Functional Improvements in Dried Egg White through the Maillard Reaction

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The effects of the Maillard reaction on the functional properties of dried egg white (DEW) were investigated. Maillard-reacted DEW (M-DEW) was prepared by storing sugar-preserved DEW (SP-DEW) at 55 °C and 35% relative humidity for 0−12 days. The M-DEW developed an excellent gelling property, and hydrogen sulfide production from heat-induced M-DEW gels decreased. Surface sulfhydryl (SH) group content of M-DEW increased while total SH group and α-helix contents decreased with increasing heating time in the dry state. Breaking strength, breaking strain, water-holding capacity, and hydrogen sulfide of heat-induced M-DEW gels significantly correlated with surface and total SH group contents in M-DEW. SDS−PAGE revealed that M-DEW proteins were polymerized in which covalent bonds were involved. The present study demonstrated that the Maillard reaction partially unfolds and polymerizes proteins of SP-DEW and, consequently, improved gelling property of SP-DEW under certain controlled conditions.

Keywords: *Dried egg white; heat-induced gels; hydrogen sulfide; Maillard reaction*

INTRODUCTION

Egg white (EW) is an important ingredient in food processing because of its variety of functional properties such as gel formation, water-holding capacity (WHC), foaming capacity, flavor, and emulsifying ability (Yang and Baldwin, 1995). Among these properties, gel formation and WHC are utilized mainly in surimi products (Chang-Lee et al., 1989; Park, 1994; Reppond et al., 1995) and other meat products (Dawson et al., 1990) to improve textural properties. However, the undesirable flavor of hydrogen sulfide generated by heating EW restricts EW addition to surimi and meat products.

The Maillard reaction (nonenzymatic browning) occurs during processing or storage of protein foods containing reducing carbohydrates or carbonyl compounds (Cheftel et al., 1985). It has been reported that protein glycosylation with reducing sugar effectively improved functional properties of food proteins. Notable improvements in emulsifying properties of ovalbumin (Nakamura et al., 1992) and dried EW (DEW) (Kato et al., 1993) were observed by the attachment of polysaccharides through the Maillard reaction. Saeki (1997) also reported that carp myofibrillar protein conjugated with glucose through the Maillard reaction had improved solubility and emulsifying properties.

EW contains glucose, but commercial DEW is desugared to prevent browning and loss of solubility by the Maillard reaction during pasteurization or storage (Sebring, 1995). However, the effects of the controlled Maillard reaction between EW protein and glucose on the functional properties, especially gelling properties, of EW are still unknown.

Our objective was to investigate possible applications of the Maillard reaction to improve functional properties of sugar-preserved DEW (SP-DEW). The relationship between gelling properties and protein conformation or microstructure of heat-induced gels was also examined.

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MATERIALS AND METHODS

Sample Preparation. SP-DEW was prepared by spray-drying 10 kg of commercially separated EW with 40 g of citric acid. Prior to desugarization, the EW contained 0.34% (w/v) glucose. For comparison, desugared DEW (D-DEW) was prepared in the same manner as SP-DEW after glucose was removed by adding 10 g of baking yeast and fermenting at 40 °C for 7 h. The solids content was determined by drying samples to a constant weight at 110 °C (AOAC, 1990). To react protein with glucose, SP-DEW and D-DEW (6.5% water content) were incubated at 55 °C and 35% relative humidity (RH) for 0, 2, 4, 6, 8, 10, and 12 days. An environmental chamber (PL-2FP, Tabai Espec Corp., Tokyo, Japan) was used to control temperature and RH. Immediately following incubation, the Maillard-reacted DEW (M-DEW) was kept at 4 °C until used. For pH measurement, glucose content measurement, and preparation of heat-induced gels, a sample solution with 10% solids content was prepared by mixing DEW with distilled water at 3600 rpm for 3 min using a table blender (National MX-X51, Matsushita Electric Industrial Co., Ltd., Osaka, Japan). The glucose contents of EW and sample solutions were measured with a glucose assay kit (Glucose B-testwako, Wako Pure Chemical Industry, Ltd., Osaka, Japan) using glucose oxidase.

