Effect of Dry Heat and Mild Alkaline Treatment on Functional Properties of Egg White Proteins

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Physicochemical properties exhibited by the dried egg white protein molecule during the dry heat treatment at low water activity were assessed. Gelling properties of dried egg white proteins were greatly improved by heating in the dry state at a mild alkaline pH (under 9.50). The surface hydrophobicity increased with heating time, and sulfhydryl−disulfide exchange was accelerated at a mild alkaline dry heating. The proteins were deamidated by this treatment with no effect on solubility and hydrolysis. After 15 days of storage of the proteins in the dry state, the deamidation rate reached 12.2%. However, dry heating at higher pH (>9.50) caused detrimental effect on egg white proteins, resulting in the formation of insoluble aggregates by polymerization. Increasing solubility due to the deamidation of the denatured proteins and the reaction can be an important factor for forming excellent firm gels of dried egg white proteins. Thus, the combination of dry heating and deamidation under controlled pH values offers a new approach to improve the functional properties of food proteins and to study the structure−function relationship.

Keywords: *Egg white; gelation; elasticity; dry heating; pH; deamidation; alkali treatment*

INTRODUCTION

Dry heating (storage in a hot room under controlled temperature and moisture content conditions) is one of the most promising approaches for improving the gelling properties (gel strength, elasticity, and water holding capacity) of dried egg white (DEW) proteins (Kato *et al*., 1989, 1990a; Mine, 1996). The structure−function relationship of the dry-heated DEW proteins related to their protein denaturation during the dry heating and gelling properties is of great interest in the development of protein functionality. The partially unfolded conformation formed by dry heating may be attributed to the increase in the gelling properties of DEW proteins (Kato *et al*., 1990a). Formation of low molecular weight and narrow molecular distribution of the aggregate found during the subsequent heating for gelation also contributed to the excellent gel formation of the proteins (Kato *et al*., 1990b; Mine, 1996). In addition, I found a mild alkaline dry heating (under pH 9.50) is also an effective method to obtain firm and elastic gels of DEW proteins for a short period of time (3−5 days) without the loss of water solubility (Mine, 1996). The mild alkaline dry heating of DEW proteins resulted in the acceleration of protein denaturation as detected by circular dichroism (CD) and differential scanning calorimetry (DSC) analysis. The polymerization of DEW proteins was also enhanced by the treatment leading to sulfhydryl−disulfide interchange. According to the above results, an important question occurs: why is the water solubility of denatured DEW proteins not affected by the mild alkaline dry heating, although the denaturation and polymerization of the proteins were increased by this treatment compared to those of unadjusted pH DEW proteins? It is also unclear how the physicochemical properties of egg white proteins might be affected during dry heating in low water activity and mild alkaline pH values. Investigation of these physicochemical properties of the proteins would provide better understanding for structure−function properties of food proteins. For this paper, some physicochemical properties of DEW proteins occurring at a mild alkaline dry heating were studied, and their significance related to the gelling properties of DEW proteins will be discussed.

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MATERIALS AND METHODS

Preparation of DEW Proteins. Following treatment by the decarbohydration of a glucose oxidase catalase enzyme system, the egg white liquid was adjusted to pH 6.0, 7.1, and 7.6 with 1 N HCl or 1 N NaOH, respectively, before spray-drying (Hill and Sebring, 1986). Spray-drying was done using a pilot plant dryer with an exhaust temperature 65−70 °C. The DEW with three pH levels before spray-drying was stored at 75 °C for various periods of time (days) in dry state (8.5% moisture content, a little higher than that of commercial spray DEW). Periodically, samples of DEW were taken from the incubator and cooled at room temperature. These samples were used for testing the physicochemical and functional properties.

