No unfolding
Unfolding at pH 1.5
Unfolding att pH 2.5
Unfolding att pH 3.5
Unfolding att pH 10.5
Unfolding att pH 11.5
Unfolding att pH 12.5

(a)


Refolding at pH 4.5
Refolding at pH 5.5
Refolding at pH 6.5
Refolding at pH 7.5
Refolding at pH 8.5
Foam Stability
1.1
0.9
0.7
0.5
No unfolding
Unfolding at pH 1.5
Unfolding at pH 2.5
Unfolding at pH 3.5
Unfolding at pH 10.5
Unfolding at pH 11.5
Unfolding at pH 12.5

(b)



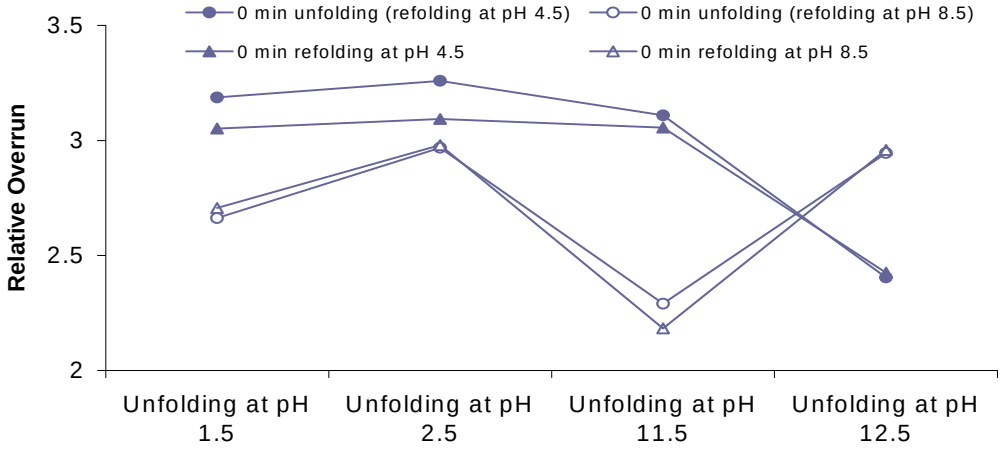
(c)

Figure 5. The foaming properties of egg white proteins after the pH-induced unfolding and refolding regime in the presence of calcium (2.5% w/v in 100 mM NaCl and 10 mM $CaCl_2$). The controls in the figures are samples that directly adjusted to pH 4.5, 5.5, 6.5, 7.5 or 8.5 from their native pH. (a) Relative overrun (b) foam stability (c) liquid drainage

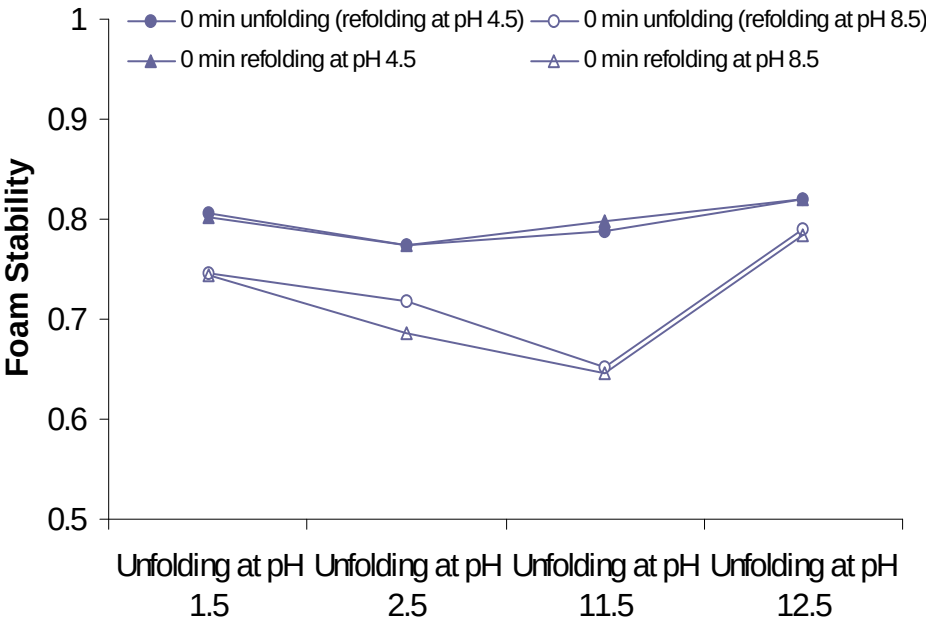
The influence of unfolding and refolding times on the foaming properties of egg albumen

It was investigated if the unfolding and refolding times were key factors affecting the foaming properties of egg albumen treated by the unfolding and refolding regime. Many proteins are known to go through several different intermediate stages during unfolding and refolding some of which are more stable than others. Some proteins can actually be trapped in a misfolded structure on refolding (Kumar and others 2000) if that structure has a more favorable energy than the native state under given solution conditions. The conversion from one state to another is not only dependent on the solution conditions (e.g. pH, ionic strength, salt type and temperature) but also time. Some proteins have been found to take on different structures when different speeds of unfolding and refolding are applied (Shen and Hermans 1972; McPhie 1982; Kristinsson and Hultin 2004). For example, Kristinsson and Hultin (2004) reported that longer holding times at low pH led to less recovery in native structure and lower protein solubility of trout hemoglobin. Different levels of refolding could thus lead to different structures with different functionalities. For egg albumen, the 60 min unfolding time and 45 min refolding time was compared to immediate unfolding and refolding (~0 min) in the absence of calcium. The foaming capacity, foam stability and liquid drainage of the treated samples are shown in Figure 6. The data for the ~0 min unfolding treatment and ~0 min refolding treatment were very similar to those of the corresponding pH-treated egg albumen samples with 60 min unfolding and 45 min refolding. This may suggest the egg albumen proteins were unfolded very fast to a certain form and also refolded (partially) very rapidly to a certain structure. The data overall suggest the unfolding or

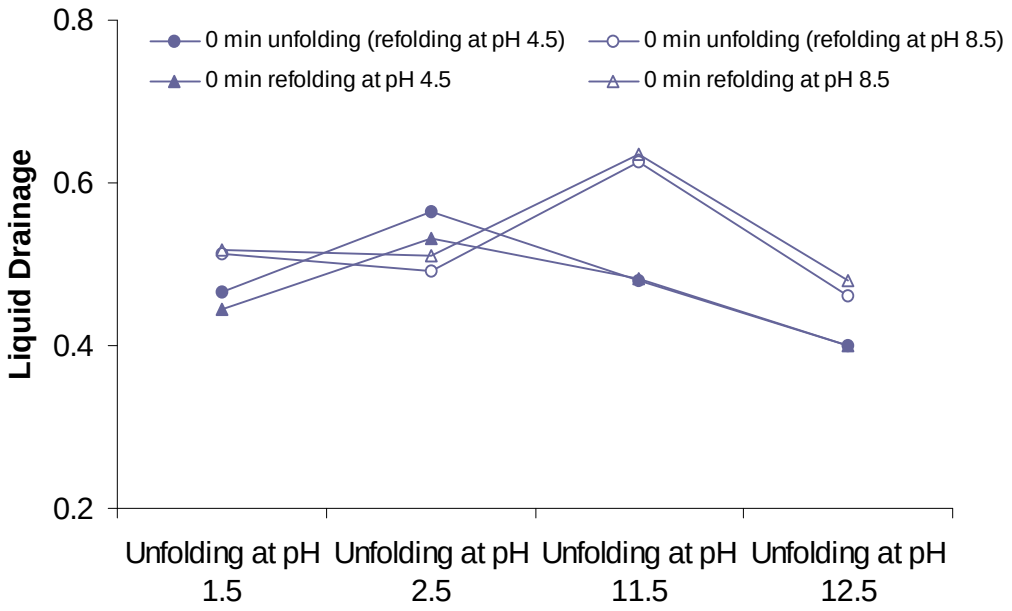
refolding time are not key factors influencing the improvements in foaming properties. This provides great flexibility in potential industrial applications of the pH-treated egg albumen.



(a)



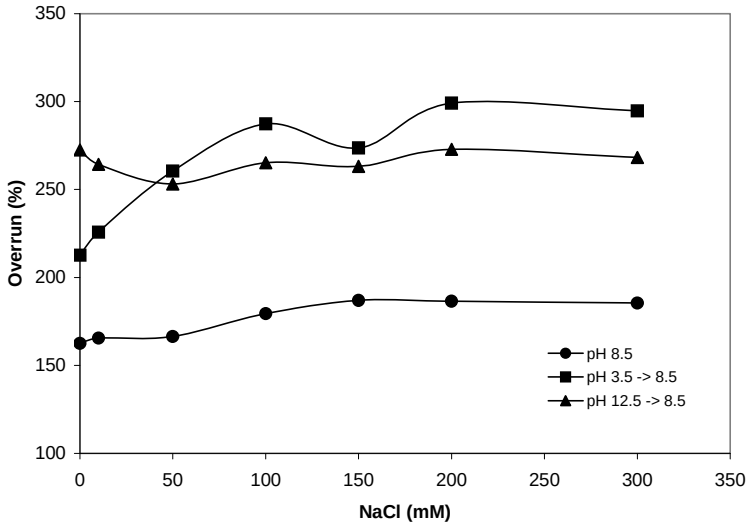
(b)



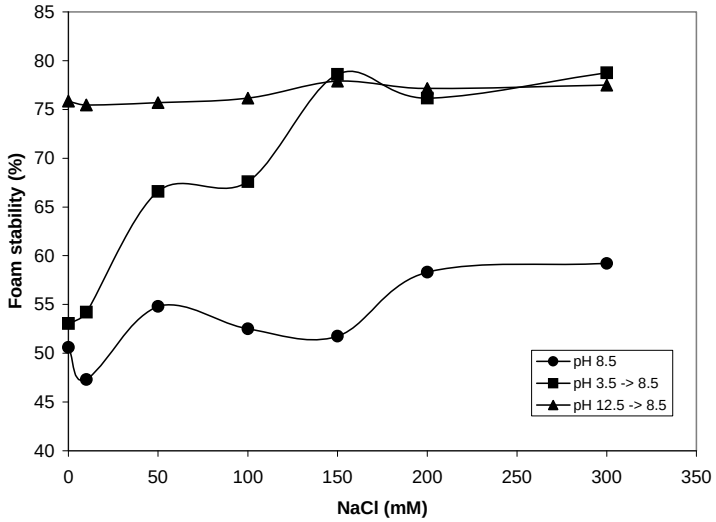
(c)

Figure 6. The influence of unfolding and refolding time on the foaming properties of egg albumen proteins. (a) Foaming capacity; (b) Foam stability; (c) Liquid drainage.