Properties of DEW. Hunter *L*, *a*, *b*, and Δ*E* values of DEW were measured using a colorimeter (ND-1001DP, Nippon Denshoku Industries Co., Ltd., Tokyo, Japan). DEW was placed uniformly into a cylindrical cell (diameter = 30 mm, height = 12 mm) at a thickness of 10 mm and measured. Available lysine content of DEW was determined according to the method of Hernandez and Alvarez-Coque (1992) using *o*-phthalaldehyde and *N*-acetyl-L-cysteine. The concentration of sulfhydryl (SH) groups in DEW was determined using Ellman's reagent (Ellman, 1959). The SH groups were measured in the absence (surface SH groups) or in the presence (total SH groups) of 0.25% SDS. Protein concentration was determined according to the method of Lowry et al. (1951). Ovalbumin (grade V, minimum 98%, Sigma Chemical Co., St. Louis, MO) was used as the standard.

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Native polyacrylamide gel electrophoresis (PAGE) and SDS−PAGE both in the absence and in the presence of 2-mercaptoethanol were carried out by following the method of Laemmli (1970) using an electrophoresis unit (STC-808, TEFCO, Tokyo, Japan). For native PAGE, an 8% precast separating gel was used. For SDS−PAGE, a 4−20% precast gradient gel was used. Protein components were stained with Coomassie Brilliant Blue R-250 and destained in methanol/acetic acid/water (20:10:70 v/v/v).

To investigate the secondary structure of proteins, DEW was dissolved in 50 mM phosphate buffer, pH 7.0, and filtered with a Millipore filter (pore size = 0.45 μm). Protein concentration was adjusted to 0.05% (w/v). Circular dichroism (CD) spectra were measured on a spectropolarimeter (J-720, JASCO, Tokyo, Japan) using a 1 mm cell at 25 °C in the far-ultraviolet region (200−250 nm). The data were expressed as mean residue ellipticity (deg·cm^2·dmol^{-1}).

Preparation of Heat-Induced Gels. Cylindrical gels (diameter = 30 mm, height = 30 mm) were prepared by heating sample solutions at 80 °C for 40 min in a vinyl chloride plastic casing using a water bath (LT-480, ADVANTEC, Tokyo, Japan). The pH of sample solutions was adjusted to 9.0 with 2 N NaOH because the pH of commercial EW is ∼9. After heating, the gels were immediately cooled in ice water and equilibrated to ambient temperature (24 ± 1 °C).

Functional Properties of Gels. Breaking strength and breaking strain of heat-induced gels were measured at ambient temperature (24 ± 1 °C) using a rheometer (NRM-2010J-CW, Fudoh, Tokyo, Japan) equipped with a spherical plunger (diameter = 8 mm) at a crosshead speed of 60 mm/min. WHC of gels was calculated from $W_1 \times 100/W_0$, where W_0 was the initial gel weight and W_1 was the gel weight after being laid on five layers of filter paper (No. 2, diameter = 110 mm, ADVANTEC) at ambient temperature (24 ± 1 °C) for 60 min.

Hydrogen sulfide from gels was measured using lead acetate paper. A gel was placed in a jar with lead acetate paper covering the lid, and the color that developed after 30 min at ambient temperature (24 ± 1 °C) was measured using a colorimeter (ND-1001DP, Nippon Denshoku Industries, Co., Ltd., Tokyo, Japan). The amount of reacted hydrogen sulfide with lead acetate was expressed as $H_0 - H_1$, where H_0 was the Hunter L value of noncolored paper and H_1 was the Hunter L value of colored paper.

