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Effect of pH Adjustment during Production of Egg White Powder on Foaming and Gelling Properties

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Abstract Egg white powders (EWPs) were produced after pH adjustment (pH 6-9) of fresh egg white, followed by spray-drying, and foaming and gelling properties of EWPs were examined. EWP produced after pH adjustment to 6.5 (EWP-6.5) resulted in significantly higher foaming ability and gel hardness than control and other pH-adjusted EWP. Significant increases in surface –SH content and surface hydrophobicity of EWP-6.5 coincided with improved foaming ability and gel hardness. Significantly higher consistency index for reconstituted EWP-6.5 indicates unfolding of egg white protein was substantially increased in EWP-6.5. Decreased α-helix content in EWP-6.5 was confirmed by circular dichroism spectral analysis. These results indicate pH adjustment prior to spray-drying leads to structural changes in egg white proteins, significantly affecting major functionalities of EWP.

Keywords: egg white powder, foaming, gelling, structure, unfolding

Introduction

Egg albumen is one of the widely used ingredients in food formulations. Although fresh liquid egg white has been commonly used in bakery application, the use of dried whole egg or egg white products in the food industry has steadily increased, especially in developed countries due to advantages over traditional liquid eggs in terms of convenience, safety, and uniformity of products (1, 2).

The primary functional attributes of egg white are foaming and gelling properties, which are mainly governed by the egg white proteins (3). In the production of egg white powder (EWP), these proteins are exposed to various physical, thermal, and chemical stresses, all of which affect the functional properties of egg white.

Hegolle *et al.* (4) reported that heat treatment over denaturation temperature of the purified egg white protein such as lysozyme improved the foaming ability and stability by increasing surface hydrophobicity and flexibility of the proteins. However, data obtained from the isolated proteins cannot be directly applied for the prediction of functional properties of EWP, because protein-protein interactions cannot be ruled out in heterogeneous egg white protein systems (5). In addition, heating of egg white over denaturation temperature cannot be applied in the production of EWP, because heating above 60°C causes aggregation of egg white proteins.

As another factor affecting the functionalities of EWP, our study focused on the effect of pH, because pH can modulate functionalities by inducing conformational changes in proteins. Although pH-induced unfolding and refolding regimes have been tested to modify the functional properties of cod muscle proteins (6), data on the functional properties of EWP are not yet available.

Therefore, the objectives of this study were to investigate the effect of pH adjustment prior to spray-drying on the foaming and gelling properties of EWP and to examine the physicochemical changes in egg white proteins induced by pH treatment.

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Materials and Methods

Materials Fresh hen eggs were kindly provided by Join poultry farm (Youngin, Korea), and egg whites were carefully collected from fresh eggs after the removal of chalaza. Freeze-dried egg white was prepared as a control. All chemicals used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of pH-adjusted egg white powder After clarification, pH of the fresh egg white was adjusted from 6.0 to 9.0 (no adjustment) using 1 N HCl and spray-dried using a pilot plant dryer (Samjin Engineering, Seoul, Korea) with respective inlet chamber and outlet air temperatures of 130 and 90°C. The pH and moisture content of fresh egg white were 9.0 and 90%, respectively. The average moisture content of spray-dried EWPs was 2.8% with no significant variations among the samples.

Foaming capacity and stability Foam was prepared following the method described by Kim and Imm (7). The EWPs were reconstituted in double-distilled water (11%, w/v), and 80 g each sample was subjected to foam testing chamber and whipped using an electronic heavy duty stirrer (Ika, Nara, Japan) at 1,200 rpm for 2 min. The foaming capacity was calculated as the overrun using following equation:

Overrun (%) = $(W_S - W_F)/ W_F \times 100$

where, W_S is the weight of sample (100 mL) and W_F is the weight of foam (100 mL).

After foaming, samples were transferred into a drainage testing unit, and the weight of liquid drained from the foam was continuously recorded using a digital balance (Ohaus Co., Pine Brook, NJ, USA). The drainage weight at 40 min was reported as foam stability. Foaming ability and stability were determined in triplicates.