Influence of salt concentration on the foaming properties of pH treated egg albumins
It was also of interest to study if salt concentration would have an impact on the foaming properties of the egg albumins. Figure 7 gives an example of the results for the different salt levels. It was found that salt levels from 0-300 mM NaCl had little impact on the foam overrun (%) for control (pH 8.5) and alkali treatment (e.g. pH 12.5→ 8.5), while increased salt concentration did increase the overrun for the acid-treated egg albumens (e.g. pH 3.5→8.5). Similar results were obtained for foam stability, which was most greatly affected by the acid treatment. The data thus suggest great flexibility for the alkali treatment in particular, as not only are they not particularly time sensitive but also not too salt sensitive, and thus strong stable foams can be formed over a range of salt concentrations. The salt sensitivity of the acid treated samples may on the other hand be used to produce different textures products, since varying foams can potentially be made.



(a)



(b)

Figure 7. The influence of ionic strength (NaCl concentration) on the foam stability of egg albumin. Control (pH 8.5), acid treated (adjusted to pH 3.5 for 1 hour followed by adjustment to pH 8.5) and alkali treated (adjusted to pH 12.5 for 1 hour followed by adjustment to pH 8.5) are shown.

Change in surface and total sulfhydryl contents of the egg albumen after the pH-induced unfolding and refolding regime

Due to the insolubility of Ellman's reagent at acidic pH, only the surface and total sulfhydryl contents of egg albumen samples after the pH treatments of "pH 1.5, 2.5, 11.5 or 12.5 → 8.5"

were determined and compared to those of the control (pH 8.5). For each treatment, three samples were taken for the analysis: a. egg albumen solution after refolding at pH 8.5 and before foaming (identified as "Total"); b. the liquid fraction of egg albumen sample after holding the sample for 30 min after foaming (identified as "F. L."); c. the drained liquid fraction of the foam part after separating the foam part from F. L. for 18-24 hr (identified as F. F.). The contents of surface and total -SH groups in the egg albumen samples are shown in Table 1 (no calcium). The surface -SH content of the control (pH 8.5) was 8.19 μM/g protein, which is higher than the values reported by Howell and Taylor (1995). The authors reported no surface -SH in egg albumen or ovalbumin and claimed that their results were based on the fact that the -SH groups were located internally in the proteins (Howell and Taylor 1995). Although many of the egg albumen proteins have disulfide linkages, ovalbumin is the only protein in egg albumen with -SH groups and has 4 known –SH groups (Tatsumi and others 1998). The -SH groups are in hydrophobic regions of ovalbumin and thus are known to react poorly to sulfhydryl reacting agents in its native state (Doi and others 1989). The fact that we found surface -SH groups in the control might reflect structural modification of the egg albumen proteins due to the pH adjustment from their native pH (the egg albumen solution in 100 mM NaCl was at pH 7.0-7.3) to pH 8.5. It is also possible that the preparation method used to make the egg albumen could have induced a slight structural modification of the proteins, thus exposing more -SH groups.

Table 1. The surface and total sulfhydryl group contents (μM/g protein) of the egg albumin proteins after the pH-induced unfolding and refolding regime in the absence of calcium[a].

	pH 8.5 (control)		pH 1.5→8.5		PH 2.5→8.5		pH 11.5→8.5		pH 12.5→8.5	
	Surface –SH	Total –SH	Surface –SH	Total –SH	Surface –SH	Total –SH	Surface –SH	Total –SH	Surface –SH	Total –SH
Total	8.19	39.89	72.77	34.55	30.90	40.40	9.83	35.99	24.83	25.40
F. L.	10.79	39.46	39.56	35.32	19.73	35.44	7.36	36.36	38.19	38.64
F. F.	8.42	40.34	26.55	38.49	11.42	36.66	5.45	33.67	10.85	9.33

[a]Total: egg albumin solution after refolding at pH 8.5 and before foaming; F.L.: the liquid fraction of egg albumin sample after holding the sample for 30 min after foaming; F.F.: the drained liquid fraction of the foam part after separating the foam part from F.L. for 18-24 hr.

The total -SH content of the control was however somewhat lower (39.89 μM/g protein) than the reported 47.9 μM/g protein for egg albumen at neutral pH (Howell and Taylor 1995). Compared to the total -SH content of the control, the total -SH contents of the samples after unfolding and refolding were generally lower. The difference reflects forming of disulfide bonds during the unfolding and refolding treatment. The statistical analysis shows that the total surface –SH content of proteins after pH 12.5→8.5 treatment was significantly lower than those of the other treatments and control, which did not significantly differ from each other. The surface -SH contents of egg albumen proteins after unfolding and refolding were greatly increased in all cases except for the pH 11.5 unfolding treatment compared with those of the control. Due to an unknown reason, the surface sulfhydryl content of the egg albumen after the treatment of "1.5→8.5" was calculated out to be higher than their total sulfhydryl content in the "Total' and "F. F." fractions, which is obviously not correct. The interfering factors are unknown. Taking out the incorrect surface –SH reading of the proteins after pH 1.5→8.5 treatment, the surface –SH of

the proteins after pH 2.5→8.5 and pH 12.5→8.5 treatments was significantly higher than that of the control and the proteins after pH 11.5→8.5 treatment. There is no significant difference either between the surface –SH of the proteins after pH 2.5→8.5 and pH 12.5→8.5 treatments or between the surface –SH of the control and the proteins after pH 11.5→8.5 treatments. The increase of surface sulfhydryl content suggests only partial refolding of the egg albumen proteins leaving the otherwise buried -SH groups more accessible. The great increase in surface -SH content (except for the pH 11.5→8.5 treatment) coincided with the improvement in foaming properties of these proteins, which suggests that more exposure of -SH groups might favor the improvement of foaming properties of egg albumen. The decreased level of surface -SH groups with relatively high level of total –SH groups in the liquid fraction drained from the foam (F.F.) suggests this fraction contained proteins with native -SH group characteristics (compare with the "total" of control in table 1) so that the undrained foam fraction would have contained the proteins with increased surface -SH groups. The increased surface -SH group would then likely have caused disulfide bonding on foaming (Kitabatake and Doi 1987; Lechevalier and others 2003) leading to improved foam stability.

The pH 12.5→8.5 treatment which gave the largest improvement in foaming properties in these studied samples led to an increase in the surface sulfhydryl content of the proteins before foaming about 3 times that of the control. In addition, the surface sulfhydryl content and the total sulfhydryl content of the proteins after the pH 12.5→8.5 treatment but before foaming were similar and about 37% less than the total sulfhydryl content of the control. This suggests that a large portion of the sulfhydryl groups were exposed to the surface after the pH treatment and a considerable amount of the original sulfhydryl groups formed disulfide bonds during the treatment. The very low total -SH content (compared to the other treatments) of its drained liquid fraction (F.F.) suggests extensive S-S bonds may have formed in the undrained foam since even the least foam stabilizing proteins (i.e. the proteins in F.F.) had high levels of S-S formation. For the other treatments (pH 1.5→8.5, pH 2.5→8.5 and pH 11.5→8.5), the total sulfhydryl contents of the samples before foaming were not as low as that of the pH 12.5→8.5 treatment which means less formation of disulfide bonds.

It is worth noting that the egg albumen proteins after the pH 11.5→8.5 treatment showed significantly improved foaming capacities (Figure 4) despite their surface sulfhydryl content before foaming was about the same as that of the control. This shows that the relationship between foaming properties and surface sylfhydryl content is not straightforward and improvement of foaming properties is thus not necessary caused by the significant increase of surface sulfhydryl content or disulfide bonding. Hammershoj and others (1999) pointed out that the role of -SH groups and formation of disulfide bonds in protein foams is ambiguous since the foamability of different proteins are affected differently by these factors. The improvement of foaming properties seen is most likely a result of comprehensive changes of protein conformation, rather than the change of –SH group alone. The conformational change may include change of surface hydrophobicity and flexibility which could therefore be more important for improvement of foaming. The direct role of the exposed -SH groups, if any, is being investigated. The contents of surface and total -SH groups in the pH unfolded and refolded egg albumen samples in the presence of calcium are shown in Table 2, which shows similar patterns as those in the corresponding samples in absence of calcium.

Table 2. The surface and total sulfhydryl group contents (μM/g protein) of the egg albumin proteins after the pH-induced unfolding and refolding regime in the presence of calcium.

	pH 8.5 (control)		pH 1.5→8.5		PH 2.5→8.5		pH 11.5→8.5		pH 12.5→8.5	
	Surface –SH	Total –SH	Surface –SH	Total –SH	Surface –SH	Total –SH	Surface –SH	Total –SH	Surface –SH	Total –SH
Total	8.33	47.47	106.28	43.64	54.23	49.10	7.95	37.30	37.53	38.89
F. L.	13.62	48.11	32.3	36.64	20.05	41.49	10.12	37.03	32.2	34.68
F. F.	8.42	48.88	10.33	37.64	14.67	43.74	6.84	33.72	13.76	13.45

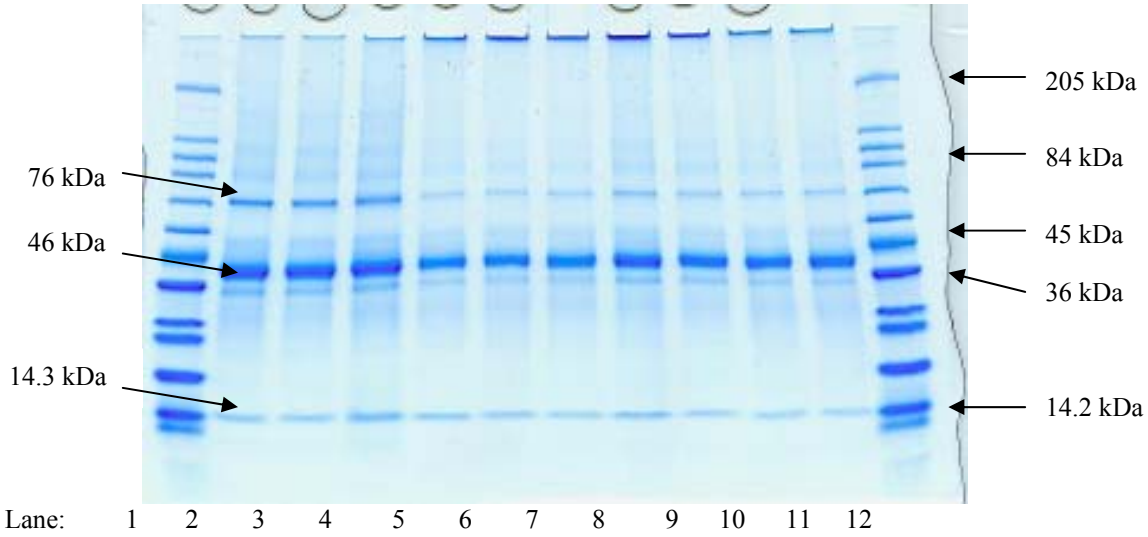
SDS-PAGE analysis of the egg albumen proteins after the pH-induced unfolding and refolding regime