Microstructure of Gels. The microstructure of gels was determined by scanning electron microscopy (SEM). Gel samples (1 mm^3) were fixed in 2.5% glutaraldehyde (0.1 M cacodylate buffer, pH 7.3), postfixed in 1% osmium tetraoxide (0.1 M cacodylate buffer, pH 7.3), and dehydrated. The samples were freeze-dried (−20 °C) with a freeze-dryer (ID-2, Eiko Co., Ibaraki, Japan), mounted, sputter-coated with gold−palladium, and examined in a Hitachi FE-SEM S-900 (Hitachi, Ltd., Tokyo, Japan) at 5 kV.

Statistical Analysis. All experiments were replicated twice. Duncan's multiple-range test (Maxwell and Delaney, 1990) was used to detect significance of differences ($p < 0.05$) among means.

RESULTS AND DISCUSSION

Preparation of M-DEW. The moisture content of SP-DEW and D-DEW remained relatively constant (6.5−7.2%) during the 12 days of heating treatment. Browning of ND-DEW increased with increasing heating time as evidenced by a gradual decrease in L value and a gradual increase in a, b, and ΔE values (Figures 1 and 2). Available lysine (Figure 3) and glucose (Figure 4) contents of SP-DEW decreased dramatically for the first 4 and 6 days, respectively, of heating treatment and remained constant thereafter. These results indicate that heating induced the Maillard reaction in SP-DEW and that EW protein−glucose complexes were formed early during the heating treatment. In agreement, ovalbumin−glucose complexes were formed in the early stage of the Maillard reaction, whereas browning and protein polymerization occurred in the advanced stage of the Maillard reaction (Kato et al., 1981, 1988).

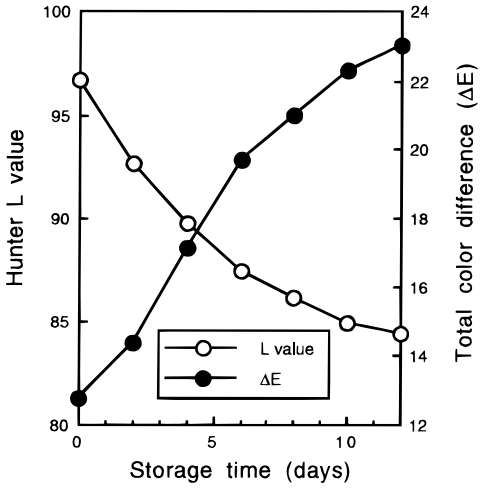

Figure 1. Changes in Hunter L value and total color difference (ΔE) of SP-DEW with heat treatment.

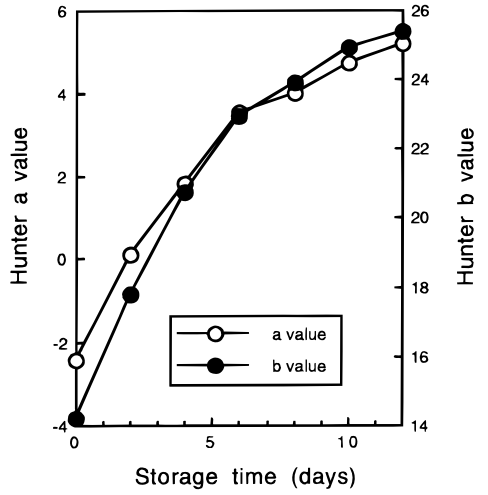

Figure 2. Changes in Hunter a and b values of SP-DEW with heat treatment.

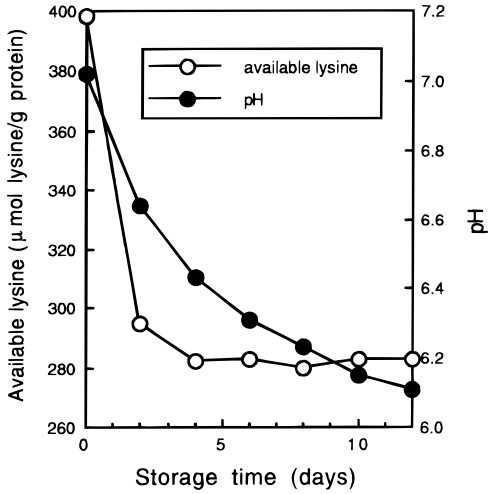

Figure 3. Changes in pH and available lysine content of SP-DEW with heat treatment.