Solubility The solubility of EWP was determined by the method of Mine (8) with slight modifications. EWP (1%, w/v) was reconstituted in 100 mM sodium phosphate buffer (pH 7.2) for 30 min at room temperature and centrifuged at 15,000×g for 30 min. The supernatant was filtered through a membrane filter (0.45 μm; Sartorius AG 37070, Goettingen, Germany), and protein concentrations before and after centrifugation were determined by the Lowry method (9). Solubility was calculated in triplicate as follows:

Solubility (%) = (protein content of supernatant / protein content of sample) × 100

Sulfhydryl content The concentration of sulfhydryl groups (SH group) in EWP was determined using Ellman's reagent (10). For the determination of surface SH group, 1 mL egg white solution (1%, w/v) was added to 4 mL Tris-glycine buffer (100 mM, pH 8.0). After addition of 125 μL 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) solution (4 mg/mL in Tris-glycine buffer), the egg white solution was incubated at 25°C for 10 min, and absorbance of the sample was measured at 412 nm using a spectrophotometer (Amhersham Bioscience, Stockholm, Sweden). For the determination of total SH group, egg white solution was added to Tris-glycine buffer containing 2% sodium dodecyl sulfate (SDS) and kept at 40°C for 15 min in a water bath to allow the protein to unfold, thus making all SH groups accessible to DTNB. The SH content was calculated as follows:

$\mu M\ SH/g = 73.53A_{412}\,(D/C)$

where, A_{412} = absorbance at 412 nm, C = sample concentration in mg/mL, and D = dilution factor (5.125).

Surface hydrophobicity Surface hydrophobicity of EWP was determined by the method of Lechevalier *et al.* (11) with slight modifications. EWP was solubilized in sodium phosphate buffer (20 mM, pH 7.0) at different concentrations (40-200 ug/mL), and 5 uL of 8 mM 8-anilino-1-naphthalene sulfonic acid (ANS) solution was added to 3 mL sample protein solution. ANS fluorescence intensity (emission spectra) was measured using a spectrofluorometer (Kontron Instrument, Milan, Italy) at 485 nm after excitation at 374 nm. The slope of the fluorescence intensity as a function of protein concentration was calculated and used as an index of surface hydrophobicity.

Viscosity The EWPs were reconstituted (11%, w/v) in double-distilled water, and viscosity of egg white solutions were determined by a rotational rheometer (AR2000; TA Instrument, New Castle, DE, USA) equipped with a cone-plate geometry (40 mm dia., 1° angle). Measurements were performed at shear rates from 0.3 to 1500 (1/sec). The temperature of samples was maintained at 25°C during the measurements. The flow behavior index (n) and consistency index (K) values from power law model were used to describe the rheological behavior of egg white solutions.

Gel preparation The EWPs were reconstituted (20%, w/v) in double-distilled water and degassed in a vacuum desiccator (KnF Laboratory, Freiburg, Germany). The samples were poured into gelatin casing (30 mm dia., Teepak, Lisle, IL, USA) and tightly sealed. The egg white gels were set in an 80°C water bath (SangWoo, Seoul, Korea) for 40 min and cooled at 25°C for 24 hr. Cylindrical specimens (30 mm dia. × 20 mm ht.) were prepared for texture profile analysis.

Texture profile analysis (TPA) A double compression test was performed using a Texture Analyzer (TA-XT2; Stable Micro System, Surrey, UK) by the method of Bourne (12) with some modifications. Cylindrical-shaped gels were uniaxially compressed twice to 50% deformation by a flat compression plate (75 mm dia.) at a crosshead speed of 0.5 mm/sec. From the texture profile analysis curves, hardness, cohesiveness, springiness, and gumminess were calculated by Texture expert software (Ver. 3.7). At least three determinations were made for each gel.

Circular dichroism analysis Circular dichroism (CD) analysis was carried out to assess the conformational changes of reconstituted EWP. EWP (0.06%, w/v) was reconstituted in 10 mM sodium phosphate buffer (pH 7.2) and filtered through a filter membrane (pore size, 0.45 μm). CD spectra were measured by a spectropolarimeter (Model J-715; Jasco, Tokyo, Japan) using 1 mm cell in the far-ultraviolet region (190-260 nm) at 21°C. The resolution was 1 nm, and scan speed was 50 nm/min. CD spectra were expressed as mean residue ellipticities (deg cm^2/dmol).