Measurement of Gelling Properties. Preparation of DEW protein gels was carried out as follows: 10% protein solution of each sample in 50 mM phosphate buffer (pH 7.40) was put into 30 mm diameter poly(vinylidene chloride) tubes. Then the tubes were sealed and immersed in a water bath at a temperature of 75 °C for 60 min. Then, the tubes were taken from the hot bath and cooled in ice water. The samples were kept at 4 °C overnight before testing. The quality of the gels was assessed by measuring their gel strength (g/cm) and deformation (cm). Sample gel was cut to 30 mm in length. Each gel piece was set on the detachable table of a Rheometer (Fudo Kogyo K.K., Tokyo, Japan) equipped with a spherical plunger ($\varnothing = 8$ mm). The amount of compression was 20% of original height. Compression on the sample piece was executed with a table speed of 60 mm/min. Elasticity was obtained by deformation (g × cm) (Mine, 1996). The measurement was repeated eight times on each sample gel, and the average was calculated. Water-holding capacity (WHC) was determined as follows. The DEW protein gels (30 mm diameter and 30 mm height) were placed on two layers of filter paper (Whatman No. 1, 11 cm diameter) at room temperature for 90 min. The percentage of syneresis of the gels was measured and used as an index of WHC.

S0021-8561(97)00158-1 CCC: $14.00

Measurement of Solubility. Solubility of DEW proteins was measured by determining the protein concentration in the sample (0.2%) in 100 mM phosphate buffer (pH 7.2) after 30 min of stirring at room temperature followed by centrifugation (5000g × 30 min). The serum was filtered by a membrane filter (0.45 μm). Protein concentration was determined according to the Lowry method (Lowry *et al*., 1951). Percentage of protein solubility (PS) was calculated as follows.

$$\text{PS (\%)} = \frac{\text{protein content of sample}}{\text{protein content of control}} \times 100$$

The spray-dried egg white protein was used as a control.

Measurement of Surface Hydrophobicity (S_0). Surface hydrophobicity in the dry-heated DEW proteins was determined using *cis*-parinaric acid (Sigma, St. Louis, MO) as a fluorescence probe as described by Kato and Nakai (1980). Ten microliters of an ethanol solution of *cis*-parinaric acid was added to 2.0 mL of 0.1% protein solution in 0.01 M phosphate buffer (pH 7.4). The mixture was excited at 325 nm, and the relative fluorescence intensity was measured at 420 nm in a spectrophotofluorometer (Model RF-540, Shimadzu, Japan). The relative fluorescence intensity was adjusted to 1.0 when 10 μL of *cis*-parinaric acid solution was added to 2.0 mL of the buffer in the absence of protein. The initial slope (S_0) of fluorescence intensity versus protein concentration was used as a measure of surface hydrophobicity.

Measurement of Sulfhydryl Groups. Measurement of sulfhydryl groups was performed by using Ellman's reagent (Beveridge *et al*., 1974). To 1 mL of the 1% protein solution was added 4 mL of 0.1 M Tris−glycine buffer (pH 8.0) containing 0.01 M EDTA (for surface sulfhydryl groups) and the same buffer containing 10 M urea (for total sulfhydryl groups). After incubation at 40 °C for 30 min, 125 μL of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) solution (20 mg in 5 mL of 0.1 M Tris−glycine buffer, pH 8.0) was added and then incubated at 25 °C for 10 min. The color absorbance was read at 412 nm on a Shimadzu UU-1201 spectrophotometer. The sulfhydryl residues were calculated as

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

where A_{412} is the absorbance at 412 nm, C is the sample concentration in mg/mL, and D is a dilution factor, 5.125.

Measurement of Deamidation of Proteins. Protein deamidation was analyzed by measuring the ammonia generated during the reaction. The samples were dialyzed overnight against deionized water to remove the free acids and lyophilized. Then, the sample (100 mg) was dissolved in 2.0 mL of 2 N HCl and hydrolyzed for 2 h at 110 °C. An equal volume of 24% tricholoroacetic acid solution was added to the sample to precipitate the protein and centrifuged for 5000g × 30 min. Ammonia in the supernatant was analyzed using an ammonia electrode (Fisher Scientific, Ottawa, ON). The deamidation percentage of the samples was determined from the values of ammonia as follows:

$$\text{deamidation (\%)} = \frac{\text{value of heat treated DEW proteins}}{\text{value of native proteins}} \times 100$$

Measurement of Hydrolysis. After dy heat treatment, 2.0% of DEW protein solution in 100 mM phosphate buffer (pH 7.4) was mixed with an equal volume of 24% trichloroacetic acid and filtered (Whatman No. 1). The amount of peptide and amino acid in the filtrate was determined according to the standard Kjeldahl method. Degree of hydrolysis of the proteins was indicated by the ratio of peptide content in serum against of original protein concentration.

Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was carried out as described by Laemmli (1970) using a 5−15% gradient gel (Bio-Rad, Mississauga, ON). The protein was stained with Coomassie brilliant blue R-250 (Sigma) in 10% acetic acid−30% methanol.

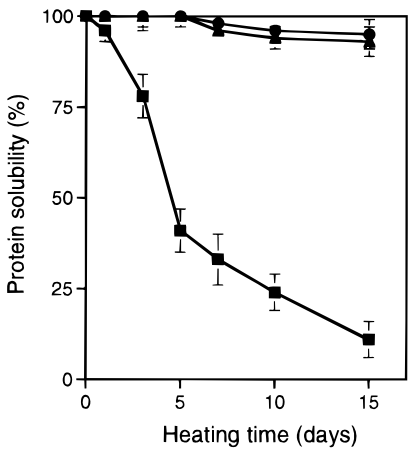

Figure 1. Changes in the solubility of spray-dried egg white proteins during dry heating at various pH values: (●) dry heated at pH 7.04 (control), (▲) dry heated at pH 9.42, and (■) dry heated at pH 10.40. Data are the average of triplicate measurements.

RESULTS AND DISCUSSION

The pH values of DEW solution in water (10% protein), which were adjusted to pH 6.0, 7.1, and 7.6 before spray-drying, were 7.04, 9.42, and 10.40, respectively. In general, liquid egg white is subjected to spray-drying at pH 5.8−6.3 and the pH of DEW powder is 7.0−7.5. The increase of pH values in DEW powder is due to the evaporation of soluble carbon dioxide in liquid egg white by spray-drying. Thus, the DEW powders with different pH values were named DEW (pH 7.04-control), DEW (pH 9.42), and DEW (pH 10.40), respectively.

Figure 1 shows the changes in the solubility of heat-treated DEW proteins at 75 °C in the dry state for various periods of time. The solubility was expressed as the ratio to the value of the nonheated sample. It was observed that there were no effects of dry heating on the solubility of DEW (pH 7.04-control) or DEW (pH 9.42) within the heating period of 7 days. Although solubility slightly decreased with heating for 15 days, the effect was not detrimental for the proteins. The solubilities of DEW (pH 7.04-control) and DEW (pH 9.42) after heating for 15 days were 95% and 93%, respectively. On the other hand, the solubility of DEW (pH 10.40) was markedly decreased with increased heating time in the dry state. Significant change in color and polymerization of DEW (pH 10.40) during the dry heating occurred when the protein was treated over 5 days in the dry state (data are not shown). Considerable off-flavors also resulted after treatment. These results indicate that the effect of heating in the dry state on the solubility of DEW protein was very small up to pH 9.42; however, the effect was detrimental at pH 10.40.

The effect of pH during the dry heating of DEW proteins on their gelling properties is summarized in Table 1. The gel strength and elasticity of DEW proteins were greatly increased by the dry heating and resulted in an excellent firm gel structure. The relative gel strength of DEW (pH 9.42) was higher than that of the control. The water-holding capacity of DEW proteins was also improved by heating in the dry state at pH 9.42. The transparency of DEW proteins was also increased by this treatment (data are not shown). However, the gel of DEW (pH 10.40) proteins was weak because of low solubility of the proteins. These phe-

Table 1. Gelling Properties of Dry Heated Egg White Proteins at Various pH Values[a]

sample[b,c]	gel strength (g/cm)	elasticity (g × cm)	WHC[d] (%)
stored at pH 7.04			
0 days	123 ± 7	98 + 5	9.2 + 0.3
7 days	655 ± 12	202 ± 18	6.2 ± 0.4
stored at pH 9.42			
0 days	143 ± 6	104 ± 7	8.8 ± 0.6
7 days	847 ± 16	475 ± 19	4.1 ± 0.4
stored at pH 10.40			
0 days	118 ± 17	101 ± 7	9.7 ± 0.5
7 days	98 ± 7	77 ± 4	32.1 ± 3.4

[a] Data represent averages of eight determinations with standard deviation. [b] Samples were heated in dry state at 75 °C. [c] Ten percent protein in 50 mM phosphate buffer (pH 7.4) was heated at 75 °C for 60 min. [d] Water-holding capacity.