SP-DEW at pH 7.02 (Figure 3) was prepared in the present study to slow the Maillard reaction, which progresses more rapidly at alkaline pH (Kato et al., 1974). The pH of M-DEW solutions decreased as heating

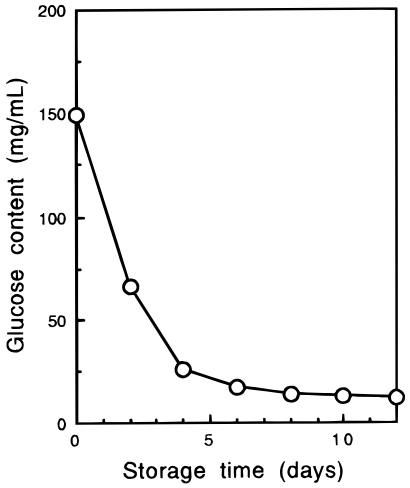

Figure 4. Changes in glucose content of SP-DEW with heat treatment.

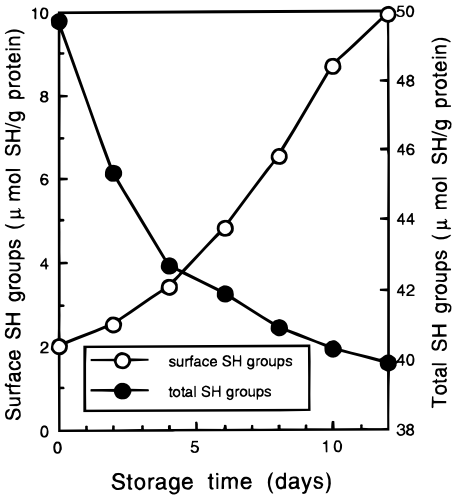

Figure 5. Changes in concentration of surface and total sulfhydryl (SH) groups of SP-DEW with heat treatment.

time increased, probably due to the decrease of available lysine (Figure 3).

L, *a*, *b*, and ΔE values, available lysine, glucose, and pH of D-DEW remained unchanged ($p > 0.05$) during the 12 days of heating treatment (data not shown). Similarly, color, available lysine, solubility, amino acid composition, and secondary structure of freeze-dried ovalbumin did not change after storage at 50 °C and 65% RH for 18 days (Watanabe et al., 1980). These data indicate that the Maillard reaction did not occur in these conditions.

Properties of DEW Proteins. Surface SH groups increased ($p < 0.05$) and total SH groups decreased ($p < 0.05$) in SP-DEW as heating time increased (Figure 5). This result suggests that the SP-DEW proteins were unfolded through the Maillard reaction, which was also supported by the fact that α-helix content in SP-DEW decreased with increasing heating time (Figure 6). Surface and total SH groups and α-helix content of D-DEW remained unchanged ($p > 0.05$) throughout the 12 days of heating (data not shown).

In native PAGE profiles of heat-treated SP-DEW, the ovalbumin and ovotransferrin bands moved to the anode side with increasing heating time (Figure 7). This result suggests that glucose attached to amino groups of SP-DEW proteins decreased positive charges on the protein