Statistical analysis All analytical measurements were performed in triplicates, and the data were analyzed using Minitab (Ver. 13.1, Minitab Inc., State College, PA, USA). When analysis of variance (ANOVA) revealed significant differences at $p<0.05$, the data were further analyzed using Tukey's multiple comparison test.

Results and Discussion

Foaming properties of EWP produced after pH adjustment The foaming properties of reconstituted EWPs (11 %, w/v) were evaluated. EWP produced after pH adjustment to 6.5 (EWP-6.5) had significantly higher ($p< 0.05$) foam overrun than other EWPs (Fig. 1). The foam overrun of EWP-6.5 was higher than that of the freeze-dried egg white control (EWP-control), whereas other pH treatments resulted in similar or lower foam overrun compared to the control. In addition, all EWPs produced after pH

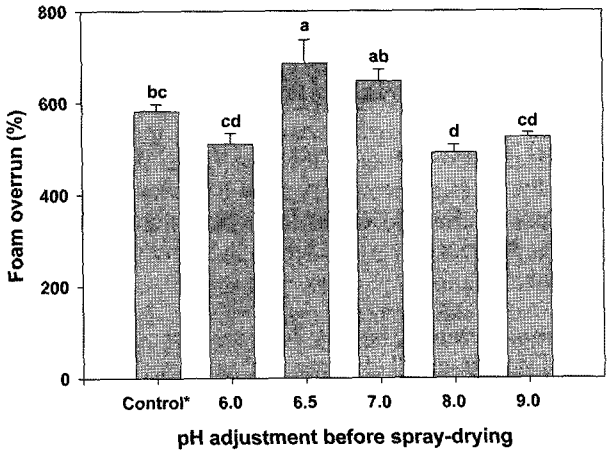

Fig. 1. Foam overrun of egg white powder produced after pH adjustment. Control*: freeze-dried egg white powder (pH 9.2). Means with different letters are significantly different at $p<0.05$.

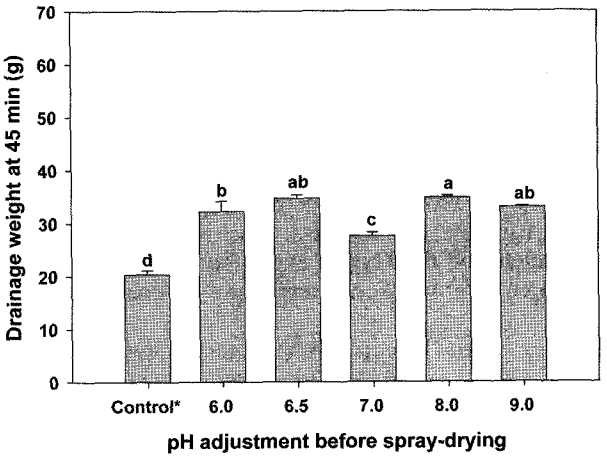

Fig. 2. Foam stability of egg white powder produced after pH adjustment. Control*: freeze-dried egg white powder (pH 9.2). Means with different letters are significantly different at $p<0.05$.

treatments showed lower foam stability (greater drainage weight) than EWP-control (Fig. 2). EWP-7.0 showed relatively higher stability than others.

These results suggested that pH adjustment of egg white prior to spray-drying significantly influenced the foaming ability of EWP, whereas the conditions of spray-drying (drying temperature and atomization, etc.) adopted in this study did not significantly affect the foaming ability.

The formation of foam is initiated by adsorption of proteins into air/water interface, and this phenomenon is thermodynamically favorable due to the hydrophobic patches of protein surface driving this process (13). Once proteins are adsorbed, they are denatured at the interface, and films of denatured proteins are generated with rearrangement of protein layers (14). Based on this context, foaming ability is closely related to the easiness of protein adsorption into the interface, while foaming stability is rather dependent on the integrity of protein network that is able to stabilize the foam. This also indicates that foam formation and stabilization are governed by different

