

Final Report to MPRP
**Enhancing Foaming Properties of Egg Albumen by Using Modified
Soy and Egg Proteins**

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Summary

To improve foaming properties of egg white albumen, soy protein isolate (SPI) and egg proteins were modified to make basic proteins, i.e. proteins with isoelectric point (pI) in the alkaline range. SPI and egg yolk proteins were successfully modified to have pI of 10, and sonication was shown to increase protein dispersibility after the ethyl esterification reaction. However, only the addition of sonicated and modified SPI (SMSPI) showed improvement of foaming properties in the 5% egg albumen model system with 0.4% egg yolk addition (as-is basis) for lipid contamination simulation. Therefore, SMSPI was used in making angel food cake to examine if the cake performance reduction due to yolk contamination of the egg albumen would be restored or overcome by adding such alkaline protein. Cake performance was improved when cream of tartar was used together with the modified soy protein, but not by SMSPI alone. The basic protein and cream of tartar seemed to have certain degree of synergistic effect on the height and volume of the final angel cake. In conclusion, basic soy protein can be made and added to egg albumen to improve its foaming properties and cake performance.

Introduction

Yolk contamination of egg white protein reduces albumen's foaming properties, as shown by Wang and Wang (2009a). We previously modified soy protein isolate (SPI) by methyl esterification (Wang and Wang 2009b) and this modified soy protein improved the foaming capacity and foam stability of both egg white and yolk-contaminated egg white protein in model systems. The objectives of this research were to synthesize ethyl esters of SPI and egg white and yolk proteins and to evaluate foaming properties by the standard whipping method and in an angel food cake system.

Methods and Materials

Preparation of basic proteins from SPI, EW (egg white), and EYP (egg yolk protein) by esterification: The proteins were dispersed in pure ethanol at two concentrations (2% and 5% w/v). Concentrated hydrochloric acid (HCl) was added to give a final concentration of 0.1 and 0.5 M HCl in ethanol. The esterification reaction was conducted at ambient temperature (25 °C) with stirring for 72 h.

Sonication treatment to improve protein dispersibility: Samples were treated with sonication for 10 min at setting 9 (power output of 540 W at 20 KHz) with Pulsar on 60 s every 2 min using a Misonix XL Sonicator.

Foaming properties of egg white as measured by whipping method: A whipping method described by Wang and Wang (2009a) was used and two foaming parameters were calculated from the data collected.

Foam expansion (FE), % = $100 \times (\text{total volume of foam and liquid} - \text{initial volume of liquid}) / \text{initial volume of liquid}$

Foam liquid stability (FLS), % = $100 \times (\text{volume of liquid drained from foam after 30 min} / \text{initial volume of liquid})$

Effect of yolk addition and modified proteins on foaming of fresh egg white protein: For each 50 gram of fresh egg white, 4%, 8%, and 16% (relative to dry protein content) of SMSPI or SMEYP (sonicated and modified egg yolk protein). At each concentration of added protein, an equivalent amount of egg white dispersion was added as controls. In order to compare the effect of added protein on the foaming of yolk-contaminated egg white, identical samples were prepared with 0.4% yolk addition to the albumen (as-is basis).

Preparation and measurement of angel food cake: Angel cakes were prepared from one-half a standard recipe (Penfield and Campbell, 1990). A completely randomized design was applied to prepare three replications of 8 treatments each (cakes with and without SMSPI, egg yolk and/or cream of tartar). The 24 cakes were assigned random order, prepared and baked over several days. Volume and pH of the foam were measured. For the finished cake, the height (cm) was measured at the center. Volume of the cakes was measured by using the rapeseed method. The texture of the cakes was measured using a Texture Analyzer.

Results and Discussion

Effect of protein type and concentration, and hydrochloric acid concentration on protein modification: For EW, no signs of pl change were observed, therefore, EW was eliminated from the following studies. For EYP and SPI, the pl of both proteins treated 0.5M hydrochloric acid had changed pl (to about 10). Therefore, in the subsequent study, protein concentration of 5% for the esterification reaction was used. The dispersibility for these two modified proteins was improved by sonication as shown in figure 1.

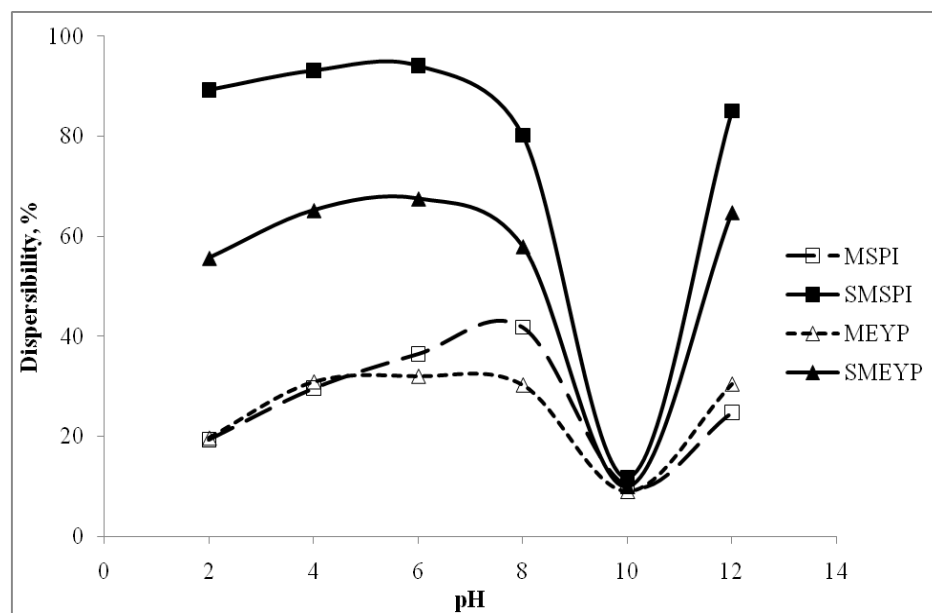


Figure 1. Dispersibility of modified proteins and the effect of sonication on protein dispersibility change. MSPI, modified SPI; MEYP, modified egg yolk protein; SMSPI, sonicated and modified SPI; SMEYP, sonicated modified egg yolk protein.

Effect of protein concentration (adding modified protein vs. simply higher albumen concentration) on the foaming of pure and yolk-contaminated egg white protein: For SMSPI, the effect of protein concentration on foaming of egg white protein is shown in Figure 2 and 3. As shown in Figure 2, addition of SMSPI to pure egg white did not significantly improve FE as compared with its EW controls at the same concentration until the addition was 16%. Similar results were observed for the egg white samples with 0.4% yolk addition (Figure 2). Foam expansion of all treatments decreased with the addition of yolk. However, with the addition of both egg white protein and SMSPI, the FE was improved. At higher concentration, the improvement with SMSPI was much more significant than with egg white protein. The main effect of the improvement is about 9%. For FLS, as shown in Figure 3, a significant improvement was observed when only 8% SMSPI was added. The improvement of foam stability with SMSPI was much more significant than with the same level addition of egg white. The main effect of the improvement is about 64%.