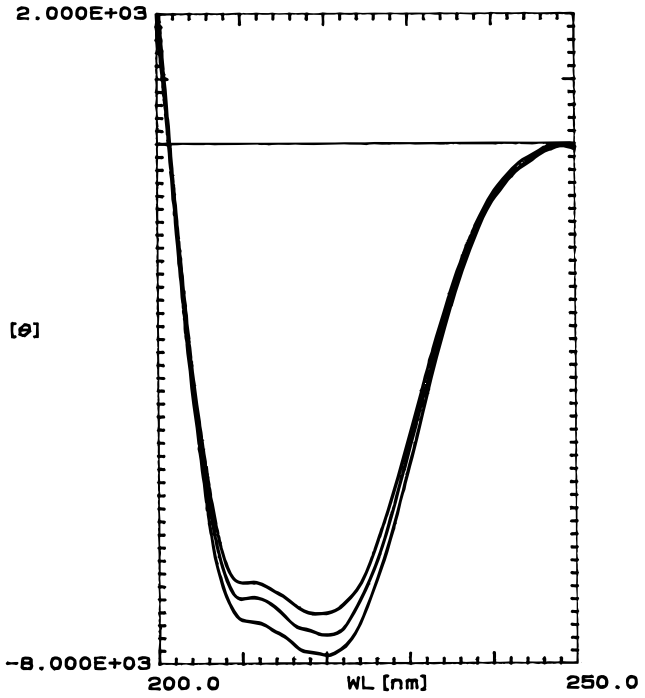

Figure 6. Circular dichroism spectrum of SP-DEW that was nonheated (bottom curve), heated for 6 days (middle curve), or heated for 12 days (top curve).

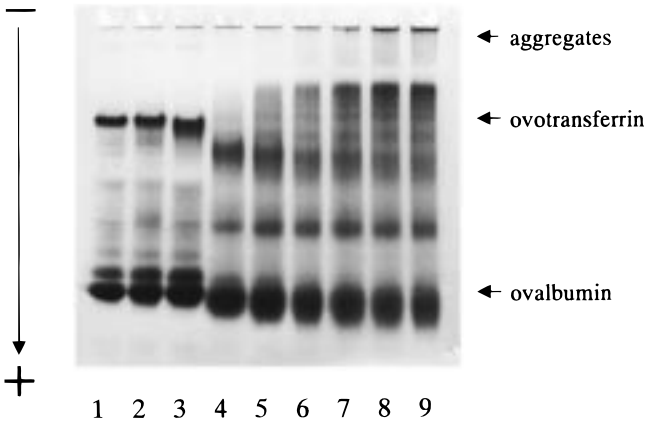

Figure 7. Native PAGE patterns of nonheated D-DEW (lane 1), D-DEW heated for 12 days (lane 2), nonheated SP-DEW (lane 3), and SP-DEW heated for 2, 4, 6, 8, 10, and 12 days (lanes 2−7, respectively).

surface. SP-DEW proteins polymerized with heating treatment, and the extent of polymerization increased with increasing heating time (Figure 7). In SDS−PAGE profiles of SP-DEW (both with and without 2-mercaptoethanol), mobility of ovalbumin, ovotransferrin, and lysozyme bands decreased after 2 days of heating and thereafter decreased gradually (Figure 8). This may be due to molecular weight increases by glucose attachment to these proteins. Polymerization (aggregate formation) occurred even in the presence of 2-mercaptoethanol, which cleaves disulfide bonds (Figure 8). Therefore, it is suggested that covalent bonds other than disulfide bonds were involved in the polymerization.

Kato et al. (1987) reported that 3-deoxyglucosone, an intermediate product of the Maillard reaction, was the cross-linker responsible for the glucose-induced polymerization of proteins such as ovalbumin and lysozyme. Ovalbumin stored with glucose at 50 °C and 65% RH for >6 days has been reported to aggregate through Maillard reaction-induced intermolecular cross-linking