To supplement the data on sulfhydryl contents, SDS-PAGE analysis was conducted on the egg albumen proteins with or without reducing agents. The SDS-PAGE patterns of egg albumen proteins after the pH 1.5→8.5, pH 2.5→8.5 and pH 11.5→8.5 treatments were similar (Figures 7 and 8). The intensities of ovalbumin (46 kDa), ovotransferrin (76 kDa) and lysozyme (14.3 kDa) of these samples were somewhat less than those of the control. A band of high molecular weight aggregates that could not enter the gel appeared on the top of each lane of the egg albumen samples after the unfolding and refolding treatments, but the bands of high molecular weight aggregates were very light for the samples of the control (Figure 8). This suggests that the aggregates were most likely stabilized by disulfide bonds. When mercaptoethanol was applied to the samples, the bands of the aggregates disappeared which confirmed that the aggregates were formed by disulfide bonds (Figure 9). This is consistent with previous findings by Ingadottir and Kristinsson (2003). The SDS-PAGE analysis of egg albumen proteins after the pH 12.5→8.5 treatment showed extensive aggregation of proteins, which is consistent with the significant decrease of total sulfhydryl group content (Table 1). Ovalbumin and ovotransferring were possibly both crosslinked via S-S bonds according to the SDS-PAGE analysis, but not lysozyme (at least to a much lesser extent). In the presence of mercaptoethanol, the bands of the aggregates lightened with the reappearance of the band of ovalbumin but interestingly not the ovotransferrin. Interactions other than S-S bonds should thus be responsible for the aggregation of ovotransferrin. The crosslinking or aggregation of the egg albumen proteins caused by the pH 12.5→8.5 treatment could thus be in part responsible for the great improvement on the foaming capacity of the proteins and the stability of the foams generated. This is interesting since the presence of S-S bonds prior to foaming has been found to reduce foamability of proteins such as lysozyme (Kato and others 1994) and bovine serum albumen (Yu and Damodaran 1991) due to the restriction of protein flexibility. This does not appear to be the case collectively for the egg albumen proteins. The pattern of SDS-PAGE analysis on the treated samples in the presence of calcium was almost identical to that of the treated samples in absence of calcium.

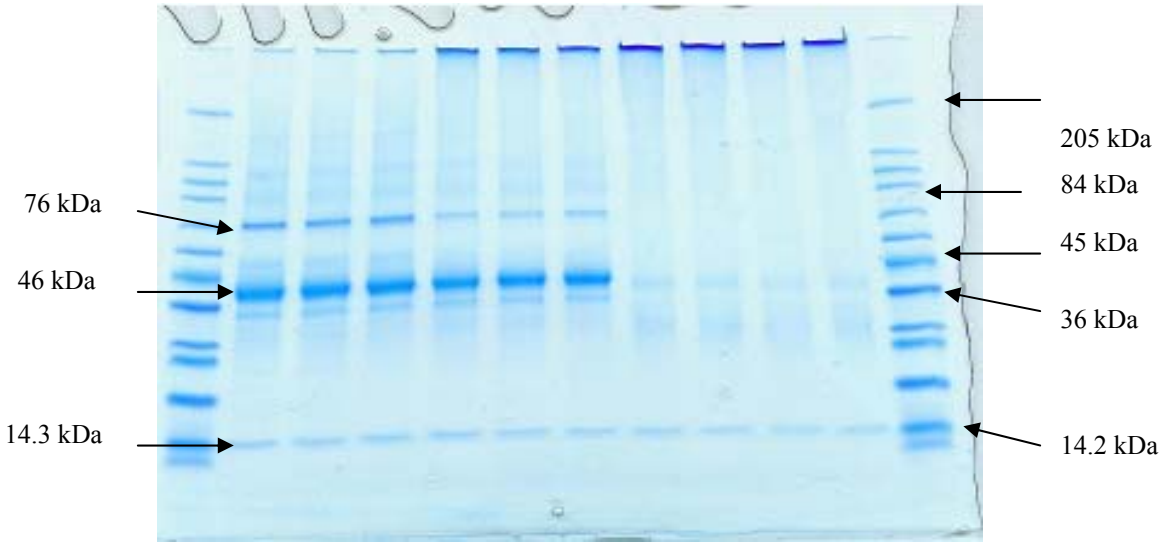
Surface hydrophobicity change of the egg albumen proteins after the pH-induced unfolding and refolding regime

Since changes in sulfhydryl contents alone could not explain improvements in foaming properties, the change of protein surface hydrophobicity was studied. A relationship exists between surface hydrophobicity of proteins and their surface active properties (Damodaran 1997). In this study, we chose the uncharged fluorescent probe PRODAN to interact with the hydrophobic patches of the egg albumen proteins. The uncharged probe helps to exclude the

interference of electrostatic binding of probes such as ANS (aniline-1-naphthalene-8-sulfonate) to proteins (Alizadeh-Pasdar and Li-Chan 2000). Due to the high turbidity of egg albumen proteins refolded to acidic foaming pH values (especially pH 4.5 and 5.5), the surface hydrophobicity of the egg albumen proteins was only studied for the samples with the treatments of refolding at pH 8.5 (i. e. pH 1.5, 2.5, 11.5 or 12.5 → 8.5) which are the same pH treatments as those chosen for sulfhydryl content analysis. The surface hydrophobicity of egg albumen proteins determined in this study was much higher than that of ovalbumin reported by Haskard and Li-Chan (1998). Since the egg albumen is a collection of proteins, measurement of egg albumen proteins other than ovalbumin alone is likely responsible for the difference. In addition, the emission peak we found for egg albumen in the presence of PRODAN was at 435 nm instead of the 466 nm peak for ovalbumin in the presence of PRODAN (Haskard and Li-Chan 1998).

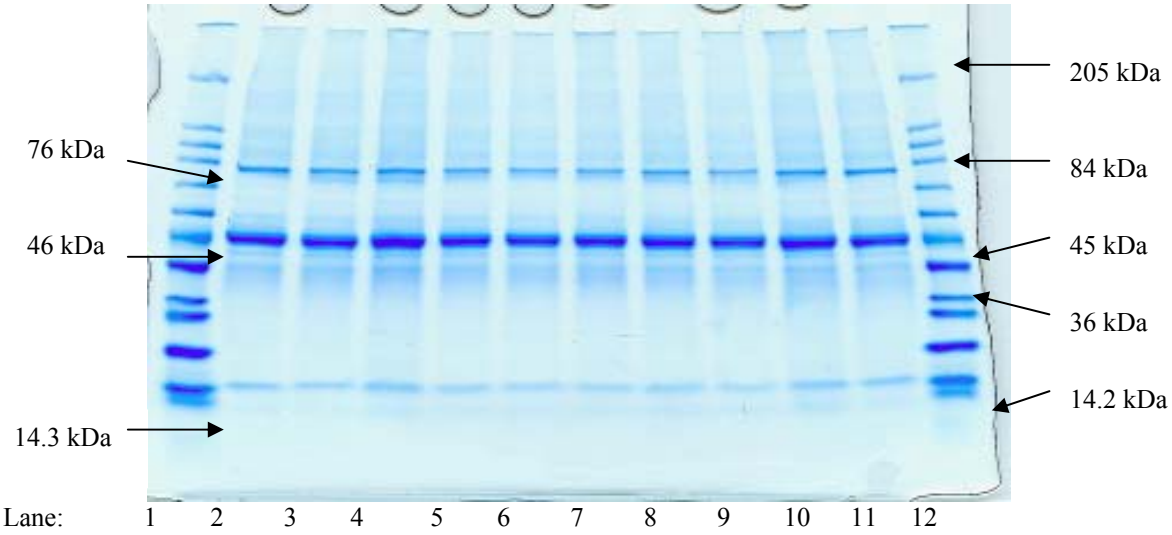


(a)

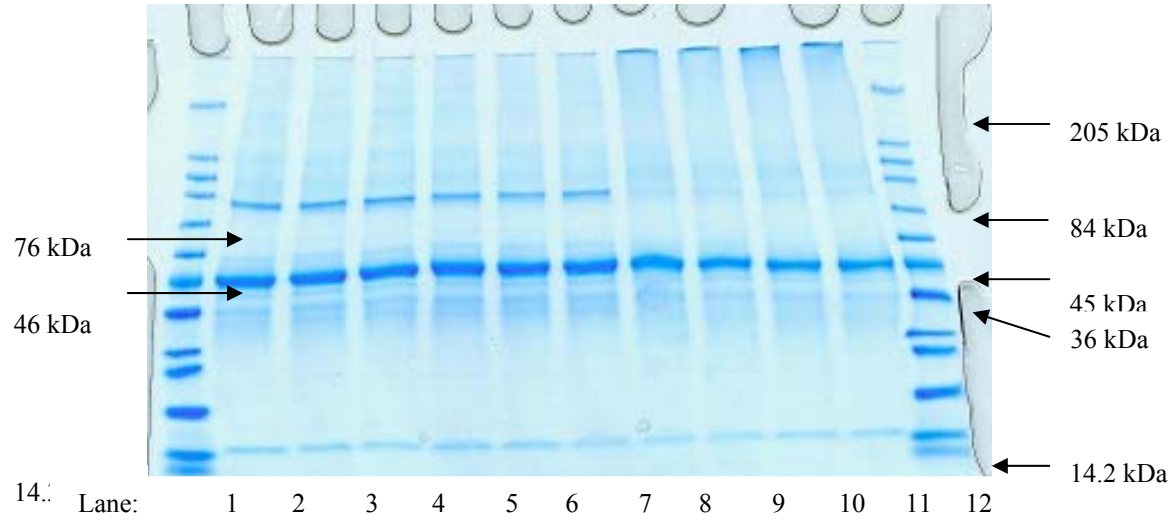


(b)

Figure 8. The protein profile of egg albumen (in absence of calcium) after pH-induced unfolding and refolding analyzed by SDS-PAGE without mercaptoethanol. (a). From left to right: Lane 1 and 12, Molecular Weight Standard; Lane 2-4, Total, F.L., F.F. of control "pH 8.5"; Lane 5-7, Total, F.L., F.F. of sample "pH 1.5→8.5"; Lane 8-10, Total, F.L., F.F. of sample "pH 2.5→8.5". (b). From left to right: Lane 1 and 12, Molecular Weight Standard; Lane 2-4, Total, F.L., F.F. of control "pH 8.5"; Lane 5-7, Total, F.L,. F.F. of sample "pH 11.5→8.5"; Lane 8-10, Total, F.L., F.F. of sample "pH 12.5→8.5". Total: egg albumen solution after refolding at pH 8.5 and before foaming; F.L.: the liquid fraction of egg albumen sample after holding the sample for 30 min after foaming; F.F.: the drained liquid fraction of the foam part after separating the foam part from F.L. for 18-24 hr.