Table 1. Surface and total SH content of egg white powder produced after pH adjustment

pH adjustment	Surface SH (μM SH/g)	Total SH (μM SH/g)	Surface SH/Total SH
Control[1)]	1.053 ± 0.036^{c}	55.595 ± 0.281^{a}	0.019^{c}
6.0	1.884 ± 0.118^{b}	52.948 ± 0.169^{c}	0.036^{b}
6.5	2.257 ± 0.129^{a}	52.453 ± 0.139^{d}	0.043^{a}
7.0	2.359 ± 0.150^{a}	54.916 ± 0.084^{b}	0.043^{a}
8.0	1.764 ± 0.026^{b}	50.037 ± 0.048^{e}	0.035^{b}
9.0	1.723 ± 0.118^{b}	47.355 ± 0.080^{f}	0.036^{b}

[1)]Freeze-dried egg white (pH 9.2).
Means in a column followed by different superscripts are significantly different at $p<0.05$.

mechanisms as reported by HammershØj *et al.* (1).

Although the improved foam overrun probably reflects comprehensive structural modifications in egg white proteins, determination of exact changes in individual egg white protein is difficult. Egg white consists of several types of proteins with a wide range of isoelectric point from 4.1 (ovomucoid) to 10.7 (lysozyme) (1), and structural modifications either in a protein or between proteins can occur during pH treatment followed by spray-drying.

Doi and Kitabatake (15) reported that highly ordered globular proteins showed low foaming ability; therefore, native ovalbumin, the major egg white protein, showed limited foaming ability. Partial unfolding of proteins in EWP-6.5 and EWP-7.0 might facilitate protein absorption into the interface, resulting in improved foaming ability. However, this structural transformation could not form rigid viscoelastic films at the interface and resulted in reduced foaming stability.

Surface SH, surface hydrophobicity, and solubility of EWP produced after pH adjustment To explain the differences in foaming ability among EWPs, surface –SH and hydrophobicity were determined, because both properties could reflect the degree of protein unfolding (16). Surface SH contents of EWP-6.5 and EWP-7.0 were significantly higher than those of other EWPs (Table 1). Total SH residues of EWPs gradually decreased with increasing pH. This trend was consistent with the result of Mine (17) that the oxidation of SH groups into disulfide bonds increased at alkaline pH than at neutral pH. When the results were expressed in terms of ratio of surface to total SH content, the ratios of EWP-6.5 and EWP-7.0 were higher than those of other EWPs.

Although many of the egg albumen proteins have S-S linkages, ovalbumin is the only egg white protein to contain four free SH groups (18). The SH groups of ovalbumin are located in the hydrophobic regions and are known to react poorly to sulfhydryl-reacting agents in their native state (19). Therefore, the increased surface –SH contents of EWP-6.5 and EWP-7.0 reflected unfolding of the ovalbumin due to the pH adjustment, and increased exposure of SH groups to the surface contributed to the improvement of foaming ability.

Surface hydrophobicity also plays an important role in

foaming ability of proteins, because it is closely related to the ability of protein to adsorb into the hydrophobic surface, which is the first step in foam formation (20). Surface hydrophobicity of egg white significantly increased during the production of EWP, with EWP-6.5 showing the highest surface hydrophobicity (Fig. 3). The increased surface hydrophobicity indicates protein unfolding and exposure of the aromatic amino acid residues, because the applied fluorescent probe, ANS, has an ability to bind aromatic side chains in the surface of proteins.

No significant differences were observed among samples; all samples showed excellent solubility (Table 2). This result indicates that changes in protein structure induced by pH treatments did not impair solubility characteristics of EWP under the tested condition. Damodaran (21) suggest that the solubility characteristics of proteins may depend on the physicochemical characteristics of the protein surface and thermodynamics of its interaction with the surrounding solvent.

Viscosity of EWP produced after pH adjustment The power law model is generally used to describe the rheological properties of solutions. Power law parameters, consistency coefficient (K) and flow behavior index (n), were obtained by fitting the shear rate versus apparent viscosity as follows:

$$\eta_a = K \cdot \dot{\gamma}^{(n-1)}$$

where, η_a is the apparent viscosity (Pa·sec), K (Pa·sec^n) is the consistency coefficient, $\dot{\gamma}$ is the shear rate (1/sec), and n (dimensionless) is the flow behavior index. EWP-6.5 had the highest consistency coefficient, while EWP-control, EWP-8.0, and EWP-9.0 showed the lowest values (Fig. 4). These results suggest that EWP-6.5 is more viscous than other EWPs when reconstituted in distilled water.