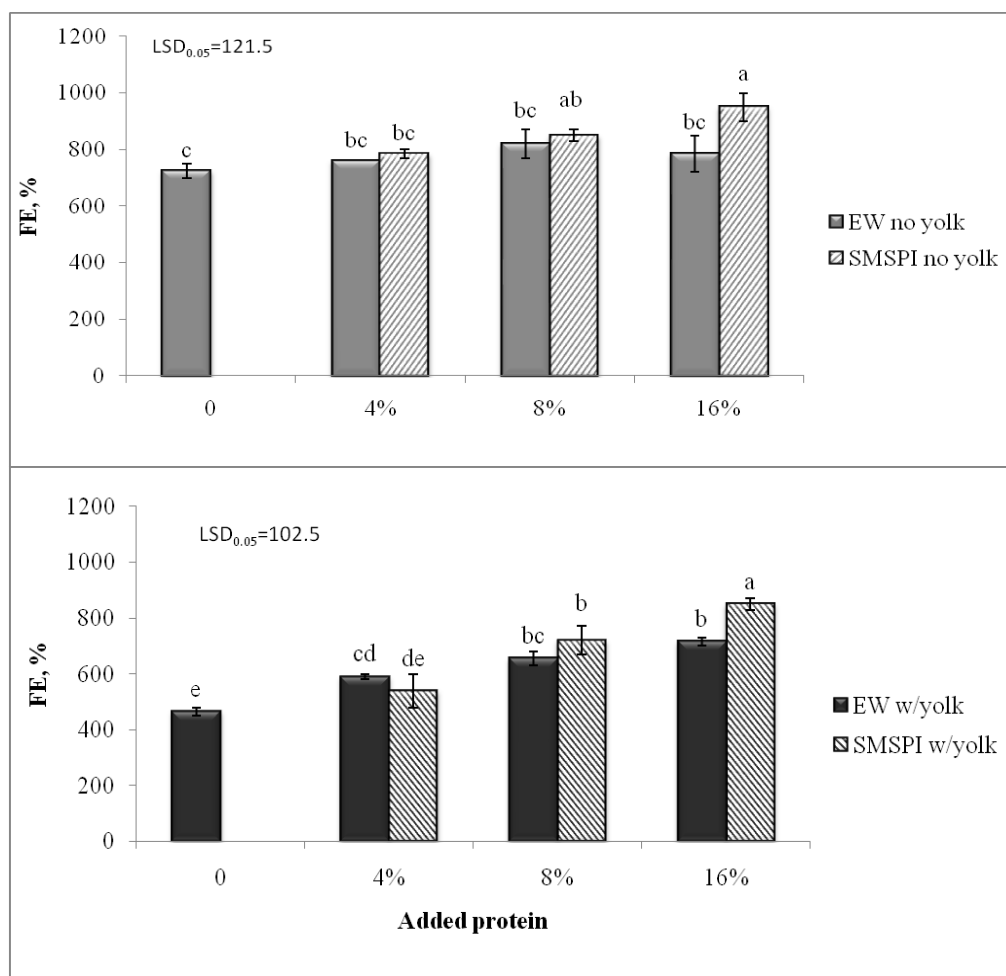


Figure 2. Foam expansion changes of 5% egg white as affected by added SMSPI as compared to EW with equivalent concentration. Upper figure represents treatments of no yolk, and lower figure represents treatments with 0.4% yolk addition. Different letters on the bars represent significant difference at $P = 0.05$ for each chart. EW, egg white; SMSPI, sonicated and modified soy protein isolate.

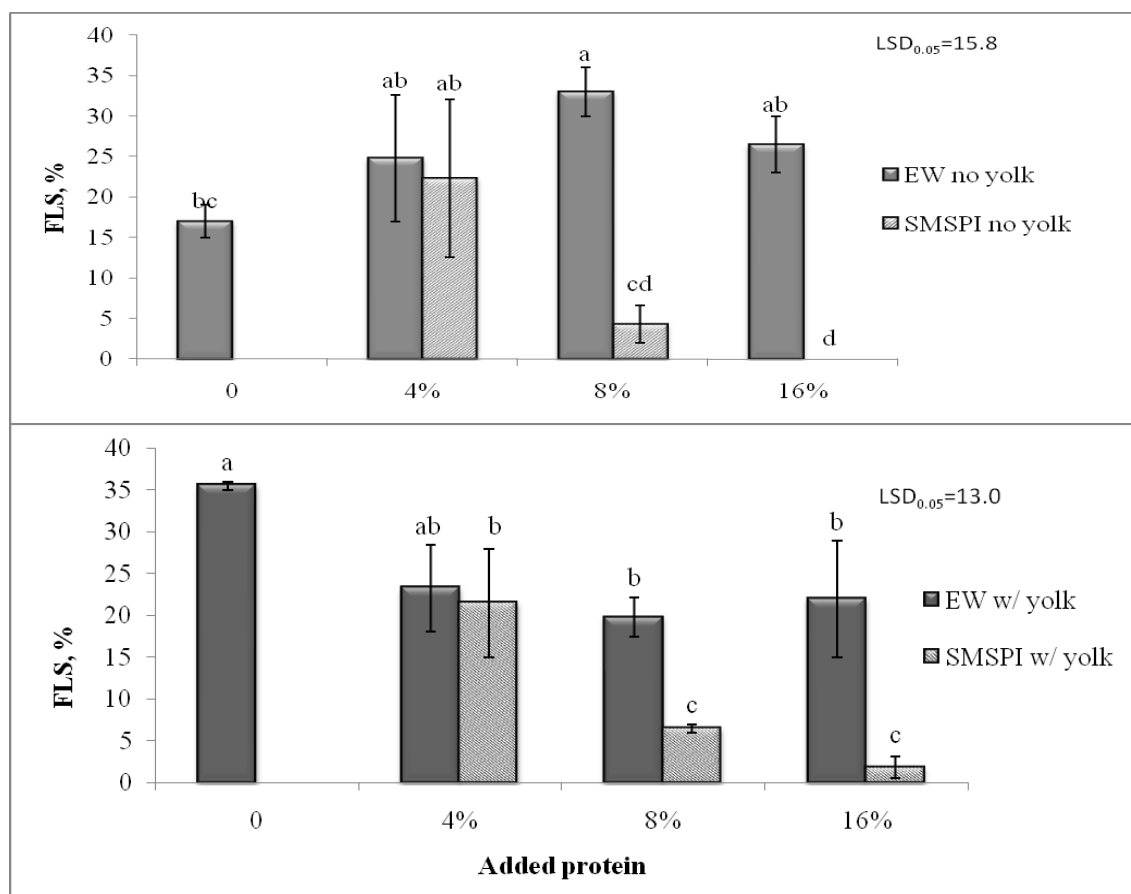


Figure 3. Foam liquid stability changes of 5% egg white as affected by adding SMSPI compared to EW with equivalent concentration. Different letters on the bars represent significant difference at $P = 0.05$ for each chart. EW, egg white; SMSPI, sonicated and modified soy protein isolate.

Evaluation of performance of angel food cake: The addition of yolk did not affect pH, but decreased foam volume as expected due to the yolk lipids. SMSPI addition increased pH of egg foam and decreased foam volume, a result different from the model system (5% egg white protein) study. Yolk addition decreased height of cakes made with and without cream of tartar. Height the cakes made without cream of tartar did not change when SMSPI was added, but the addition of SMSPI increased the height of cakes made with cream of tartar. This indicates the synergistic effect of cream of tartar with SMSPI. The volume of the cake increased with the addition of SMSPI and this is especially true with the co-addition of cream of tartar. For tenderness, the addition of SMSPI and cream of tartar resulted in the most tender cakes. In general, SMSPI countered the impact of egg yolk, but only when cream of tartar was present.