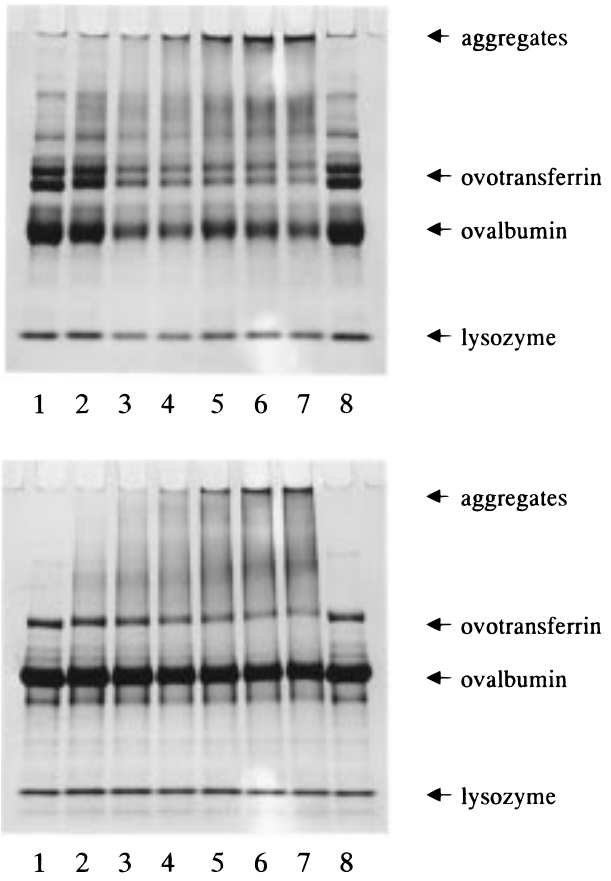

Figure 8. SDS−PAGE patterns in the absence (top) and presence (bottom) of 2-mercaptoethanol of nonheated SP-DEW (lanes 1 and 8) and SP-DEW heated for 2, 4, 6, 8, 10, and 12 days (lanes 2−7, respectively).

without involvement of disulfide bonds (Kato et al., 1981). However, in the present study, a comparison between SDS−PAGE profiles of SP-DEW heat-treated for >6 days with and without 2-mercaptoethanol (Figure 8) revealed that disulfide bonds were possibly involved in the polymerization of EW proteins. Further research is needed to elucidate the mechanism of protein polymerization through the Maillard reaction.

The PAGE pattern of D-DEW heat-treated for 12 days was the same as that of non-heat-treated D-DEW with (data not shown) and without (Figure 7) SDS. These data again demonstrate that the Maillard reaction did not occur while D-DEW was heated.

Functional Properties of Gels. Breaking strength and breaking strain (Figure 9) and WHC (Figure 10) of heat-induced M-DEW gels increased with heating time. Interestingly, heat-induced M-DEW gels became increasingly transparent as heating time increased (data not shown). Reportedly, opaque ovalbumin gels are soft and less elastic, whereas transparent ovalbumin gels are firm and elastic and had high WHC (Kitabatake et al., 1989). Protein gel networks are generally formed via noncovalent cross-linkages such as hydrophobic interactions and less frequently by covalent interactions such as disulfide bonds (Clark, 1992). However, the excellent gelling properties of heat-induced M-DEW gels in the present work may be attributed to covalent cross-linking between EW protein molecules.

There were strong correlations between the physical properties of heat-induced M-DEW gels and surface and total SH groups (Table 1). The data indicate that surface SH groups contributed to the formation of strong and

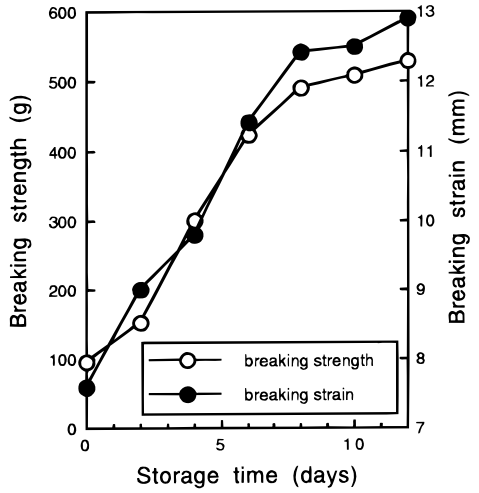

Figure 9. Changes in breaking strength and breaking strain of heat-induced gels from heated SP-DEW.