Singer *et al.* (22) reported that unfolding of globular proteins is frequently accompanied by swelling, resulting in increased hydrodynamic radii of protein molecules and greater molecular interentanglements. The increased exposure of hydrophobic amino acids found in EWP-6.5 (Table 1) led to the unfolding of egg white proteins, subsequently contributing to high resistance against flow, as suggested by previous results.

All EWP solutions showed non-Newtonian flow behavior, in which the apparent viscosity changed with the shear rate. The calculated flow behavior indices (n) of all EWP solutions were less than 1 and exhibited shear-thinning behavior (data not shown).

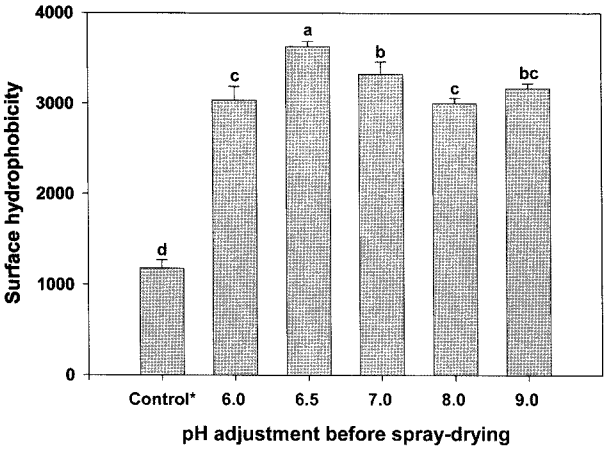

Fig. 3. Surface hydrophobicity of egg white powder produced after pH adjustment. Control*: freeze-dried egg white powder. Means with different letters are significantly different at $p<0.05$.

Table 2. Solubility of egg white powder produced after pH adjustment

pH adjustment	Solubility (%)
Control[1)]	99.47 ± 0.30[a]
6.0	98.11 ± 1.39[a]
6.5	99.50 ± 0.21[a]
7.0	98.70 ± 1.24[a]
8.0	98.79 ± 1.18[a]
9.0	99.60 ± 0.42[a]

[1)]Freeze-dried egg white (pH 9.2).
Means in a column followed by different superscripts are significantly different at $p<0.05$.

Texture profile analysis of EWP produced after pH adjustment The texture properties of pH-adjusted EWP gels (20%, w/v) were examined (Table 3). Hardness of EWP-6.5 gel was significantly higher ($p<0.05$) than those of other EWPs. The gel hardnesses of EWP-7.0 and EWP-8.0 were relatively higher than those of EWP-6.0 and EWP-9.0 gels. On the other hand, cohesiveness and springiness were not critically affected by pH treatments.

The sulfhydryl-disulfide interchange reaction has been suggested as one of the main causes for the gelation of egg whites at above 75°C (23). Margoshes (24) also reported that surface SH groups of dried egg white had high correlation with the strength of heat-induced dried egg white gels. These reports were consistent with the finding

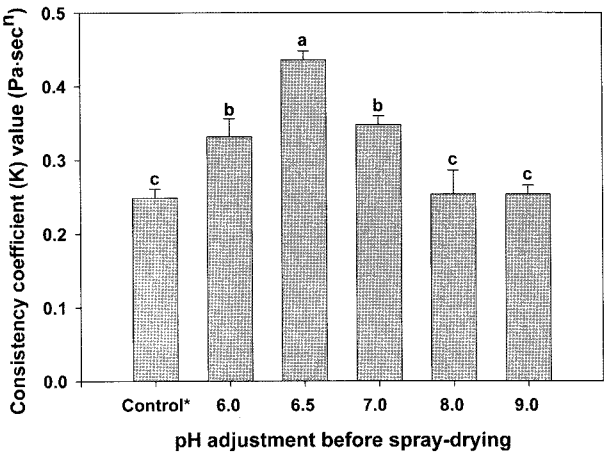

Fig. 4. Consistency index of reconstituted egg white solution. Control*: freeze-dried egg white powder. Means with different letters are significantly different at $p<0.05$.