In summary, SMSPI increased the volume of egg white foam and increased angel cake height, volume and tenderness only when cream of tartar was also included in the formula. Cream of tartar resulted in a lower pH and neutralized the charge of egg white

proteins. The modified alkaline protein was also expected to neutralize the protein charge.

Conclusion

Alkaline proteins can be made using soy protein and egg yolk protein, however, only the modified soy protein showed significant improvement in foaming properties in pure egg white proteins and in egg white that is damaged with the yolk contamination. Such modified soy protein also enhanced angel food cake performance when the egg white protein with yolk added was used for the formulation. The ease of making and application of such modified protein can potentially bring about significantly improved foaming performance of various types of proteins intended for use for making food foams. If the egg protein is contaminated by yolk to a lesser degree ($<0.4\%$), then much lower amount of modified soy protein is needed to restore foaming.

This practical approach can be adopted readily by the industry to enhance egg white foaming that is critical in many food preparations.

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Appendix – full manuscript as submitted

**Enhancing Foaming Properties and Angel Food Cake Performance by Using Modified Soy
Protein in Egg Albumen**

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Abstract

In order to improve foaming properties of egg white albumen, soy protein isolate (SPI) and egg proteins were modified to make basic proteins, i.e. proteins with isoelectric point (pI) in the alkaline range. SPI and egg yolk proteins were successfully modified to have pI of 10, and sonication was shown to increase protein dispersibility after the ethyl esterification reaction. However, only the addition of sonicated and modified SPI (SMSPI) showed improvement of foaming properties in the 5% egg albumen model system with 0.4% egg yolk addition (as-is basis) for lipid contamination simulation. Therefore, SMSPI was used in making angel food cake to examine if the cake performance reduction due to yolk contamination of the egg albumen would be restored or overcome by adding such alkaline protein. Cake performance was improved when cream of tartar was used together with the modified soy protein, but not by SMSPI alone. The basic protein and cream of tartar seemed to have certain degree of synergistic effect on the height and volume of the final angel cake. In conclusion, basic soy protein can be made and added to egg albumen to improve its foaming properties and cake performance.

Key words: Albumen; angel food cake; basic proteins; egg white; foaming properties; soy protein isolate.

Introduction

One of the major applications of egg white albumen is to make foams in various food systems. However, yolk contamination of egg white reduces albumen's foaming properties, as shown by Wang and Wang (2009a). Such contamination may not be completely avoidable as indicated by sampling of the commercial products in comparison to the laboratory hand-separated egg white for the foaming performance testing (Wang and Wang 2009a). There is no tolerance level for yolk contamination, i.e., even at the lowest testing concentration (0.01% yolk in white protein, as-is basis) the foaming was significantly reduced (Wang and Wang 2009a). Our research also showed that neutral oil, rather than the polar phospholipids in the yolk, is the main contributor for reduced foaming.

Protein creates and stabilizes foams by forming a visco-elastic film at the air-liquid interface. The film is formed by protein-protein hydrophobic interactions once the molecules migrate to the interface and unfold to expose the more hydrophobic regions. Oil, i.e., the neutral lipid, reduces foaming by binding at the hydrophobic region of the protein, preventing protein-protein interactions for film formation. Most of the egg albumen proteins are acidic proteins with isoelectric points (pI) about pH 4-5. If a basic protein is added to yolk-contaminated albumen, its positive charge would neutralize the negative charges on the acidic proteins so the electrostatic repulsion can be reduced and the protein-protein interaction may be strengthened for a more stable film. Therefore, the magnitude of negative effect of lipids may be reduced. Some studies have provided evidence that basic proteins, such as clupeine (pI of 12, from fish) and lysozyme (pI of 10, a minor egg white protein), enhanced foaming properties when added to acidic proteins (Poole and others 1984; Poole and others 1986; Phillips and others 1989; Poole 1989). However, commercial availability of these naturally occurring proteins is limited. Chemical modification to

produce basic proteins has shown some promise (Fraenkel-Conrat and Olcott 1945; Means and Feeney 1971).

We modified soy protein isolate (SPI) by esterifying its acidic groups to methyl esters (Wang and Wang 2009b). This reaction changed the pI of the protein from 4.5 to the 9.5 to 11 range, depending on the degree of esterification. Sonication treatment was shown to be very effective in increasing protein dispersibility of the alcohol denatured modified proteins (Wang and Wang 2009b). The modified and sonicated soy protein isolate improved the foaming capacity and foam stability of both egg white and yolk-contaminated egg white protein in model systems.

Our previous studies have shown great potential in creating basic proteins and their effectiveness in foaming enhancement. Several research questions still remain – can egg proteins be modified to make basic proteins for improving foaming? Can ethanol be used to replace methanol in the esterification procedure? Can the reaction be done feasibly with higher concentration of protein? Can foaming enhancement in the model system be translated to a complex food system? Therefore, the objectives of this research were to synthesize ethyl esters of soy protein isolate and egg white and yolk proteins at high substrate concentration and to evaluate foaming properties by the standard whipping method and in an angel food cake system.

Methods and Materials

Materials:

Shell eggs were purchased from a local supermarket. Egg white was manually separated from the yolk. SPI (94.5% protein) was provided by Archer Daniels Midland (Decatur, IL.) Dried egg white protein (EW) was purchased from Honeyville (Rancho Cucamonga, CA). Egg

yolk protein (EYP) was separated from the fresh egg yolk by following Palacios and Wang's procedure (2005).

Preparation of basic proteins from SPI, EW, and EYP by esterification:

The vacuum oven dried SPI, EYP, and EW were dispersed in pure ethanol at two concentrations (2% and 5% w/v). Concentrated hydrochloric acid (HCl) was added to give a final concentration of 0.1 and 0.5 M HCl in ethanol. The esterification reaction was conducted at ambient temperature (25 °C) with magnetic stirring for 72 h and the reaction was stopped by adding a few drops of deionized water. Products were cooled to 4 °C and the solid was collected by vacuum filtration. The solid phase was pre-dried in a fume hood overnight and the residual solvent and moisture were removed using a vacuum oven at ambient temperature for 24 h. The resulting products were ground using a mortar and pestle and stored in a desiccators. The modified SPI, EYP, and EW were designated MSPI, MEYP, and MEW, respectively. All treatments were repeated with total of two replicates.

Sonication treatment to improve protein dispersibility:

To determine the effect of sonication time on protein dispersibility, the MSPI and MEYP samples were suspended in deionized water at a concentration of 25 mg/mL and pH 7. They were treated with sonication for 5, 10, and 15 min at setting 9 (power output of 540 W at 20 KHz) with Pulsar on 60 s every 2 min using a Misonix XL Sonicator (Farmingdale, N.Y.). Samples were surrounded by an ice–water bath to avoid overheating. Protein dispersibility was then measured by quantifying protein in the supernatant as described in the following section. For proteins to be used for foaming test, sonication was done in a similar manner for 10 min. The sonicated MSPI, MEYP, and MEW samples were designated as SMSPI, SMEYP and SMEW; sonicated SPI was designated as SSPI. The sonicated protein dispersions were adjusted to pH 12

by adding 1 N sodium hydroxide to increase dispersibility before it was added to liquid egg white for foaming test, because such pH adjustment of the sample did not affect foaming of egg albumen (Wang and Wang 2009b).