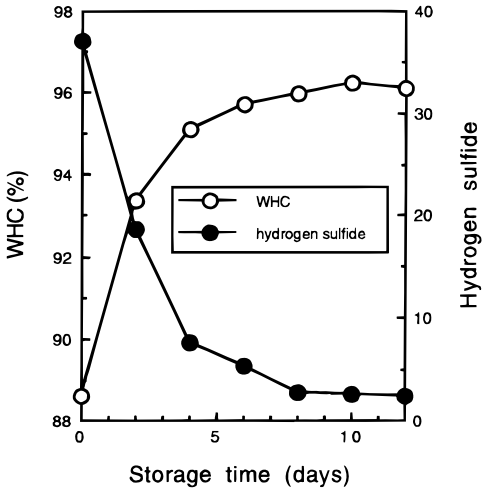

Figure 10. Changes in hydrogen sulfide production and WHC of heat-induced gels from heated SP-DEW.

Table 1. Correlation Coefficients between SH Groups of M-DEW and Functional Properties of M-DEW Heat-Induced Gels

	breaking strength	breaking strain	WHC	hydrogen sulfide
surface SH groups	0.910	0.924	0.707	−0.760
total SH groups	−0.947	−0.953	−0.978	0.989

elastic gels of high WHC. More likely, surface SH groups enhanced gel network formation by forming intermolecular disulfide bonds. As seen in the increase in surface SH groups (Figure 5) and the slight decrease in α-helix content (Figure 6), mild conformational changes were caused in SP-DEW protein molecules through the Maillard reaction, and the resulting structures may have contributed to the excellent gelling properties of M-DEW. Mine (1996) reported that DEW proteins were polymerized after 3 days of dry heating at 75 °C and that gel strength and elasticity of DEW proteins were greatly increased by the heat treatment. Because total SH groups correlated ($p < 0.05$) with gel physical properties (Table 1), polymerized forms of proteins in the dry state may be responsible for the excellent gelling properties of the M-DEW (if total SH group concentration represents the degree of intermolecular covalent bond formation). A strong correlation ($r = 0.97$) was reported between SH group concentration and gel