Table 3. Texture attributes of egg white powder produced after pH adjustment

pH adjustment	Hardness (N)	Cohesiveness	Springness	Gumminess
Control[1)]	51.97 ± 4.94^{cd}	1.10 ± 0.04^{bc}	1.00 ± 0.00^{a}	57.20 ± 6.51^{c}
6.0	55.76 ± 8.86^{bcd}	1.13 ± 0.04^{b}	1.00 ± 0.00^{a}	63.30± 11.48^{bc}
6.5	79.54 ± 7.82^{a}	1.03 ± 0.02^{c}	1.00 ± 0.00^{a}	81.66 ± 7.41^{a}
7.0	66.46 ± 6.71^{b}	1.06 ± 0.06^{bc}	1.00 ± 0.00^{a}	70.06 ± 4.02^{abc}
8.0	64.67 ± 3.03^{b}	1.04 ± 0.02^{c}	1.00 ± 0.00^{a}	67.05 ± 3.66^{bc}
9.0	52.25 ± 2.83^{d}	1.39 ± 0.05^{a}	1.00 ± 0.00^{a}	72.38 ± 4.90^{ab}

[1)]Freeze-dried egg white (pH 9.2).
Means in a column followed by different superscripts are significantly different at $p<0.05$.

of current study that EWP-6.5 showed highest surface SH content. Kato *et al.* (25) suggested that the partially unfolded structure, molten structure, is more flexible than the native form and this molten structure may reinforce the cross-linking and disulfide bonds of the unfolded molecules.

Although Handa *et al.* (26) reported that the hardness of egg white gels increased as the pH of egg white increased, this trend was not observed in our study; it should be pointed out that Handa *et al.*'s study had been conducted by controlling the pH of fresh egg white instead of EWP. This result implies that gelling properties of EWP produced by pH treatment are different from those of fresh egg white.

Circular dichroism (CD) analysis of EWP The effects of pH adjustment on the secondary structures of EWP were examined using CD spectral analysis. According to Hagolle *et al.* (27), CD spectra of EWP showed two distinct peaks at 213 and 222 nm, representing the existence of β-sheet and α-helical structures, respectively. The pH treatment induced marked changes in the CD spectra of EWPs (Fig. 5). The magnitudes of ellipticity (at 222 nm) of EWP-6.5 and EWP-7.0 were significantly lower than those of other EWPs. This result indicates that egg white proteins in EWP-6.5 and EWP-7.0 have significantly lower helical secondary structures. It is plausible that the decreased α-helical structure in egg white proteins caused exposure of hydrophobic residues to the surface (Fig. 3). Such conformational changes probably reduced the energy barrier for protein adsorption related to foaming ability. In addition, the formation of intermolecular hydrogen bond through β-sheet conformation also leads to the formation of a firm and stable gel network as reported by Kato *et al.* (28).

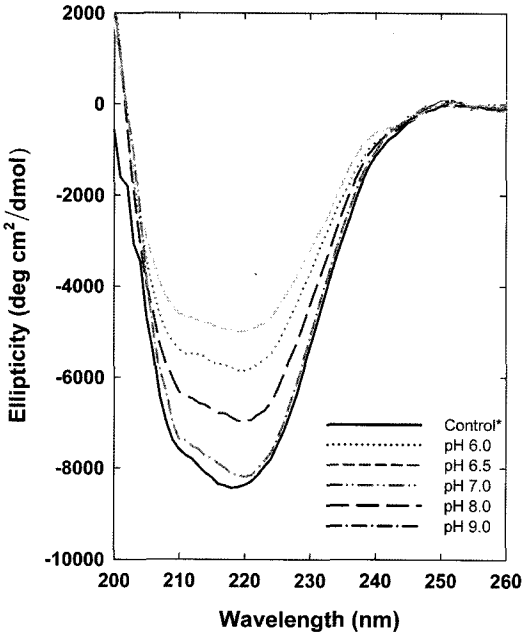

Fig. 5. Circular dichroism spectra of egg white powder produced after pH adjustment.

In conclusion, the functional properties of EWP can be modified by pH adjustment prior to spray-drying. Adjusting the pH of fresh egg white to 6.5 before drying improved the foaming ability and gel hardness of the resultant EWP (EWP-6.5). The improved functionality was closely related to the structural modifications of egg white proteins. The significant increases in surface –SH content and surface hydrophobicity of EWP-6.5 coincided with the improvement in foaming ability and gel hardness.

Acknowledgments

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