(a)

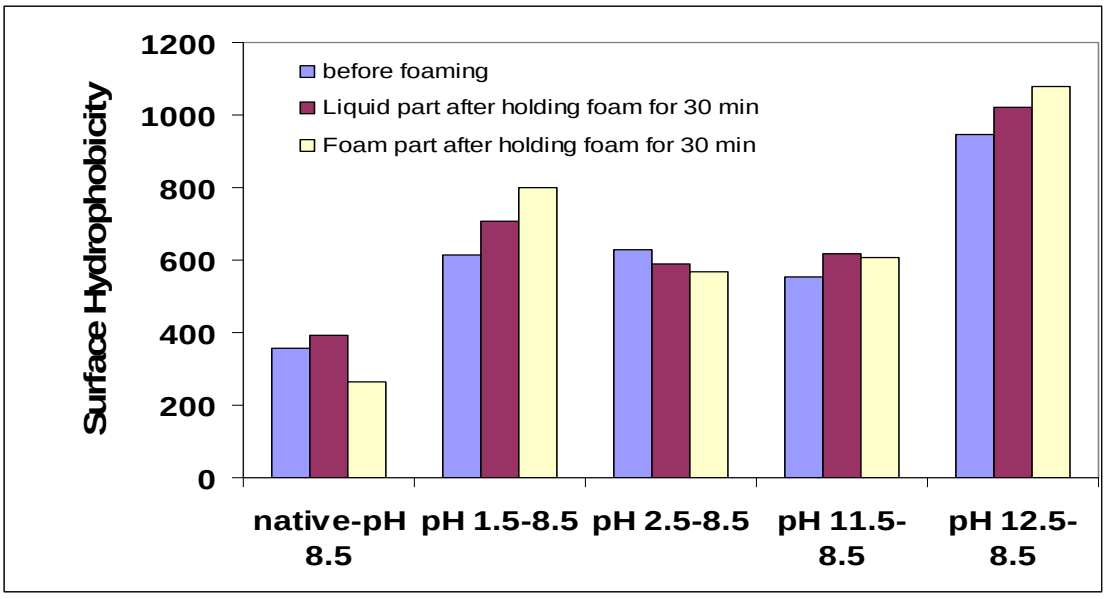


(b)

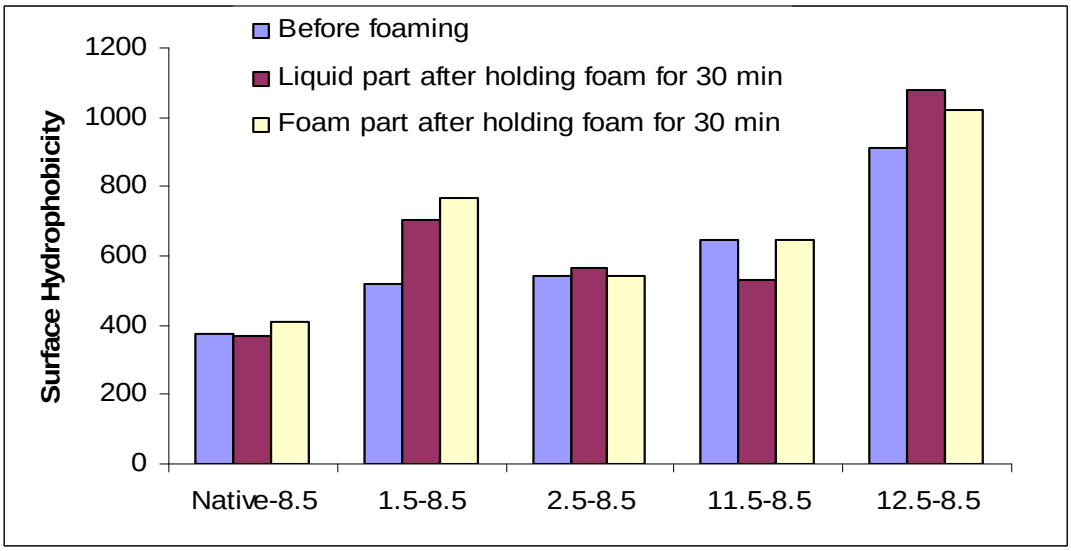
Figure 9. The protein profile of egg albumen (in absence of calcium) after pH-induced unfolding and refolding analyzed by SDS-PAGE with mercaptoethanol added. (a). From left to right: Lane 1 and 12, Molecular Weight Standard; Lane 2-4, Total, F.L., F.F. of control "pH 8.5"; Lane 5-7, Total, F.L., F.F. of sample "pH 1.5→8.5"; Lane 8-10, Total, F.L., F.F. of sample "pH 2.5→8.5". (b). From left to right: Lane 1 and 12, Molecular Weight Standard; Lane 2-4, Total, F.L., F.F. of control "pH 8.5"; Lane 5-7, Total, F.L,. F.F. of sample "pH 11.5→8.5"; Lane 8-10, Total, F.L., F.F. of sample "pH 12.5→8.5". Total: egg albumen solution after refolding at pH 8.5 and before foaming; F.L.: the liquid fraction of egg albumen sample after holding the sample for 30 min after foaming; F.F.: the drained liquid fraction of the foam part after separating the foam part from F.L. for 18-24 hr.

The surface hydrophobicity of egg albumen proteins (in absence of calcium) after pH 1.5→8.5, pH 2.5→8.5 and pH 11.5→8.5 treatments and before foaming was about twice that of the control (pH 8.5), while the surface hydrophobicity of egg albumen after the pH 12.5→8.5 treatment and before foaming was about 3 times that of the control (Figure 10a). Statistical analysis shows that the surface hydrophobicity was significantly affected by the different pH treatments ($\rho<0.05$), but not significantly different among the fractions. The surface hydrophobicity of the proteins after pH 1.5→8.5, pH 2.5→8.5 and pH 11.5→8.5 treatments was significantly higher than that of the control but did not differ among each other. The pH 12.5→8.5 treatment had significantly higher hydrophobicity than all the other treatments. This correlates well with the results of the foaming capacity of the pH-treated egg albumen proteins where the pH 12.5→8.5 treatment gave higher foaming capacity than the other treatments which had similar increases in foaming capacities amongst each other. Similar results have been obtained for the surface hydrophobicity measurements on the pH unfolded and refolded samples in the presence of calcium (Fig. 10b). These results show that the increase of surface hydrophobicity had better correlation with the improvement of foaming properties than the change in surface sulfhydryl content did. It has been demonstrated that the rate at which a protein can reduce interfacial tension between water and air is the most important factor for good foamability (Damodaran 1997). Increased hydrophobicity would normally improve the ability of proteins to rapidly adsorb to a hydrophobic surface, which is the first step in foaming. Once at the surface, the more rapidly the protein is able to undergo a conformational change, generally the better the foamability. In this step, the flexibility of the protein is important (Damodaran 1997). Lechevalier and others (2003) demonstrated that ovalbumin and ovotransferrin but not lysozyme underwent conformational changes during foaming, especially in tertiary structure. The pH-treated egg albumen proteins reported here not only have higher hydrophobicity than the control which would aid in rapid adsorption to the interface, but also presumably have a more open configuration due to their misfolded structure leading to increased flexibility. Proteins in this state, often called the "molten globular" state, are known to have higher surface activity due to more exposed hydrophobic groups and are able to stabilize hydrophobic and hydrophilic interfaces more effectively than native proteins (Dickinson and Matsumura 1994; Kristinsson and Hultin 2003b). The pH-treated egg albumen proteins are thus at an advantage over native proteins since they are already partially denatured which likely is the reason for their better foamability. It was also observed that the texture of the foams made with the pH-treated proteins was very different from that made with the native proteins. The foams generated by the pH-treated proteins were noticeably thicker. This could be due to more extensive stabilizing networks in the foams, which are in part due to S-S bonds and hydrophobic contacts. It is possible that the partially unfolded proteins have higher hydrodynamic ratios than

the native proteins (i.e. more extended structure). The high hydrodynamic ratios would increase the viscosity of the system, which could lead to improved stability. For example, adding ovomucin to egg albumen greatly increases foam stability presumably due to the high viscosity of ovomucin (Nakamura and Doi 2000).



(a)



(b)

Figure 10. The surface hydrophobicity of the egg albumen proteins after the pH-induced unfolding and refolding regime. Total: egg albumen solution after refolding at pH 8.5 and before foaming; F.L.: the liquid fraction of egg albumen sample after holding the sample for 30 min after foaming; F.F.: the drained liquid fraction of the foam part after separating the foam part from F.L. for 18-24 hr. (a) in absence of calcium (b) in presence of calcium.

Rheological properties of the foams generated from egg albumen proteins after the pH-induced unfolding and refolding regime

In this study, we used an acrylic ST X plate with a cross-hatched surface as the upper plate. This geometry is specially made for foam measurements by TA instruments. A pasting sandpaper was glued to the lower plate to act as the lower plate. To find the region of linear viscoelasticity, a strain sweep study was performed at a constant frequency of 1 Hz. Figure 9 gives an example of the strain sweep studies on the foam generated from egg albumin after native-pH 8.5 treatment in the absence of calcium. We found that the storage moduli of the foams were constant for strains up to 0.5% (Figure 11). Effect of frequency was then investigated in the linear viscoelastic region as the strain value was always kept below the critical value (for example <0.5% for the foam generated from egg albumin after native-pH 8.5 treatment). Figure 12 shows a frequency sweep study for foam obtained from egg albumin unfolded at pH 2.5 and refolded at pH 4.5 in the absence of calcium. This foam was characterized by one of the smallest values of dynamic yield stress (Table 3). Both storage moduli and loss moduli were insensitive to frequency and storage moduli were about 8 times higher than the loss moduli (Figure 12). The foam behaved like a highly elastic material (Khan et al 1988).

Steady shear rate produces steady increase of strain with time. Figure 13 shows the relationship between time of shearing and obtained shear stress values for three different foams. Static yield stress was measured as a shear stress value at which the departure from linearity of the shear stress-time profile is observed. Dynamic yield stress was the maximum noted value of the shear stress. Yield stress is one of the most important rheological properties of materials. Foams support small stress like solid materials do, but flow under shear like fluids do. This solid-like to fluid-like transition depends on the foam structure. This is particularly important, as the structure of fluid foams will influence the structure of solid foams obtained by heating or other solidification processes. Yield stress can be defined as a stress below which no flow is observed under the conditions of experiment and was proven to be very useful in a range of applications (Barnes 1999). Yield stress can be measured by applying low, constant shear. As the rheometer tries to move the spindle to meet the designated rotational speed, shear stresses are developed. Initially, the sample deforms elastically due to stretching of the foam structure. At a point, the network begins to break under increased shear stress. The development of viscoelastic effects is represented by the departure from linearity of the shear stress-time profile. At a point, the structure is fractured and a maximum stress can be obtained. At last, the stress decays to some equilibrium value as the spindle begins to rotate (Saak et al. 2001). Two yield stress values can be obtained by a shear stress-time measurement. When the shear stress-time curve starts to be nonlinear, the onset of viscoelasticity occurs and this value of shear stress is called a static yield stress, as in the experimental scale of time there is no macroscopic flow of the material. Flow of the material causes a drop in the shear stress value, as the resistance of the material dramatically decreases. This maximum value of shear stress is defined as the dynamic yield stress as the broken structure flows in a “dynamic” state. For all investigated foams, an increase in static and dynamic yield stress was observed as the foams were matured for 30 min (Figure 14, Table 3).