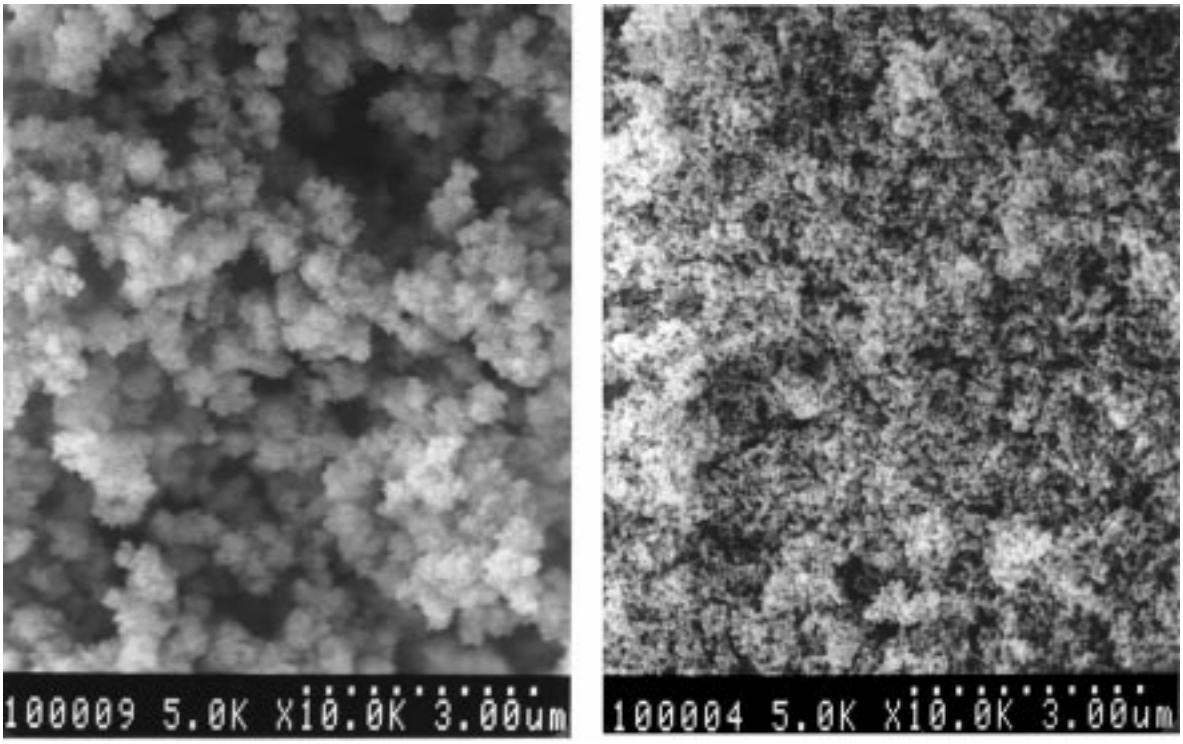

Figure 11. Scanning electron micrographs of heat-induced gels from nonheated SP-DEW (left) and SP-DEW heated for 12 days (right).

strength of heat-induced EW gels (Margoshes, 1990). Furthermore, a negative correlation ($r = -0.86$) between SH group concentration and gel strength of EW protein denatured with 0.1% SDS was reported (Margoshes, 1990).

Functional properties of D-DEW were reportedly maximized by heating in the dry state (80 °C, 7.5% moisture content) for 10 days (Kato et al., 1989). In addition, controlled heating at alkaline pH (<9.5) in the dry state (75 °C, 8.5% moisture content) for 5 days improved gelling properties of D-DEW (Mine, 1996). However, in the present study, the measured physical properties of gels from heat-treated D-DEW did not differ ($p > 0.05$) from those of gels from nonheated D-DEW and SP-DEW (data not shown). This was attributed to the lower heating temperature (55 °C) used in the present study.

Hydrogen sulfide from heat-induced gels decreased with increasing heating time (Figure 10) and significantly correlated ($p < 0.05$) with surface ($r = -0.760$) and total ($r = 0.989$) SH groups concentration (Table 1). Hydrogen sulfide is released from heated EW due to cysteine degradation (Chen and Chen, 1984). Release of hydrogen sulfide can be prevented by blocking SH groups on cysteine with carbonyl groups (Jocelyn, 1972). Apparently, the Maillard reaction during heating of SP-DEW reduced the total SH groups over time (Figure 5), thus also reducing the amount of released hydrogen sulfide (Figure 10). Germs (1973) reported that hydrogen sulfide production from EW increases at higher temperatures and more alkaline pH. Therefore, the gel pH was adjusted to 9, which is closer to the pH of commercial EW, to better detect differences of hydrogen sulfide production among samples.

Microstructure of Gels. A finer and more uniform three-dimensional network was observed in heat-induced gels of SP-DEW heat-treated for 12 days (Figure 11). This structure might contribute to improved physical properties and transparency of the gel. Transparent gels have been reported to be strong and elastic with fine and filamentous networks (Tani et al., 1995; Handa et al., 1998). Increasing repulsive forces between protein molecules through incorporation of hydrophilic groups along with Maillard reaction-induced cross-linking may improve SP-DEW gel structure, thereby improving the gelling properties.

Conclusion. The present study demonstrated that the Maillard reaction improved the physical properties and flavor of heat-induced SP-DEW gels under certain controlled conditions. In addition, strong correlations were observed between surface and total SH groups of M-DEW proteins and functional properties of heat-induced M-DEW gels. Other functional properties of M-DEW, such as flavor, foaming properties, and emulsifying properties, as well as the acceptability of M-DEW as an ingredient in processed food products merit investigation.

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