Protein solubility curve determination:

A series of 1% (w/v) protein aqueous dispersion at pH 2 to 12 was prepared. The pH of these dispersions was adjusted using either hydrochloric acid or sodium hydroxide solutions. The protein dispersions were centrifuged at $3,100 \times g$ for 10 min using an IEC Centra CL3 centrifuge (Thermo Electron Corp., Waltham, Mass.). Dispersibility was determined by quantification of the protein in the supernatant using the Biuret colorimetric protein assay with absorption wavelength at 540 nm (Gornall and others 1949). Bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Mo.) was used to establish a standard curve for protein quantification.

Foaming properties of egg white as measured by whipping method:

A whipping method described by Wang and Wang (2009a) was used. Briefly, whipping was performed at a speed setting of 10 (400 rpm) for 90 s with a KitchenAid brand mixer with a wire whip attachment. All treatments were done using 100 mL dispersions of 5% base egg albumen protein concentration. The initial foam volume was measured by gentle filling and tapping the foam into a 1 L graduated cylinder. The final liquid volume drained from the foam after 30 min of standing was measured for calculation of foaming properties. Two foaming parameters were calculated from the data collected, and all tests were done in duplicate.

Foam expansion (FE), % = $100 \times (\text{total volume of foam and liquid} - \text{initial volume of liquid}) / \text{initial volume of liquid}$

Foam liquid stability (FLS), % = $100 \times (\text{volume of liquid drained from foam after 30 min} / \text{initial volume of liquid})$

Effect of yolk addition and modified proteins on foaming of fresh egg white protein:

Protein MSPI and MEYP were further treated with sonication by the method mentioned above for 10 min, and they were designated as SMSPI and SMEYP, respectively. The solid content of fresh egg white was determined to be 10% and 5% solutions were made for the foaming test. For each 50 gram of fresh egg white, 4%, 8%, and 16% (relative to dry protein content) of SMSPI or SMEYP were added as 25 mg/mL protein dispersions at pH 12. At each concentration of added protein, an equivalent amount of egg white dispersion (25 mg/mL, pH 12) was prepared and added as controls. In order to compare the effect of added protein on the foaming of yolk-contaminated egg white, identical samples were prepared with 0.4% yolk addition to the albumen (as-is basis). Foaming properties (FE and FLS) were then evaluated by the whipping method.

Effect of chemical modification and sonication on foaming of yolk-contaminated egg white:

Protein dispersions (25 mg/mL) (SPI, SSPI, MSPI, and SMSPI) at 16% based on dry matter of egg white protein were added to 50 g of yolk-contaminated (0.4%) egg white. Total mass was adjusted to 100 gram using deionized water to form 5% base protein dispersions. Foaming properties (FE and FLS) were measured by the whipping method described above.

Preparation of angel food cake:

Angel cakes were prepared from one-half a standard recipe (Penfield and Campbell, 1990) (Table 1). The SMSPI was freeze-dried and used in the formulation. A completely randomized design was applied to prepare three replications of 8 treatments each (cakes with and without SMSPI, egg yolk and/or cream of tartar. The 24 cakes were assigned random order, prepared and baked over several days.

Fresh egg whites, separated from eggs purchased from a local supermarket, were added to 1 L glass bowls with volume measurements. Fresh yolk (0.4%) and/or SMSPI (16% relative to dry albumen weight) was added, if applicable, and stirred in with a fork until a uniform suspension was created. The pH of the egg mixture was measured. The mixture was beaten to the foamy stage with a handheld electric mixer on high to the foamy stage (many bubbles of different sizes and little liquid remaining at the bottom of the bowl). Salt and cream of tartar were added at the foamy state. The egg mixture was then beaten to the upper limit of soft peak stage (when the beaters were pulled out of the whites, the peak would just bend over slightly at the top). All foams reached this stage, but cakes with cream of tartar had softer foams, while those with no cream of tartar had drier, stiffer foams.

Volume of the foam was measured by smoothing the top of the foam inside the bowl to an even level and reading volume on the side of the bowl. The pH of the foam was also measured. The flour and $\frac{1}{4}$ of the sugar were sifted together and set aside. The remaining sugar was added to the egg whites in three portions, beating 10 sec between each addition, then beating until stiff peaks (a very stiff batter which clung to the beaters when they were lifted out) were formed. The flour-sugar mixture was sifted over the meringue in 4 portions and folded into the meringue, just enough to blend between additions. The batter was poured into an ungreased, pre-weighed 8.5 x 15 cm foil pan and the weight of batter plus pan was recorded. Cakes were baked in the same preheated (350°F) ovens for 20 min, inverted on a wire rack to cool, removed from the pans, placed in a labeled bag, and frozen until texture evaluation.

Evaluating performance of angel food cake:

In addition to the pH measurement of the egg white, with yolk or SMSPI addition (before beating and after the foams were at stiff peak before sugar addition), the height (cm) of each cake was measured at the center.

Volume of baked cakes was measured by using rapeseed to fill a 10.1 x 20.3 cm (4 x 8 inch) pan. Rapeseed in the filled pan was measured in a graduated cylinder and reported as mL. Angel cakes were wrapped in plastic and placed in the pan. Rapeseed was added to the pan with the cake, leveled, then removed from the pan and measured (mL). Volume of cakes was determined by difference and reported as cm³.

The texture of the baked angel cakes was measured using a TA.XT2 Texture Analyzer (Texture Technologies Corp and Stable Micro Systems, Ltd., Scarsdale, NY) with a 2.5-cm diameter plexi-glass probe on stainless steel plate. A Texture Profile Analysis (TPA) test at 60% compression was conducted (distance 12 mm, speed 3 mm/sec). All cakes, which had been frozen after baking, were measured in one day. Frozen cakes were cut in half and two 2-cm slices were cut from one half of each cake. Cakes were cut frozen in a plexi-glass box, with a very sharp, serrated 'Henckels' knife to prevent compression of cakes. The cakes were fully thawed (15 min) before analysis. All crumbs were removed from the slices of cake. Each cake slice (2 per treatment) was placed on its side so the probe compressed the center. Maximum force (g) and area (g X sec) of two compressions per slice were reported. Means of duplicate measurements for each treatment were reported.

Statistical analysis:

All protein modification and model system foaming samples were prepared and tested in duplicate. The effect of protein, concentration, and yolk contamination were examined using

multivariate General Linear Model (GLM) and multiple-factors analysis of variance (ANOVA) with the SAS 9.2 program.

Three replications of 8 angel cake treatments (egg white, egg white + SMSPI, egg white + 0.4% yolk, egg white + cream of tartar, egg white + yolk + SMSPI, egg white + SMSPI + cream of tartar, egg white + yolk + cream of tartar, and egg white + yolk + SMSPI + cream of tartar) were completed. A complete randomized design was used to determine the effect of yolk, SPI and/or cream of tartar in angel cakes. Analysis of variance was performed and probability of 0.05 was used to evaluate treatment significance. Main effect means were reported when no interactions were observed. When interactions occurred, individual means were reported. Fisher's Least Significant Differences (LSD) were calculated and used for mean comparison.