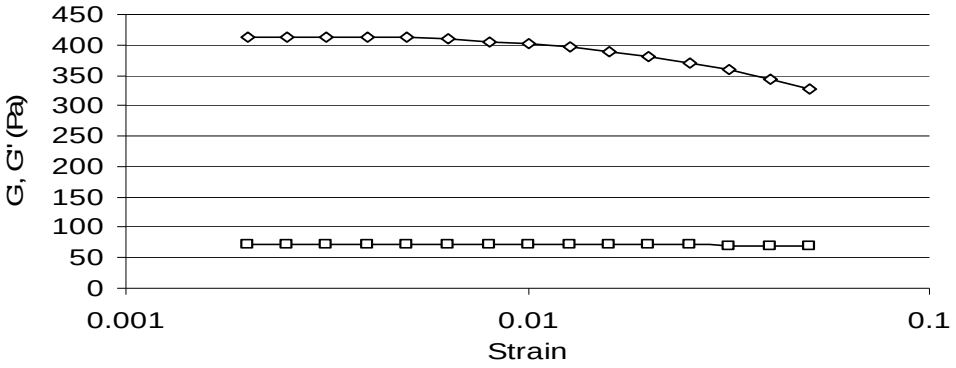


Figure 11. Strain sweep experiment for a fresh egg albumin foam formed at pH 8.5 in the absence of calcium. Storage modulus (G' -◇-) and loss modulus (G" -□-) as a function of strain are shown in the graph. Frequency was set at 1 Hz and the strain sweep was performed from 0.002-0.05.

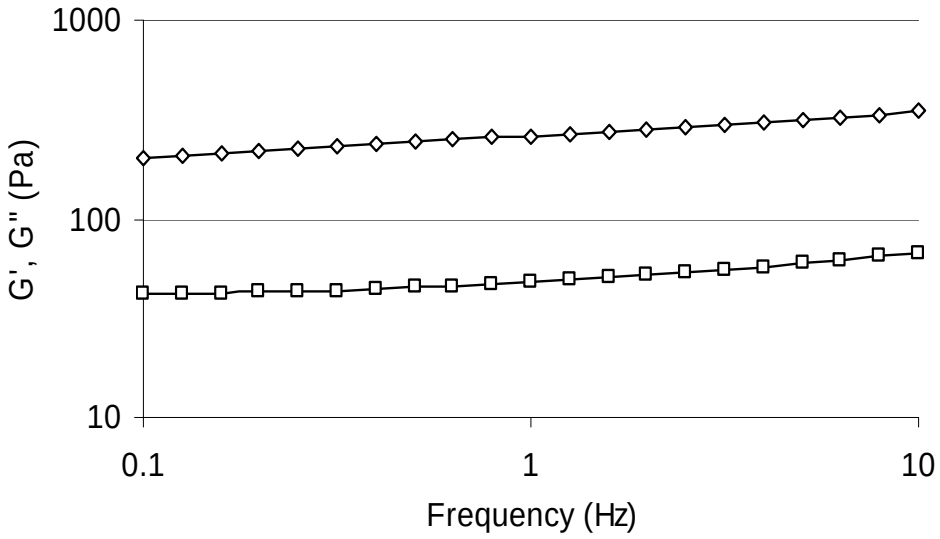


Figure 12. Frequency sweep experiment for pH 2.5→4.5 (fresh foam) in the absence of calcium. Storage modulus (G'-◇-) and loss modulus (G" -□-) as a function of strain are shown in the graph. The frequency was varied from 0.1-10 Hz and strain was set at 0.4%.

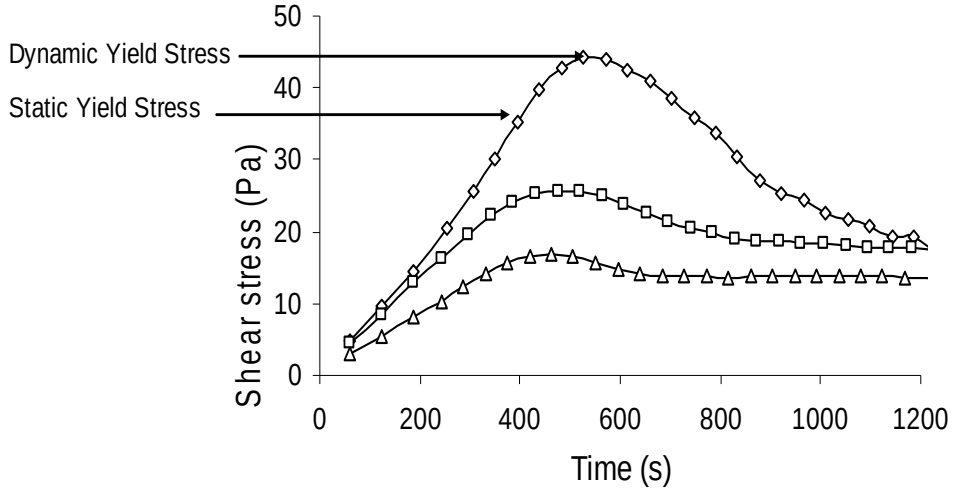


Figure 13. Shear stress as a function of steady shear for foams at different pH treatments. pH 11.5→4.5(fresh) (-△-) and pH 12.5→4.5(fresh) (-◇-) foams compared to pH 4.5 (fresh) (-□-) control foam in absence of calcium. The dynamic and static yield stresses are shown in the graph. A steady shear of 0.05 s^{-1} for 1200 s was applied to the foams.

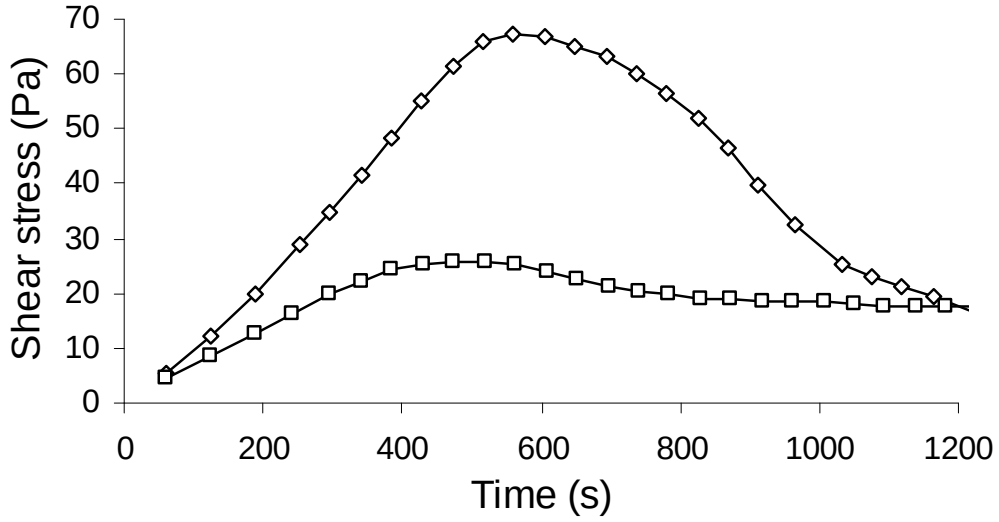


Figure 14. Shear stress as a function of steady shear for fresh (f) (-□-) compared to separated (s) (-◇-) foams. Foams at pH 4.5 in the absence of calcium are shown as an example. A steady shear of 0.05 s^{-1} for 1200 s was applied to the foams.

Table 3. Static yield stress and dynamic yield stress for fresh and separated egg albumin foams in the absence of calcium.

pH treatment	Fresh Foam		Separated Foam	
	Static Yield Stress (Pa)	Dynamic Yield Stress (Pa)	Static Yield Stress (Pa)	Dynamic Yield Stress (Pa)
4.5	22.43	25.43	54.5	67.5
1.5→4.5	26.1	31.1	59.6	73.0
2.5→4.5	13.8	17.0	50.7	58.6
11.5→4.5	11.4	14.20	51.0	58.9
12.5→4.5	35.2	44.4	83.5	100.17
8.5	3.4	5.53	30.2	37.9
1.5→8.5	36.35	47.12	91.1	122.58
2.5→8.5	29.0	33.5	66.3	90.8
11.5→8.5	16.5	18.56	48.8	61.4
12.5→8.5	39.2	43.46	66.3	88.5

As presented before, in general, less drainage and higher stability were observed for the pH-treated foams compared to the untreated foams (Fig. 4). Liquid drainage is caused by the drain of water due to gravitational force, and foam stability is affected by Ostwald ripening. These two effects have a conflicting effect of yield stress measurements (Gardiner et al., 1998). Drainage of the foam increases the air fraction and in this way increases the yield stress (liquid itself is usually a low viscosity material). Foam stability was measured as the volume after 30 min divided by fresh foam volume. Decrease in foam volume is caused partly by Ostwald ripening, thus the foam which is left is usually composed of larger bubbles. Foam composed of larger bubbles has lower yield stress. Thus, yield stress of separated egg albumen foam is predominantly determined by the drainage process. Samples with the highest drainage should have the highest relative increase in yield stress value after 30 minutes. Relative increase in yield stress was calculated as:

$$RIY = Y_s - (Y_f/Y_s)$$

where:

Y_f - yield stress of fresh foam
Y_s - yield stress of the separated foam after drainage

Values of the RIY are shown in Table 4. As liquid drainage is mainly responsible for foam stability, relative increase in yield stress should be also related to the stability of the foam. More stable foams should have lower value of RIY. Comparison of the RIY values with foam stability and liquid drainage showed in Fig 4 fully support suggested relationships, i.e. the pH-treatments yielding the highest foam stability and lowest liquid drainage had the lowest RIY.

Table 4. Relative increase in static yield stress and dynamic yield stress after separation of pH treated egg albumin foams in absence of calcium.

pH treatment	Relative Increase in Static Yield Stress	Relative Increase in Dynamic Yield Stress
4.5	0.59	0.63
1.5→4.5	0.56	0.57
2.5→4.5	0.73	0.71
11.5→4.5	0.78	0.76
12.5→4.5	0.58	0.56
8.5	0.88	0.86
1.5→8.5	0.60	0.62
2.5→8.5	0.56	0.63
11.5→8.5	0.66	0.70
12.5→8.5	0.41	0.51

The yield stresses of foams generated from pH-induced unfolding and refolding egg albumen in presence of calcium were measured immediately after their formation and shown in Table 5. The yield stresses are generally higher than those of the foams with no calcium, especially for those when the unfolding pH was at the alkali side. The calcium might interact with the egg white proteins at high pH, but the interaction is at least not totally cancelled out when the pH was reajusted back to 4.5 or 8.5. This reserved interaction then accounts for the higher yield stess.