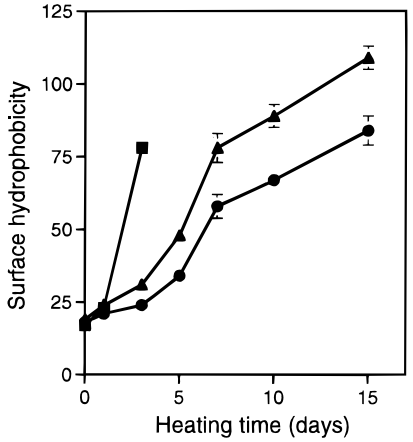

Figure 2. Relationship between surface hydrophobicity and heating time in the dry state of spray-dried egg white proteins at various pH values: (●) dry heated at pH 7.04 (control), (▲) dry heated at pH 9.42, and (■) dry heated at pH 10.40. Data are the average of triplicate measurements.

nomena are important in elucidating the structure−function relationship of food proteins on the molecular level. I have previously revealed that the increase in degree of denaturation and polymerization of DEW proteins found during the dry heating was accelerated in alkaline pH region (Mine, 1996). The degree of unfolding of the proteins upon dry heating may play a crucial role in the gelling process of the proteins. However, studies on the physicochemical properties that might occur on the protein molecules at alkaline pH values, such as sulfhydryl−disulfide exchange, surface hydrophobicity, deamidation, peptide cleavage, or changes in surface charge, are required for better understanding of the gelation mechanism of DEW proteins.

Figure 2 shows the relationship between surface hydrophobicity of DEW heating proteins and heating time in the dry sate at alkaline pH values. The surface hydrophobicity greatly increased with increased heating time in the dry state. The value of surface hydrophobicity increased 5-fold over the value of nonheated DEW proteins when heated at 75 °C for 15 days. The increasing rate of surface hydrophobicity related to heating time was larger in DEW (pH 9.42) than in DEW (pH 7.04-control). On the other hand, the surface hydophobicity of DEW (pH 10.40) was increased for a short time of period (3 days), but the proteins became insoluble because of the denaturation and polymerization of the proteins. The majority of surface sulfhydryl residues in egg white proteins exist in the interior of protein molecules and are exposed with heat denaturation (Mine *et al.*, 1990; Mine, 1992). Heat-induced

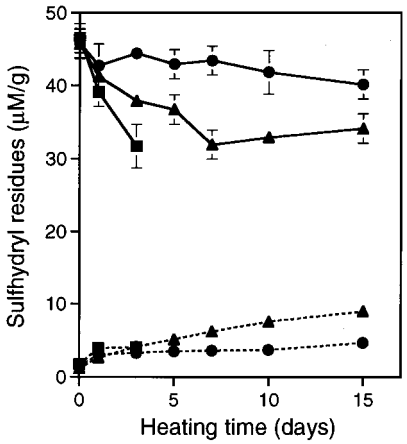

Figure 3. Changes of sulfhydryl residues in spray-dried egg white proteins during dry heating at various pH values: (●) dry heated at pH 7.04 (control), (▲) dry heated at pH 9.42, and (■) dry heated at pH 10.40; (—) total sulfhydryl level; (· · ·) surface sulfhydryl level. Data are the average of triplicate measurements.

sulfhydryl−disulfide exchange in DEW proteins upon dry heating may affect the aggregation behavior of the proteins. Figure 3 shows the changes of total and surface sulfhydryl groups in DEW proteins in the dry state for various periods at alkaline pH values. The total sulfhydryl level of nonheated DEW proteins was 45.7 ± 0.6 μM/g of dry weight, a little lower than the value found in the literature (Beveridge *et al.*, 1974). The total sulfhydryl residues of DEW proteins were gradually decreased with increased heating time in the dry state. The total sulfhydryl level of DEW (pH 9.42) proteins was decreased to 32.9 from 45.7 μM/g of control level. On the other hand, the surface sulfhydryl groups in DEW proteins were gradually increased with increased heating time. These results suggest that sulfhydryl groups in DEW proteins were exposed to the surface on the proteins and sulfhydryl−disulfide exchange reactions were accelerated by heating in the dry state. The sulfhydryl−disulfide interchange reaction was also promoted at alkaline pH values compared to that at neutral pH. The hydrophobic interaction and sulfhydryl−disulfide interchange may be an important factor for polymerization of egg white proteins.