Results and Discussion

Effect of protein type and concentration, and hydrochloric acid concentration on protein modification:

In this experiment, EW, EYP, and SPI were used at two concentrations (2% and 5%) in ethanol with two hydrochloric acid concentrations (0.1 and 0.5 M). For EW, no signs of pI change were observed as confirmed by protein solubility curves. Therefore, EW was eliminated from the following studies. For EYP and SPI, the pI of both proteins treated with 0.1M hydrochloric acid changed slightly regardless of protein concentration used. On the contrary, 0.5M hydrochloric acid changed pI of both proteins significantly at both high and low protein concentrations. The pIs of both EYP and SPI were increased to about 10. Therefore, in the subsequent study, protein concentration of 5% for the esterification reaction was used in order to maximize production capacity.

The dispersibility for these two modified proteins was much lower than the starting materials. It was expected that ethanol would denature the proteins. Therefore, sonication treatment was applied to both proteins to obtain improved dispersion of proteins.

Effect of sonication on protein dispersibility:

For the SPI used in this study, sonication improved protein dispersibility from less than 30% to 90% at neutral pH (Wang and Wang 2009b). In the process of preparing large quantities of modified protein, an efficient procedure is needed so that protein can be re-dispersed with minimal energy input. Therefore, the effect of sonication time on protein dispersibility was examined using a different batch of proteins from the proteins used for foaming performance testing. The change of protein dispersibility at different length of sonication duration is shown in Figure 1. After sonication for 10 min, both SMSPI and SMEYP showed maximal dispersibility at pH 7. Therefore, in the subsequent study all sonication treatment was done for 10 min. The solubility curves for both SMSPI and SMEYP are presented in Figure 2. A significant improvement in dispersibility is shown for both MSPI and MEYP after sonication treatment. The modified basic proteins have pI of about 10.

Effect of protein concentration (adding modified protein vs. simply higher albumen concentration) on the foaming of pure and yolk-contaminated egg white protein:

The experimental factors examined in this experiment are listed in Table 2. As a result of multiple-factors analysis of variance (ANOVA), a general linear model (GLM) was conducted for FE and FLS. FE was primarily affected by yolk contamination ($P < 0.0001$). This observation was consistent with our previous findings. The next greatest contributor to the foaming difference is the protein type ($P < 0.0001$), which significantly affected foaming properties of egg white. The FE and FLS as affected by protein type are shown in Table 3. In comparison with

the egg white control group, SMSPI showed significant improvement in both FS and FLS, whereas SMEYP showed deteriorated FE and FLS. As a general conclusion combining both FE and FLS responses, SMSPI addition was much superior to egg white control, while SMEYP was not effective in improving foaming of egg white protein (it even reduced foaming significantly). Therefore, in the further testing in angel cake system, SMEYP was not used.

For SMSPI, the effect of protein concentration on foaming of egg white protein is shown in Figure 3 and 4. As shown in Figure 3, addition of SMSPI to pure egg white did not significantly improve FE as compared with its EW controls at the same concentration until the addition was 16%. Similar results were observed for the egg white samples with 0.4% yolk addition (Figure 3). Foam expansion of all treatments decreased with the addition of yolk. However, with the addition of both egg white protein and SMSPI, the FE was improved. At higher concentration, the improvement with SMSPI was much more significant than with egg white protein. The main effect of the improvement is about 9% (Table 3).

For FLS, as shown in Figure 4, a significant improvement was observed when only 8% SMSPI was added. The improvement of foam stability with SMSPI was much more significant than with the same level addition of egg white. The main effect of the improvement is about 64% (Table 3). For both FE and FLS, no significant interactions between yolk and SMSPI concentration were noted, which indicates the presence of yolk did not affect the FE and FLS changes regarding the effect of protein concentration or type. In other words, the effect of modified SPI on enhancing foaming of pure egg white and yolk-contaminated egg white were not significantly different.

The effect of chemical modification and sonication of the protein on foaming improvement of yolk-contaminated egg white:

This experiment was conducted to identify whether protein modification or sonication played a greater role in foaming improvement. As shown Table 4, sonication treatment improved FE and FLS significantly by paired comparison (SPI/SSPI), while for modified SPI treatments, sonication only improved FLS. In the case of chemical modification, both SPI/MSPI and SSPI/SMSPI pairs showed significant improvement in FE and FLS after modification. A further multiple-factor statistical analysis showed that chemical modification is a greater contributor for the improved foaming properties.

Evaluation of performance of angel food cake:

As shown in Table 5, the addition of yolk did not affect pH, but decreased foam volume as expected due to the yolk lipids. SMSPI addition increased pH of egg foam and decreased foam volume, a result different from the model system (5% egg white protein) study. Cream of tartar decreased foam pH as expected but did not influence foam volume. The reason for the slightly reduced foam volume with SMSPI addition is unknown.

Table 6 shows the height of the cake as affected by different treatments. Yolk addition decreased height of cakes made with and without cream of tartar. Height the cakes made without cream of tartar did not change when SMSPI was added, but the addition of SMSPI increased the height of cakes made with cream of tartar. This indicates the synergistic effect of cream of tartar with SMSPI.

Table 7 presents the volume of the angel food cake as affected by the various treatments. Addition of yolk to egg white in angel cakes reduced the volume of cakes while addition of SMSPI increased volume of cakes. This is especially true with the co-addition of cream of tartar. The two volume improvements were 4 and 25% for the SMSPI alone and with the cream of

tartar, respectively. The highest cake volume resulted when both cream of tartar and SMSPI were added. This again shows the synergistic effect of the two ingredients.

Table 8 shows the tenderness of the angel food cake for all treatments. Because all four textural parameters (maximum force of compressions 1 and 2 and area 1 and 2 for the two consecutive compressions by the texture analyzer), had similar changes in responding to the treatments, only area 1, which represented the tenderness the best, is presented. The addition of SMSPI and cream of tartar resulted in the most tender cakes (lowest area of first compression curve). In general, SMSPI countered the impact of egg yolk, but only when cream of tartar was present.

The level of SMSPI addition of 16% may seem high, however, it was on dry-weight basis. This translates to 1.6% addition on albumen as-is base. The 0.4% yolk addition was used to simulate yolk contamination of the albumen for this study. This level was determined based on the findings from our previous work (Wang and Wang, 2009a), which showed that 0.4% yolk (as-is basis) led to the lowest foaming performance (reached steady state) as measured by purging method, but this level was still on the decline of performance as measured by whipping method. The 0.4% yolk addition may represent a severe case of contamination in the industry. With this level of contamination, we have shown improvement of foaming and product performance. If the egg albumen is contaminated to a lesser degree (<0.4% yolk in white), we expect a better foaming improvement and possibly with lower level of SMSPI addition. We did show from our previous study that even with 0.04% yolk contamination, foaming properties were significantly reduced, and this could be feasibly corrected by our modified alkaline protein.

In summary, SMSPI increased the volume of egg white foam and increased angel cake height, volume and tenderness only when cream of tartar was also included in the formula.

Cream of tartar resulted in a lower pH and neutralized the charge of egg white proteins. The modified alkaline protein was also expected to neutralize the protein charge. The higher solubility of cream of tartar in the cake system may have had more impact than the modified SPI, which showed effectiveness in the model system but its dispersibility and ability to interact with egg albumen may have been limited in a complex food system. However, when SMSPI was used with cream of tartar, the highest effectiveness was obtained.