Table 5. The yield stresses of foams generated from pH-induced unfolding and refolding egg albumen in presence of calcium. The foams were generated immediately after treatment on egg albumen.

pH treatment	Static Yield Stress (Pa)	Dynamic Yield Stress (Pa)
4.5	36.81	42.94
1.5→4.5	33.54	37.88
2.5→4.5	15.06	16.46
11.5→4.5	34.08	41.82
12.5→4.5	50.23	61.39
8.5	5.45	6.26
1.5→8.5	22.74	28.81
2.5→8.5	30.46	38.22
11.5→8.5	41.59	51.63
12.5→8.5	79.80	93.09

Figure 15 shows stress amplitude sweep experiment for two different foams. Below a critical value of the oscillatory stress, a plateau in storage and loss moduli was observed even for the weakest foam obtained at pH 8.5 (compare Table 3). A critical value of oscillatory stress marks an upper limit of the linear viscoelastic properties of the foams. Below this value the sample was in the nonlinear viscoelastic region and most often its structure is broken. At this value there was sudden drop in moduli. After drainage of the foam for 30 min, an increase in critical value of oscillatory stress was observed (Figure 16). Higher stress was needed to disrupt the structure of the foam after drainage. This observation is in agreement with increase of yield stress value after 30 min (Table 3). Plotting of the relationship between shear stress and strain and between oscillatory stress and strain on the same chart confirms that there is an agreement between dynamic yield stress and critical stress amplitude (Figure 17). Both values show stress, which causes disruption of the foam structure.

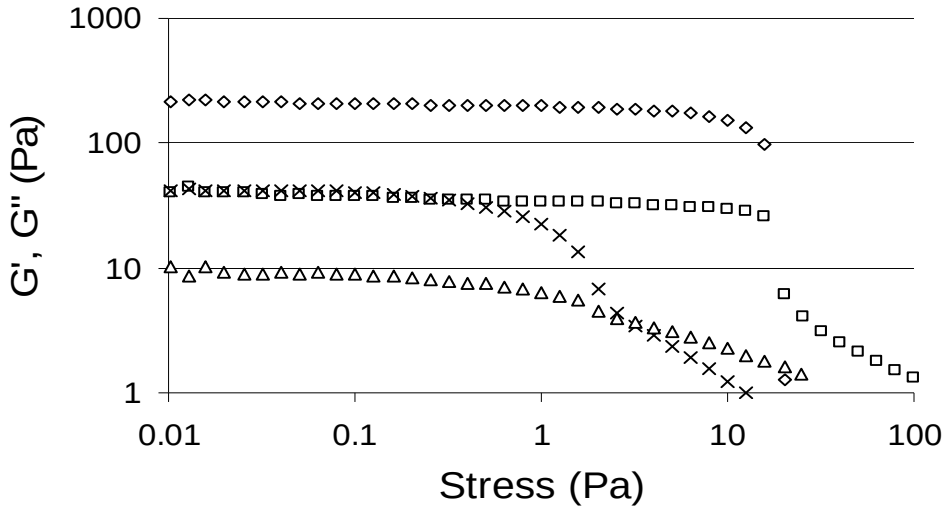


Figure 15. Stress amplitude sweep for the foams from the pH-treated egg albumins (pH 11.5→8.5, in absence of calcium) compared to a foam from untreated egg albumin (pH 8.5, in absence of calcium). The oscillatory stress sweep was done from 0.008-100 Pa at a 1.0 Hz frequency. Storage modulus (G') and loss modulus (G") as a function of strain are shown in the graph. ◇ : G' for pH 11.5→8.5 (fresh). □ : G" for pH 11.5→8.5 (fresh). × : G' for pH 8.5 (fresh). △ : G" for pH 8.5 (fresh).

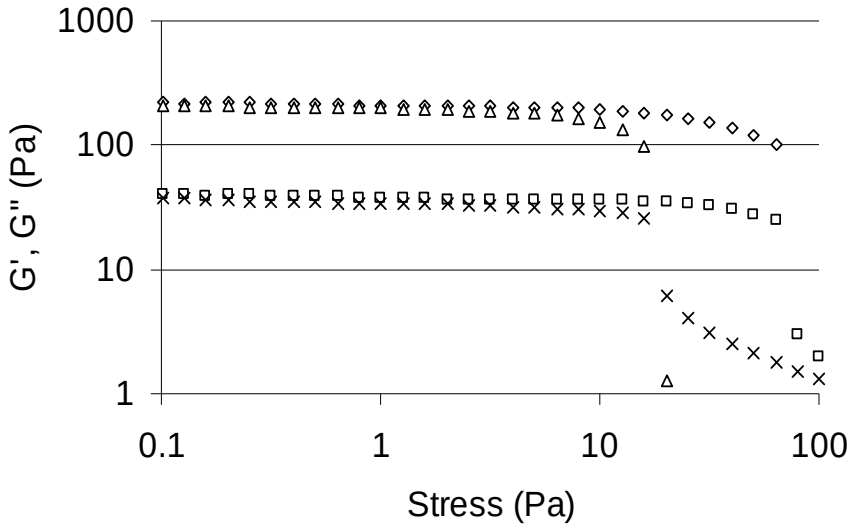


Figure 16. Stress amplitude sweep for one of the fresh and separated foam (pH 11.5→8.5 treatment, in absence of calcium). The oscillatory stress sweep was done from 0.008-100 Pa at a 1.0 Hz frequency. Storage modulus (G') and loss modulus (G") as a function of strain are shown in the graph. ◇ : G' for pH 11.5→8.5 (separated). □ : G" for pH 11.5→8.5 (separated). △ : G' for pH 11.5→8.5 (fresh). × : G" for pH 11.5→8.5 (fresh).

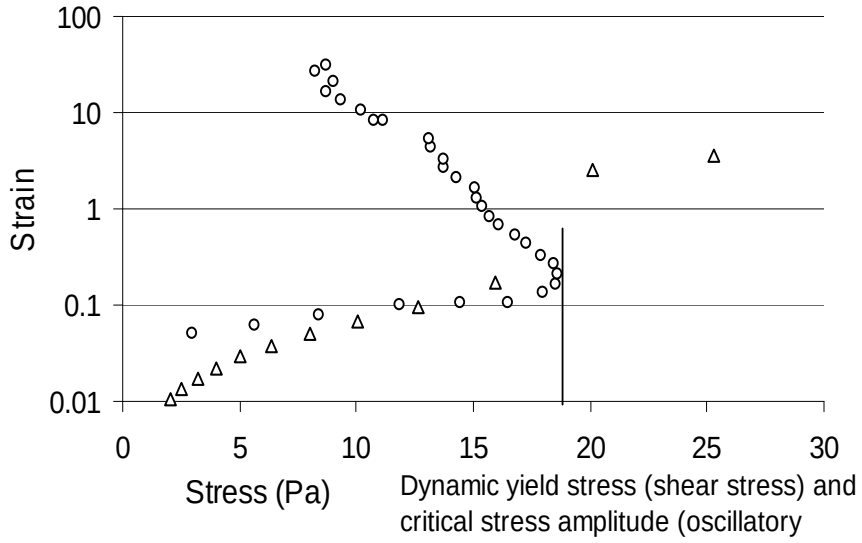


Figure 17. Relationship between stress and strain from the oscillatory and shear measurements for fresh foams made from egg albumins subjected to the pH 11.5→8.5 treatment in absence of calcium. △ : Oscillatory stress. ○ : Shear stress.

Table 6 shows storage modulus and phase angle at the plateau region for fresh and separated egg albumen foams generated from pH-treated egg albumen in absence of calcium. It is interesting to note that samples with different static and dynamic yield stress had similar values of G'. One would have expected higher yield stress values to have corresponded to higher G', which was not always the case. An example is the pH treatment 12.5→8.5. The fresh foams had a G' of 363.2 Pa while the separated foams had a lower G' of 239.1 Pa (Table 6). However, the values of static and dynamic stress were higher for the separated foam compared to the fresh foam, or 66.3 and 88.5 compared to 39.2 and 43.6, respectively (Table 3). For samples with different static and dynamic yield stress (e.g. sample 12.5-8.5, in Table 3), even lower values of G' were observed for separated foams in comparison to fresh foams of the same pH treatments" G' equals 363.2 in comparison to 239.1 for separated foam. Thus, there was no correlation between storage moduli or phase angle and dynamic or static yield stress of the analyzed foams. These findings are supported by a study by Govindasamy-Lucey et al. (2004) which investigated the manufacture of Parmesan cheese and found that storage modulus values at cutting of the curd were similar, but yield stress values differentiated. Similarly, Vaikousi et al. (2004) observed for barley (1 -> 3,1 -> 4)-beta-glucans gels that higher molecular weight samples exhibited higher yield stress while the dynamic storage modulus decreased with increasing molecular size of the polysaccharide.

Table 6. Storage modulus (G'; Pa) and phase angle (δ; °) in the linear region of the oscillatory stress sweep for fresh and separated egg albumin foams generated from pH unfolded and refolded egg albumen in absence of calcium.

	Fresh Foam		Separated Foam	
pH treatment	G'	δ	G'	δ
4.5	331.6	11.09	332.9	11.82
1.5→4.5	360.9	11.26	418.7	10.89
2.5→4.5	231.9	11.04	297.4	11.21
11.5→4.5	378.1	11.71	399.3	11.04
12.5→4.5	612.0	11.71	742.6	11.24
8.5	41.49	12.24	197.0	9.31
1.5→8.5	584.6	8.38	382.4	9.33
2.5→8.5	376.9	8.91	270.5	9.31
11.5→8.5	210.5	10.36	216.7	10.06
12.5→8.5	363.2	9.57	239.1	9.54

Table 7 represents values of stress amplitude at the onset of nonlinear viscoelastic behavior. Stress values were in some cases the same, as the stress sweep was performed using 10 stress values per decade. Stress amplitude values were higher for separated foams. Considering, that measurements were performed using 10 values of stress per decade, there was high correlation (R^2=0.89) between stress amplitude at the unset of nonlinearity and dynamic yield stress (Fig. 18). The foams generated from pH unfolded and refolded egg albumen in presence of calcium showed similar readings of G' at the plateau region and stress amplitude at the onset of nonlinear viscoelastic behavior compared to the foams subjected to same pH treatment but in absence of calcium.

Table 7. Stress amplitude (Pa) at the onset of nonlinear viscoelastic behavior for fresh and separated egg albumin foams generated from pH unfolded and refolded egg albumen in absence of calcium.

pH treatment	Fresh Foam	Separated Foam
4.5	6.35	10.07
1.5→4.5	6.35	10.07
2.5→4.5	4.00	8.00
11.5→4.5	4.00	10.07
12.5→4.5	10.07	15.96
8.5	0.50	6.35
1.5→8.5	10.07	20.09
2.5→8.5	8.00	12.68
11.5→8.5	6.35	10.07
12.5→8.5	10.07	12.68

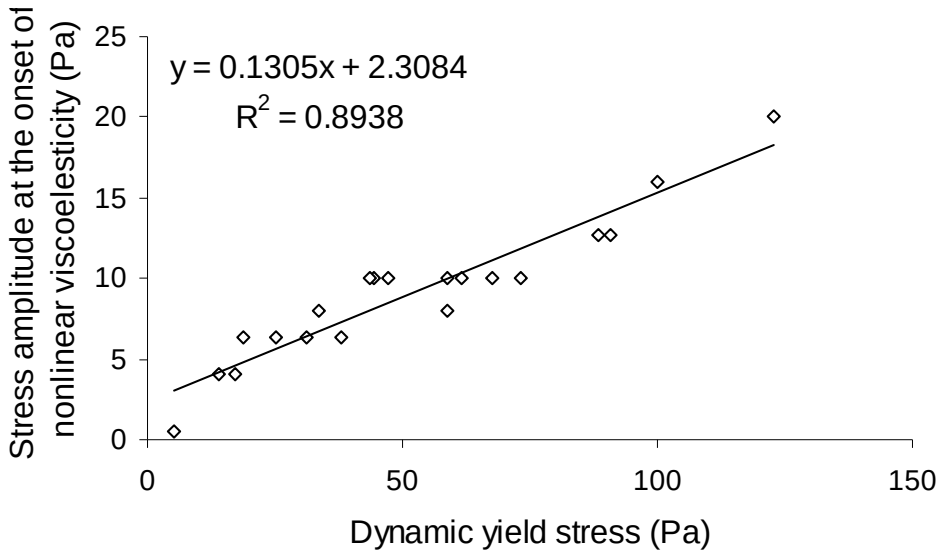


Figure 18. Correlation between dynamic yield stress and stress amplitude at the onset of nonlinear viscoelasticity for all the studied foams in absence of calcium.