An important question was raised regarding why the solubility of DEW (pH 9.42) proteins was not affected by heating in the dry state, although proteins were denatured and formed polymers with molecular masses of 200−375 kDa upon alkaline dry heating (Mine, 1996). Protein solubility is an important attribute for functional properties of food proteins. For example, to obtain optimum functionality in foods that require gelation, emulsification, and foaming properties, a highly soluble protein is desirable. Bigelow (1967) suggested that the solubility of a protein is related to the average hydrophobicity of the amino acid residues and the charge frequency of the protein. An increase in the charge density of a protein usually increases the aqueous solubility of the substance, as a result of enhanced intermolecular repulsion, alteration in viscosity, increased hydration, and decreased aggregation. Acid and alkali have been used extensively to increase solubility. However, these treatments of proteins cause not only detrimental denaturation of proteins but also cleavage of peptide bonds (Han *et al.*, 1983). Although several papers have been published on heating of DEW in the dry state, these papers looked at gelation, solubility, hydrophobicity, denaturation, electrophoresis,

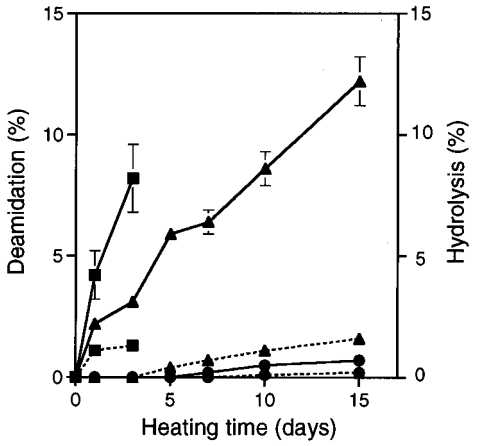

Figure 4. Deamidation and hydrolysis percentages of spray-dried egg white proteins during dry heating at various pH values: (●) dry heated at pH 7.04 (control), (▲) dry heated at pH 9.42, and (■) dry heated at pH 10.40; (—) deamidation curves; (· · ·) hydrolysis curves. Data are the average of triplicate measurements.

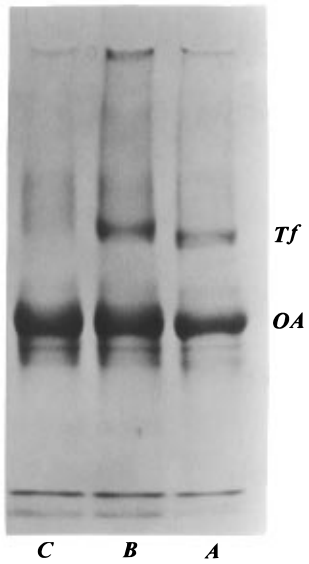

Figure 5. SDS−PAGE patterns of spray-dried egg white proteins in the dry state at various pH values: (A) native egg white proteins; (B) dry heated at pH 7.04; (C) dry heated at pH 9.42. Tf, ovotransferrin; OA, ovalbumin.