Conclusion

Alkaline proteins can be made using soy protein and egg yolk protein, however, only the modified soy protein showed significant improvement in foaming properties in pure egg white proteins and in egg albumen that is damaged with the yolk contamination. Such modified soy protein also enhanced angel food cake performance when the egg white protein with yolk added was used for the formulation. The ease of making and application of such modified protein can potentially bring about significantly improved foaming performance of various types of proteins intended for use for making food foams.

Acknowledgement

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Figure Captions:

Figure 1. Effect of sonication time on protein dispersibility at pH 7. Different letters on each data series represent significant difference at $P = 0.05$. SMSPI, sonicated and modified SPI; SMEYP, sonicated modified egg yolk protein.

Figure 2 Dispersibility of modified proteins and the effect of sonication on protein dispersibility change. MSPI, modified SPI; MEYP, modified egg yolk protein; SMSPI, sonicated and modified SPI; SMEYP, sonicated modified egg yolk protein.

Figure 3. Foam expansion changes of 5% egg white as affected by added SMSPI as compared to EW with equivalent concentration. Upper figure represents treatments of no yolk, and lower figure represents treatments with 0.4% yolk addition. Different letters on the bars represent significant difference at $P = 0.05$ for each chart. EW, egg white; SMSPI, sonicated and modified soy protein isolate.

Figure 4. Foam liquid stability changes of 5% egg white as affected by adding SMSPI compared to EW with equivalent concentration. Different letters on the bars represent significant difference at $P = 0.05$ for each chart. EW, egg white; SMSPI, sonicated and modified soy protein isolate.

Table 1. Formula for angel cakes made with yolk, SMSPI and/or cream of tartar

Ingredient	Quantity, g
Cake Flour	15.50
Sugar	42.50
Egg white, fresh	40.50
Salt	0.20
Cream of Tartar*	0.60
Egg Yolk*, fresh	0.16
SMSPI *	0.65

* Not added to all treatments. SMSPI, sonicated modified soy protein isolate.

Table 2. Experimental design for the effect of yolk and protein type and concentration on foaming of 5% egg albumen

Classes	Levels	Values
Replicate	2	1, 2
Yolk	2	A (no yolk), B (yolk)
Protein	3	EW, SMSPI, SMEYP
Concentration	3	4%, 8%, 16%

SMEYP, sonicated modified egg yolk protein; SMSPI, sonicated modified soy protein isolate; EW, egg white.

Table 3. Foaming properties as affected by main factor of protein type

	Egg white protein control	SMEYP	SMSPI
Foam Expansion (FE),%	720 ± 17 b	638 ± 17 c	782 ± 17 a
Foam Liquid Stability (FLS),%	25.0 ± 1.6 B	31.0 ± 1.6 A	9.0 ± 1.6 C

Different letters for the same parameter represent significant difference at P = 0.05.

SMSPI, sonicated modified soy protein isolate. SMEYP, sonicated modified egg yolk protein.

Table 4. Foaming of yolk-contaminated egg white as affected by addition of 16% SPI

	SPI	SSPI	MSPI	SMSPI
Foam Expansion (FE),%	420 ± 10 c	680 ± 0 b	710 ± 30 ab	765 ± 35 a
Foam Liquid Stability (FLS),%	34.5 ± 4.5 A	15.0 ± 5.0 B	12.0 ± 3.0 B	2.5 ± 2.5 C

Different letters for the same parameter represent significant difference at P = 0.05.

SSPI, sonicated soy protein isolate. SMSPI, sonicated modified soy protein isolate.

Table 5. Effect of egg yolk, SMSPI, and cream of tartar addition on pH and volume of foams for angel cakes

Addition to Cake	pH foam before beating	pH of foam at stiff peak before sugar addition	Foam volume (cm ³)
No Yolk	9.23	7.78	237.5 ^a
Yolk	9.19	7.71	218.8 ^b
No SMSPI	9.05 ^b	7.54 ^b	239.6 ^a
SMSPI	9.37 ^a	7.95 ^a	216.7 ^b
No Cream of Tartar	9.26 ^a	9.13 ^a	225.0
Cream of Tartar	9.16 ^b	6.36 ^b	231.3

Means followed by different letters within each type of addition and parameter are significantly different at P = 0.05.

There were no interactions among factors so main effect means are reported.

SMSPI, sonicated modified soy protein isolate.

Table 6. Effect of egg yolk and SMSPI addition on height of angel cakes made without and with cream of tartar

	Cake height (cm) at center No Cream of Tartar	Cake height (cm) at center Cream of Tartar
No Yolk	3.24 ^c	4.22 ^a
Yolk	3.03 ^d	3.59 ^b
No SMSPI	3.09 ^c	3.71 ^b
SMSPI	3.18 ^c	4.10 ^a

Means followed by different letters within each addition (yolk or SMSPI) are significantly different at P = 0.05.

There were interactions between yolk*cream of tartar and SMSPI*cream of tartar so individual means are reported.

SMSPI, sonicated modified soy protein isolate.

Table 7. Effect of egg yolk, SMSPI and cream of tartar addition on volume of angel cakes

Addition	Volume (cm ³)	
No Yolk	320.8 ^a	
Yolk	289.8 ^b	
No SMSPI	298.8 ^b	
SMSPI	311.9 ^a	
	No SMSPI	SMSPI
No Cream of Tartar	283.8 ^c	279.0 ^c
Cream of Tartar	313.7 ^b	344.8 ^a

Means followed by different letters within each addition are significantly different at P = 0.05.

There was an interaction between SMSPI*cream of tartar. SMSPI, sonicated modified soy protein isolate.

Table 8. Effect of egg yolk and cream of tartar addition on texture of angel cakes made without and with SMSPI

	Area 1 (g-s) No Cream of Tartar	Area 1 (g-s) Cream of Tartar
No Yolk	683.4 ^b	459.3 ^a
Yolk	755.4 ^b	757.2 ^b
No SMSPI	679.2 ^{bc}	662.7 ^b
SMSPI	759.6 ^c	553.9 ^a

Means followed by different letters within each addition (yolk or SMSPI) are significantly different at $P = 0.05$.

For all texture measurements, there were interactions between yolk*cream of tartar and SMSPI*cream of tartar so the individual means are shown.

SMSPI, sonicated modified soy protein isolate.