The data clearly shows that most of the foams generated from egg albumin after the pH-induced unfolding and refolding regime had firmer texture than the foams generated from egg albumin not subjected to the pH treatments. The increase in firmness was especially notable for egg albumin refolded to pH 8.5 after either low or high pH unfolding. At this pH, the control had a very weak structure while the foams from pH-treated egg albumin (with a final pH of 8.5) were even firmer than the control at pH 4.5 (which is an ideal foaming pH for egg albumin). Presence of calcium at 10 mM increased the yield stresses of foams where the unfolding was done at alkali pH values. The data thus complement the findings that not only the foaming capacity and foam

stability, but also the texture of the foams may be improved by treating egg albumin with the pH-induced unfolding and refolding regime. This finding is meaningful to the egg processing industry as egg albumin after the pH-induced unfolding and refolding regime can be used in applications where regular egg albumin perform poorly for foaming. In addition, it also shows that the measurement of yield stress of foams by increase of shear rate, using a geometry which prevents a slippage, is a good method to determine their firmness. Changes in viscoelastic properties of foams can be measured by stress amplitude sweep and these results are in agreement with (shearing obtained dynamic yield stress). Tabilo-Munizaga and Barbosa-Canovas (2005) found that the yield stress provided satisfactory detection of differences in firmness of yogurts and was a good predictor of the sensory firmness perceived by panelists, it is possible that measurement of rheological properties using this method can be used to supplement the sensory panel research on the egg albumin foams in the future.

PART II

Rheological and Sensory Properties of Angel Food Cake and Meringue with pH Unfolded and Refolded Egg Albumen

INTRODUCTION

Egg white foam is responsible for the structure of angel food cake, meringue, soufflés and puffy omelets, to name a few important foods. When egg white is beaten, air bubbles are trapped in liquid albumen. After heating, the air cells expand and the egg protein coagulates around them, giving permanence to the foam. If egg whites are under beaten, the volume of the finished product will be less than desired. Over beaten whites lack elasticity and they can not expand properly when heated. Angel food cake and meringue does not contain egg yolks or any other fat, making it a good choice for low fat or low calorie dessert presentations.

To improve foaming properties of egg white, different procedures have been used (Kato et al. 1989, Morr et al. 2003). Lee and Chen (2002) evaluated papain treatment and desugarization of egg white solids on angel food cake. Darkening was reduced in desugarized egg white and solubility, foaming capacity and cake volume performance improved with increasing amounts of papain. Arunepanlop et al. (1996) studied partial replacement of egg white proteins with whey protein isolate (WPI) on the appearance, structure, texture and sensory properties of angel food cakes and concluded, that up to 25% of EWP could be replaced with WPI in angel cakes without adversely affecting physical and sensory properties. Pernell et al. (2002) compared the quality of angel food cakes made from egg white or whey protein foams. Cake expansion during baking was shown to be a function of protein concentration regardless of protein type. Heat treating whey protein or adding xanthan gum increased cake volume, but not to the extent of egg white protein. Cakes containing egg white protein became more elastic at 60-85°C compared with those containing whey protein, indicating physical differences in the heat-set protein foam network associated with protein type.

Mleko, Liang and Kristinsson (2005), as previously mentioned in this report, investigated rheological properties of foams made from egg albumen proteins after pH-induced unfolding and refolding treatment. All pH treatments led to firmer foams than control foam (which was not subjected to pH induced unfolding and refolding) at pH 8.5. At pH 8.5 the control foam had a very weak structure while pH-treated foams had values that were even higher than the values for the control at pH 4.5 (which is an ideal foaming pH for egg albumen). It was shown that an increase in yield stress of foams after drainage is related to foam stability and liquid drainage. This study demonstrated that unfolding egg albumin at low or high pH followed by refolding leads to a substantial increase in foam firmness and gives the foam different properties than foams from untreated egg albumen.

The aim of this work was to apply foams obtained by egg albumen unfolding and refolding treatment to angel food cake and meringue production, to test their rheological properties as well as sensory properties.

MATERIALS AND METHODS

Materials

Egg albumin powder of the same lot number was purchased from Fisher Scientific (Pittsburgh, PA). Other chemicals were purchased from Fisher Scientific (Pittsburgh, PA) and Sigma Chemical Co. (St. Louis, MO). Powdered sugar (Dixie Crystals, Sugar Land, TX) and bleached cake wheat flour (Swan's Down, Archer Daniels Midland Co., Decatur, IL) were purchased from a grocery store.

Methods for rheological studies

a) Egg albumen treatments and foam formation

Egg albumen solutions were prepared (2, 4 or 6 g of protein in 55 ml 100 mM NaCl solutions). The egg albumen solution was adjusted to pH 1.5, 4.5, 8.5 or 12.5 using 2 M HCl or 2 M NaOH and then held 60 min to unfold the proteins. After holding the solutions were readjusted to pH 4.5 or 8.5, and held for 45 min to partially refold the proteins. Controls were egg albumen samples not subjected to a low or high pH treatment but were adjusted directly to pH 4.5 and 8.5 and held for 45 min before whipping and untreated sample at native pH (6.25). The samples were then whipped with a BIO Homogenizer (M133/1281-0, Biospec Products Inc., Bartlesville, OK) with a foaming disk attachment at speed 2 in 600 ml glass beakers for 1 min. After 15 s of whipping 18 g of powdered sugar was added and the foam was additionally whipped for 45 sec. The foams were transferred by a rubber spatula into a flat container and a mixture of 19 g of sugar and 16 g of flour was gently folded using the spatula. Foam obtained from dispersion unfolded at pH 1.5 and refolded at pH 8.5 was investigated immediately after whipping and after 45 min and 90 min. The same whipping procedure was applied to combinations of ingredients (only egg albumen dispersion; egg albumen dispersion and sugar; egg albumen dispersion and flour; flour and sugar; flour).

In the text we will use the following coding of the samples: pH of unfolding→pH of refolding. This means that for example a sample 12.5→4.5 is a sample unfolded at pH 12.5, and then refolded at pH 4.5.

b) Rheological measurements

All rheological measurements were performed using a parallel geometry of a TA Instrument AR 2000 controlled stress rheometer (TA Instruments, New Castle, DE). An acrylic ST X-Hatch (40 mm diameter) plate was used with a cross-hatched surface. The foam sample was put carefully on the lower plate and after lowering instrument to a measurement gap of 2.4 mm., any excess sample protruding beyond the upper plate was removed. To determine the linear viscoelastic region a frequency sweep (0.1-10 Hz) and a stress sweep (0.5-1.0 Pa) were performed and frequency of 0.5 Hz at 0.8 Pa was selected for all measurements. Samples were heated from 21 ^{o}C to 150 ^{o}C at 8.5 ^{o}C per min and then cooled down to 21 ^{o}C. At 21 ^{o}C oscillatory stress sweep was performed on obtained angel food cake samples in the range 0.8-5000 Pa at 0.5 Hz frequency. All samples were prepared and tested in duplicate. Charts represent an average values of two replications.

Methods for angel food cake and meringue experiments

a) Egg albumen treatments

Fresh eggs were acquired locally. Egg whites were carefully separated from yolk and subjected to the different high and low pH treatments previously discussed. All samples were held at 20-22°C. After the pH treatments the egg albumens (now at either pH 4.5 or pH 8.5) were used to make angel food cake and meringue according to the following procedures:

1. Angel food cake:

   A sample consisting of twelve egg whites was whipped in a Kitchen Aid mixer and ½ tablespoon of salt added. After 2 min, when an initial foam had formed, 1 ½ teaspoon of vanilla extract and ½ teaspoon of almond extract was added. Then 1/3 cup sugar was added and the contents mixed for an additional 6 min. The time required for a "soft peak" to form was recorded for each treatment. Then 1 ¼ cup sugar and 1 cup sifted flour was "folded" into the foam, and the foam spread into a tube pan and cooked at 375°F for 30 min. After cooking and cooling to room temperature the angel food height was recorded for each treatment. Each angel food cake sample was also subjected to a sensory panel where panelists were asked to rate the texture and taste using a hedonic scale.

2. Meringue

   A sample consisting of 3 egg whites was whipped in a Kitchen Aid mixer and 150 g sugar slowly added. The contents were mixed for 8 minutes, and the time required for a "stiff peak" recorded for each treatment. The foam was then spread onto a baking sheet (2

cm thickness) and baked at 250°F for 55 min. The meringue was then subjected to a sensory panel, as described above.

RESULTS AND DISCUSSION

A) Rheological properties

Fig. 1 represents changes in G' at heating of cake batters from 21°C to 150°C. There were big differences in cake batters rheological properties. Samples: 12.5→8.5 and 1.5→8.5 had the highest G' (i.e. firmness/stiffness). Native sample (pH 6.8) and sample pH 8.5 had the lowest G'. Heating caused a drop in G' and then a constant increase. Pernell et al. (2002) suggests, that the decrease in G' reflects the combined effects of an expanding protein network and changes within that network. The increase in G' is associated with gelatinization of the starch in flour.