and changes in free amino groups (Kato *et al.*, 1989, 1990a; Mine, 1996). Deamidation and hydrolysis of the peptide bonds can also be achieved by alkali treatment. Deamidation of proteins occurs below pH 3.0 (acid catalyzed) and above pH 8.0 (base catalyzed) at rates dependent upon the H^+ or OH^- concentration, temperature, adjacent amino acid residue (R), and predominant pathway (Riha *et al.*, 1996). However, there is no information regarding the deamidation of proteins during dry heating at low water activity. Figure 4 shows the deamidation percentage of DEW proteins during dry heating at alkaline pH values. Little deamidation occurred in the control DEW proteins in the dry state for 15 days. On the other hand, deamidation was observed in the case of alkaline dry heating. The deamidation of DEW (pH 9.42) proteins gradually proceeded with increased heating time and reached 12.2% in 15 days. In the case of DEW (10.40), the deamidation in the proteins was accelerated in a short time (3 days); however, it became insoluble due to the aggregation of the proteins (Mine, 1996). Proteolysis was evaluated by the measurement of small peptide in the filtrates in the presence of 12% trichloroacetic acid or SDS−PAGE. The large peptide resulting from the cleavage of a small number of peptide bonds was not detected in the dry-heated DEW(pH 7.04-control) and DEW (pH 9.42) proteins (Figure 4). The SDS−PAGE was performed to assess the hydrolysis of DEW proteins (Figure 5). No peptide bond hydrolysis was observed in the electrophoresis analysis of DEW proteins. However, some of the aggregate remained undissociated in the stacking gel in DEW (pH 7.04-control) proteins in the presence of 2-mercaptoethanol (Figure 5, lane B), indicating that soluble aggregates were formed between egg white proteins by hydrophobic interaction. In addition, the band of ovotransferrin was not observed in the case of DEW (pH 9.42) proteins (Figure 5, lane C). The previous paper revealed that protein denaturation of egg white proteins was accelerated in alkaline pH region detected by CD analysis. DSC analysis also showed that ovotransferrin was very sensitive to alkaline dry heating (Mine, 1996). It is hard to consider that only ovotransferrin caused peptide cleavage during the treatment. In this light, ovotransferrin predominantly may form soluble aggregates at alkali pH values. These results suggest that cleavage of a small number of peptide bonds and hydrolysis of DEW proteins were not observed by a mild alkaline dry heating. However, ovotransferrin might be more sensitive to denaturation than other proteins and form polymers by hydrophobic interaction and sulfhydryl−disulfide interchange in the dry state at pH 9.42.

Deamidation offers an effective way to enhance the functional properties of food proteins, thus making them more useful to the industry. For example, with even low levels of deamidation (2−6%), the functional properties of proteins can be significantly improved (Wu *et al.*, 1976; Matsudomi *et al.*, 1985; Hamada and Marshall, 1989). The deamidation causes better solubility, water-binding capacity, foam expansion, emulsion capacity, and viscosity compared to those of the unmodified product (Shih and Kalmar, 1987; Shih, 1987). Deamidation of food proteins may be carried out either by chemical deamidation, known as acid solubilization, or by enzymatic deamidation. It is well-known that mild acid treatment of proteins results in the deamidation of glutamine and asparagine residues with a concomitant cleavage of specific peptide bonds (Han *et al.*, 1983). A mild acid hydrolysis may bring about increased solubility of proteins mainly due to the higher electrostatic repulsion as a result of the deamidation of glutamine and asparagine (Matsudomi *et al.*, 1985; Shih and Kalmar, 1987). It is also reported that the deamidation of egg white proteins occurred with protease treatment under controlled conditions (alkaline pH) without proteolysis (Kato *et al.*, 1987). The deamidation by protease is effective for improving the functional properties of egg white proteins. Matsudomi *et al.* (1986) also found an increase in the solubility and emulsifying ability of gluten after treatment with chymotrypsin. Motoki *et al.* (1986) used the enzyme transglutaminase to specifically deamidate of α_{s1}-casein. No significant conformational changes were observed, and the solubility of the protein was found to increase at acidic pH conditions. In the light of these facts, the DEW protein can be deamidated by dry heating in a low water activity at mild alkaline pH values without proteolysis. A mild alkaline dry heating may bring increased solubility of DEW proteins, mainly due to the higher electrostatic repulsion as a result of deamidation of glutamine and asparagine in the proteins. The

sulfhydryl−disulfide exchange reaction and hydrophobic interaction of DEW proteins were also enhanced by this treatment. These reactions can be important factors for improving the functional properties of food proteins.

CONCLUSION

The combination of dry heating and deamidation under controlled pH values offers a new approach to improve the functional properties of food proteins and to study the structure−function relationship. Although proteins were denatured and formed soluble aggregates during dry heating, the solubility of proteins was not affected by this treatment. This may be due to the deamidation of proteins at alkaline pH values (under pH 9.50), and it can be an important factor for improving gelling properties of egg white proteins in mild alkaline dry heating.

Further studies on the deamidation condition with respect to the effect of water activity, dry-heating temperature, and the presence of oxygen to identify the optimal heating condition for the mild alkaline deamidation of the DEW proteins would be required. Evaluation of the formation of lysinoalanine would be also desired to optimize this alkaline heat treatment to food protein processing.

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Received for review February 27, 1997. Accepted June 13, 1997.[⊗] I gratefully acknowledge the Ontario Egg Producer's Marketing Board (Ontario, Canada) for their financial support of this work.

JF970158B

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1997.