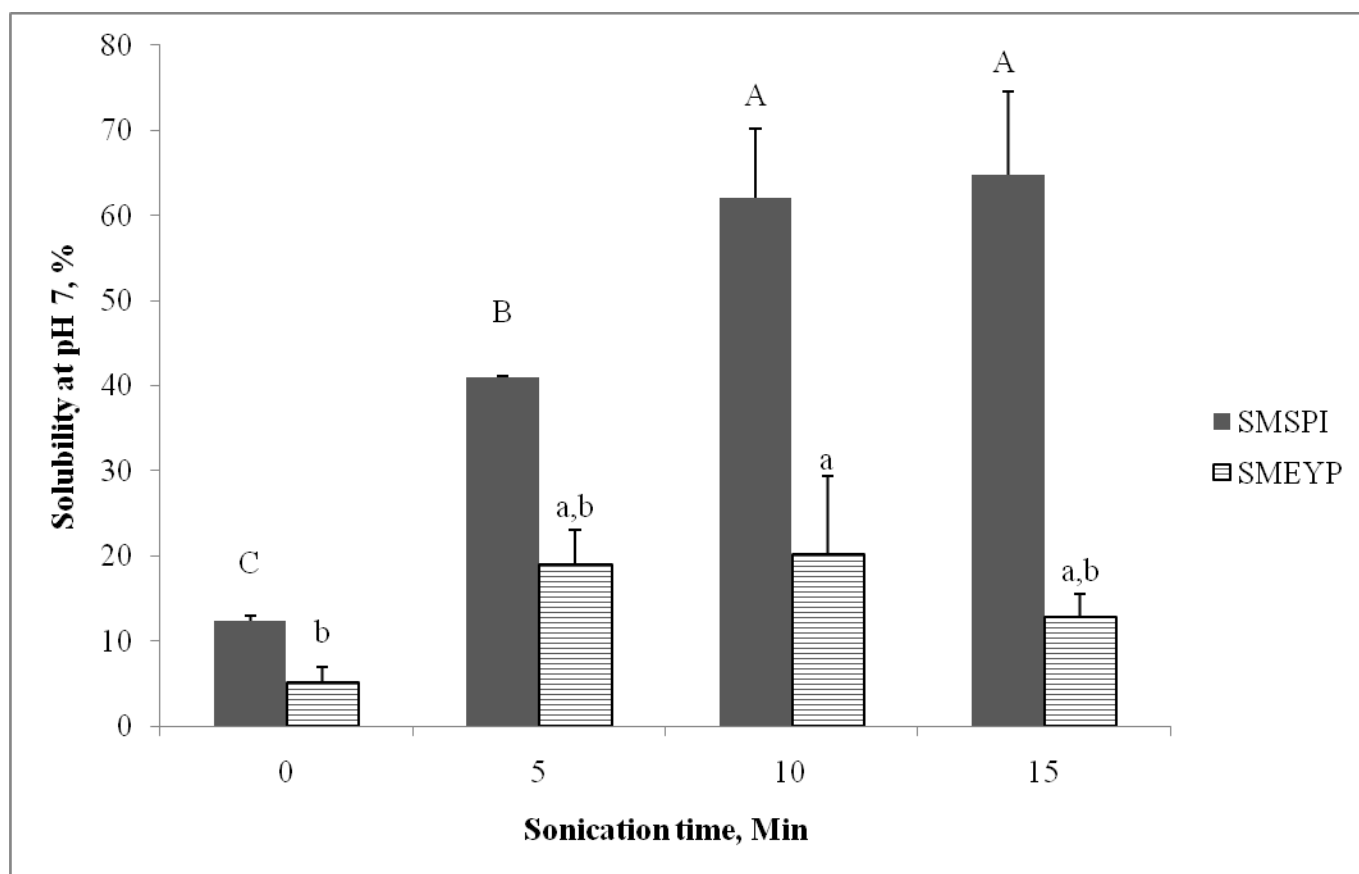


Figure 1. Effect of sonication time on protein dispersibility at pH 7. Different letters on each data series represent significant difference at $P = 0.05$. SMSPI, sonicated and modified SPI; SMEYP, sonicated modified egg yolk protein.

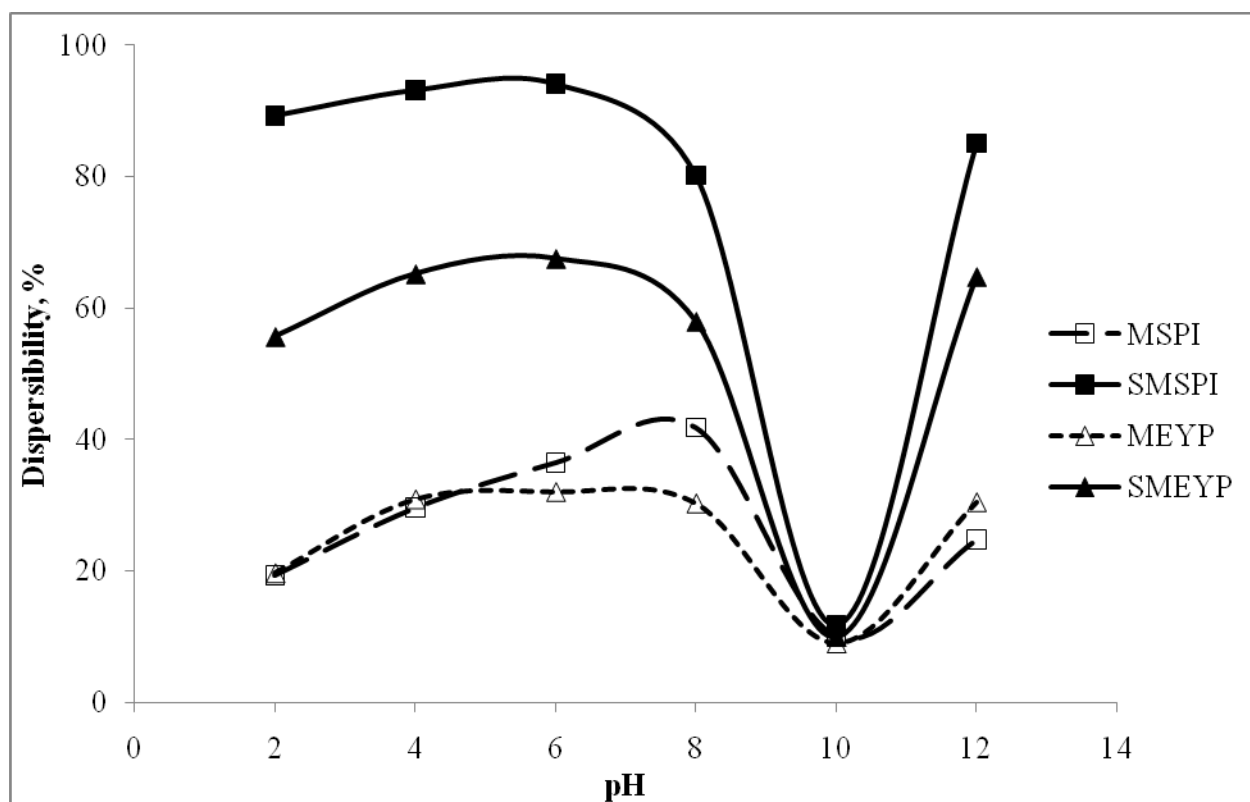


Figure 2. Dispersebility of modified proteins and the effect of sonication on protein dispersibility change. MSPI, modified SPI; MEYP, modified egg yolk protein; SMSPI, sonicated and modified SPI; SMEYP, sonicated modified egg yolk protein.