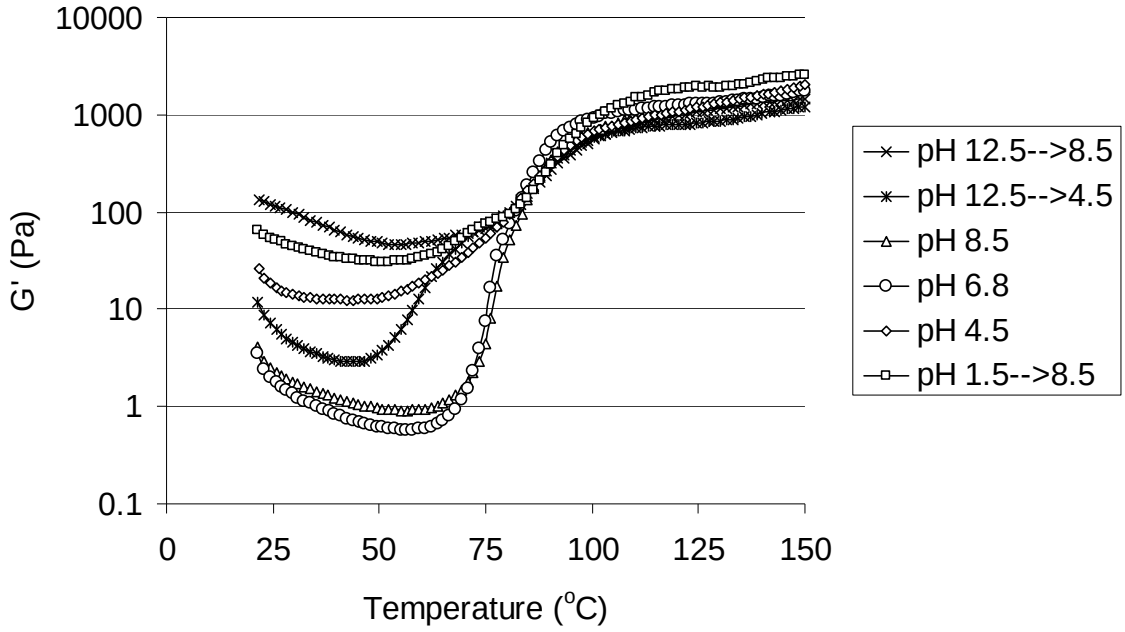


Figure 1. Changes in storage modulus for angel food cake batters heated from 21 to 150 °C obtained from different egg albumen unfolding/refolding procedures (y-axis shown in logarithmic scale to focus on lower temperatures)

Fig. 3 indicates changes in G' at heating of different cake batter ingredients. For flour an increase in G' was observed at 60°C. Adding sucrose to the flour increased starch gelatinization temperature up to 82°C. There was different behavior of the cake batters at the heating from 21-150°C (Fig. 2). All the samples had nearly the same G' value at around 78°C and after this point differences in G' development were noted. For samples with final pH 8.5 and 6.8, the highest increase in G' on heating was noted. Interestingly, there was no clear relationship between G' value of cake batters before heating and G' value of angel food cake at 150°C. This means that strength of the cake batter foam is not necessarily correlated with the strength of the final product after heating.

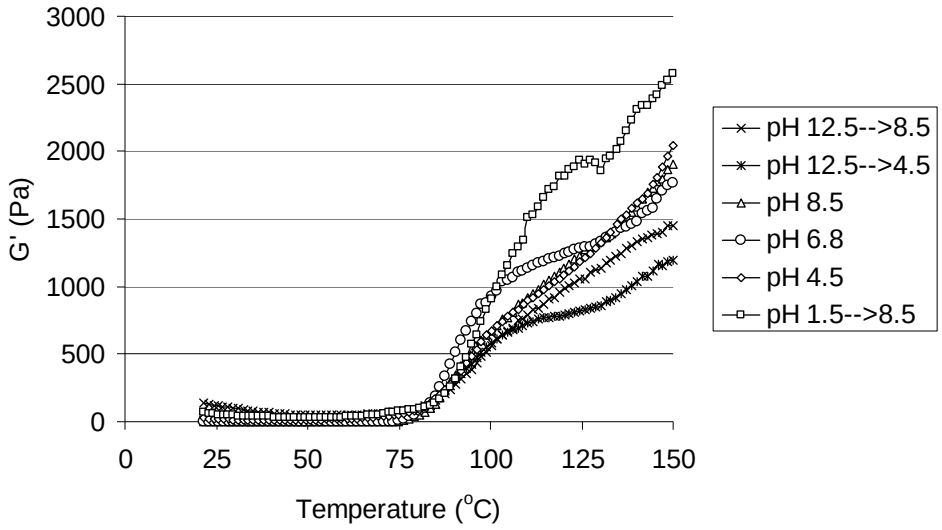


Figure 2. Changes in storage modulus for angel food cake batters heated from 21 to 150 °C obtained from different egg albumen unfolding/refolding procedures (focused on higher temperatures)

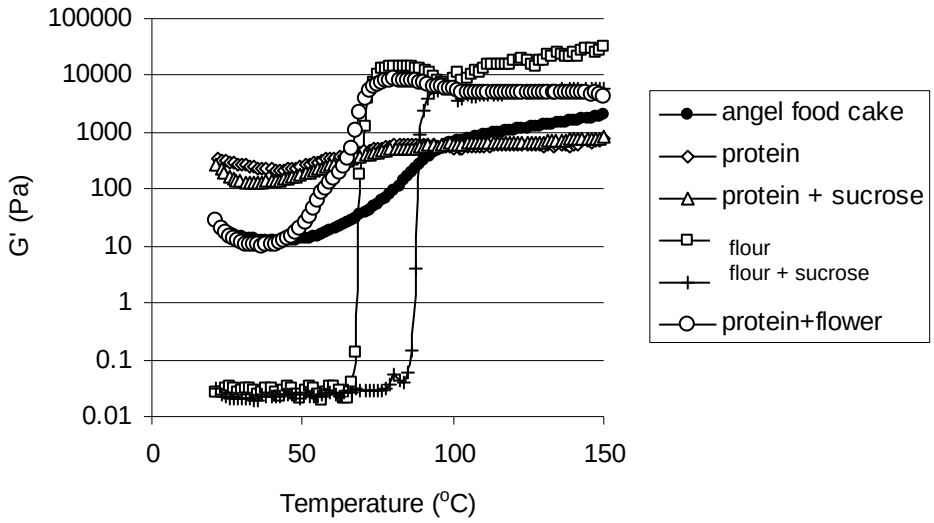


Figure 3. Changes in storage modulus for a product obtained from pH 4.5 egg albumen dispersion and for different combinations of angel food cake ingredients batters heated from 21 to 150 ^{o}C

Cooling down from 150-21^{o}C caused a rapid increase in G' with the highest values noted for cakes obtained from samples 1.5→8.5, 8.5 and 12.5→4.5 (Fig. 4). Oscillatory stress sweep of the final product revealed the strongest structure for angel food cake obtained from foams made using the 12.5→4.5 and 1.5→8.5 treatments, which is in agreement with the highest values of G' noted for their cake batters. The results clearly suggest that the pH unfolding refolding procedure resulted in a stronger final product. It can be a result of better foaming properties as found earlier by Mleko et al (2005) and Liang and Kristinsson (2005) and/or by their better gelling properties (Kristinsson, unpublished findings). The unfolding and refolding procedure could also possibly modify the interactions between starch and egg albumen proteins.

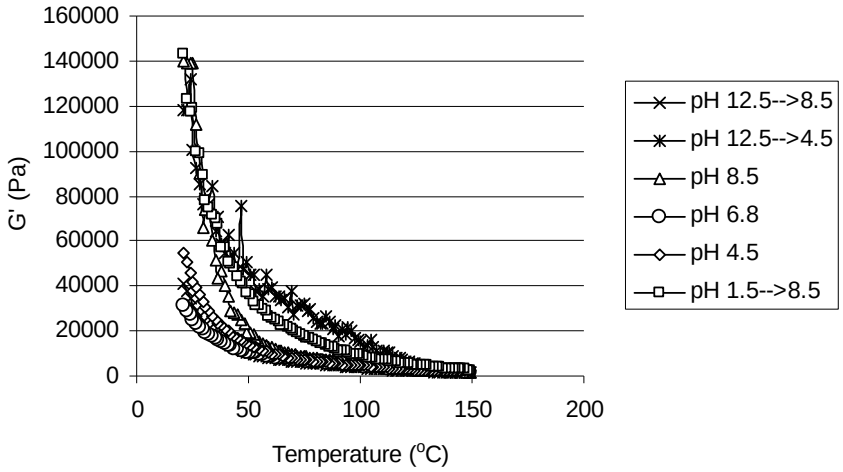


Figure 4. Changes in storage modulus for angel food cake cooled from 150 to 21 °C obtained from different egg albumen unfolding/refolding procedures

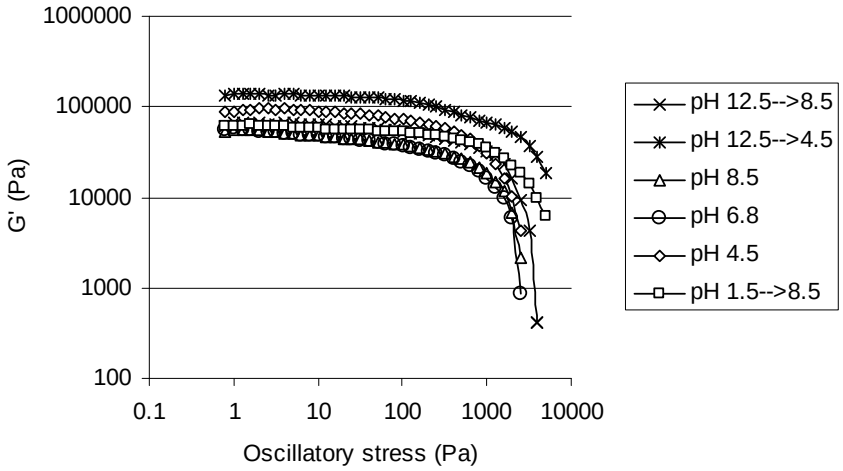


Figure 5. Oscillatory stress sweep for angel food cake obtained from different egg albumen unfolding/refolding procedures

B) Angel food cake and meringue properties

The studies done on the role of the pH treated proteins in actual food products where egg white foaming is important, did corroborate much of the data acquired on the more basic studies on the properties of the proteins. Table 1 shows results on angel food cake obtained for the pH treatments 12.5→4.5 and 12.5→8.5 and the pH 4.5 and 8.5 controls.

Table 1. Effect of pH treated egg albumins on the properties of angel food cake

Treatment	Volume increase*	Texture$^{\Psi}$	Ranked preference$^{\pi}$	Time to reach "soft peak"
pH 4.5 control	153	3	2	7.7 min
pH 12.5→4.5	213	1.6	1	3.4 min
pH 8.5	100	4.5	4	6.0 min
pH 12.5→8.5	127	5.5	2/3	5.3 min

*Value is normalized, with the sample with the smallest volume increase having a value of 100

$^{\Psi}$1= very soft/fluffy to 9 = not soft/fluffy

$^{\pi}$1= most preferred to 4 least preferred

In this set of results the cake with the albumin from the pH 12.5→4.5 was most preferred, as it had the most "fluffiness" and had a desirable softness, yet firmness to it. The foams at pH 8.5, were still liked by panelists, but were found to have a "stickier" less "fluffy" texture. It is evident that the volume increase is closely connected to the texture score. Furthermore, of the samples in Table 8, it was noted that the 12.5→4.5 treatment had the whitest color of them all, while the other 3 samples did not differ noticeably in color. Noticeable differences were also found in color of the meringue samples, but not connected to pH unfolding or refolding treatment, but rather the final pH. Samples at pH 8.5 (regardless of pH treatment or not) had a more beige color than samples at pH 4.5. Meringues could be successfully made at both pH 4.5 and 8.5, regardless of pH treatment. It was also universally noted that the surface of the meringues (which is hard while the interior is soft) made with pH treated egg albumen was less brittle than the surface of the pH 4.5 and 8.5 controls. Furthermore the time required to reach a "soft peak" was reduced for the pH treated samples.

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