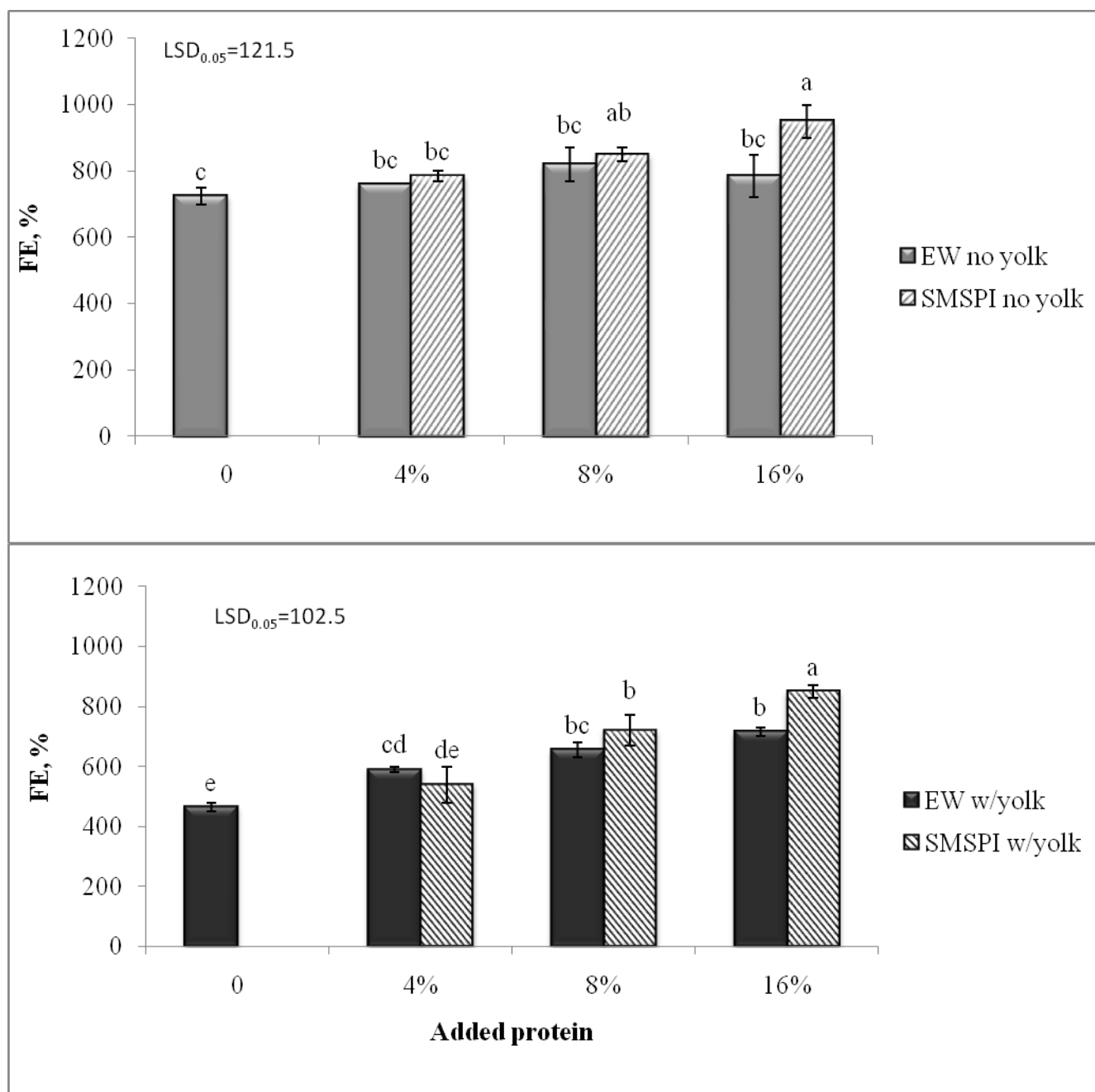


Figure 3. Foam expansion changes of 5% egg white as affected by added SMSPI as compared to EW with equivalent concentration. Upper figure represents treatments of no yolk, and lower figure represents treatments with 0.4% yolk addition. Different letters on the bars represent significant difference at $P = 0.05$ for each chart. EW, egg white; SMSPI, sonicated and modified soy protein isolate.

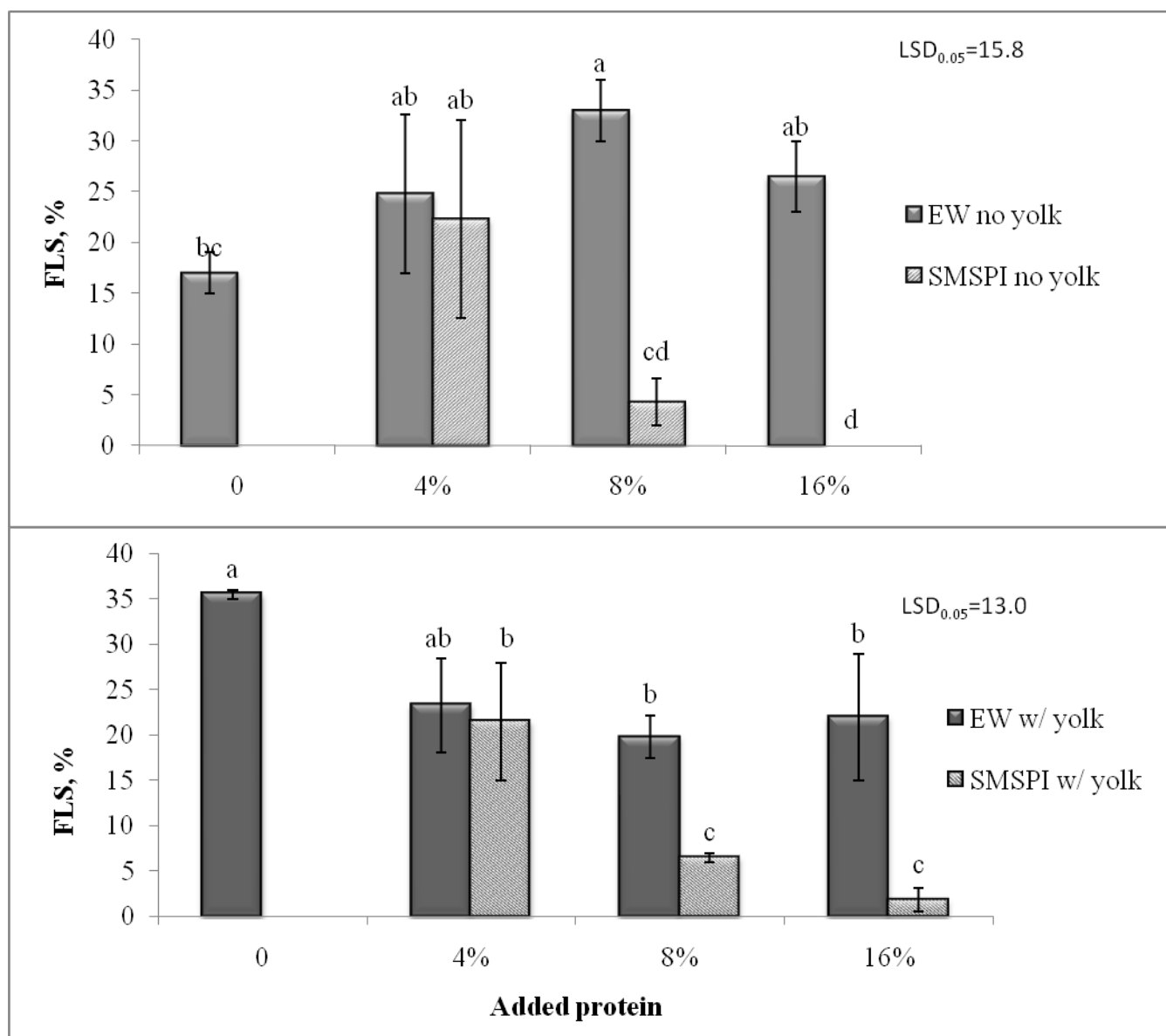


Figure 4. Foam liquid stability changes of 5% egg white as affected by adding SMSPI compared to EW with equivalent concentration. Different letters on the bars represent significant difference at $P = 0.05$ for each chart. EW, egg white; SMSPI, sonicated and modified soy protein isolate.