

Inhibiting Effects of Egg White Dry-Heated at 120 °C on Heat Aggregation and Coagulation of Egg White and Characteristics of Dry-Heated Egg White

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Dialyzed and freeze-dried egg white (FDEW) was dry-heated at 120 °C for up to 6 h. The inhibiting effects of the dry-heated egg white (DHEW) on the heat aggregation and coagulation of egg white (as 10% FDEW solution) and characteristics of the DHEW were examined. From the changes in turbidities and soluble protein contents of supernatant in various mixtures of 10% FDEW and DHEW solutions induced by heating (60 °C, 5 min), it was found that the inhibiting capacity increased with increases in the dry-heating time (DHT). The FDEW proteins were denatured with a mild conformational change (not secondary but tertiary structure) with the increase in DHT and aggregated partially. However, the more transparent solutions of DHEW containing soluble aggregates according to DHT were also obtained after heating. The transparency according to DHT came to be scarcely affected by the NaCl concentration and the dilution with diluents containing SDS, urea, and 2-mercaptoethanol. These findings suggest that the heat aggregations and coagulations of ovotransferrin and lysozyme in the FDEW were inhibited by their bindings with the soluble aggregates in DHEW.

Keywords: Egg white; dry-heating; heat coagulation; heat aggregation; ovalbumin; ovotransferrin

INTRODUCTION

In the processing of egg white powder, glucose is generally removed by microbial fermentation or enzyme action before spray-drying to prevent the Maillard reaction, and the spray-dried egg white is heat-stored at 55–65 °C for 3–5 days to reduce the microbial load (Bergquist, 1995). The effect of this high-temperature storage of spray-dried powder on physical changes and the thermal resistance of *Salmonella* organisms are well characterized (Parkinson, 1977). Egg white powder can be stored at elevated temperatures of 71 °C for 8 h, 82 °C for 2 h, and 90 °C for 1 h to eliminate *Salmonella* without significant impairment of whipping and angel food cake-making properties (Smith, 1964). Controlled heating in dry state of egg white (for example, 80 °C for 5–10 days) was found to be effective to improve significantly functionalities such as gelling, emulsifying, and foaming properties and water holding capacity without any loss in solubility (Kato et al., 1989, 1990a,b; Mine, 1996, 1997). We also showed that when the solutions of spray-dried (60–70 °C) and then dry-stored (55–65 °C, 3 days) egg whites were reheated at 60 °C for several minutes, coagulation hardly occurred (Xu et al., 1997). This phenomenon was also found to be mainly attributable to changes of the egg white proteins occurring in the process of heating in the dry state. It was concluded that spray-dried egg whites dry-heated at 120 °C formed transparent solutions without any coagulation on heating at the industrial pasteurization temperature of 60 °C for 3.5 min and had an inhibiting effect on heat coagulation of fresh egg white (Xu et al., 1998).

Thus, thermal uncoagulable egg white can be manufactured easily by mixing egg white with spray-dried

egg white dry-heated at 120 °C. The egg white dry-heated at a high temperature of 120 °C would be effective as a new food material. In this paper, we first compared the inhibiting capacity of dry-heated egg whites (DHEWs) treated at 120 °C for various times up to 6 h on the aggregation and coagulation of egg white, which occurred by heating the various mixtures of 10% freeze-dried egg white (FDEW) solution and 10% DHEW solution, and then determined the changes of FDEW proteins during dry-heating at 120 °C and DHEW proteins in the heating at 60 °C. A possible mechanism by which DHEW inhibited the heat aggregation and coagulation of egg white proteins is discussed.

MATERIALS AND METHODS

Preparation of Samples. As described in the previous paper (Xu et al., 1998), the dialyzed, centrifuged, and freeze-dried egg white [FDEW; pH 7.4, moisture content 4.5%] was placed in a sealed vial and dry-heated in the oven at 120 °C for various durations of up to 6 h, and these dry-heated egg whites (DHEWs) and FDEW were used as samples.

Determinations of Change in Moisture Content in FDEW during Dry-Heating. The weight in FDEW during dry-heating was determined by a TG/DTA 300 thermal analyzer (Seiko Instruments Inc., Tokyo, Japan) as follows. FDEW (25 mg) was directly weighed on the microbalance in the thermal analyzer and kept at 30 °C for 15 min. Temperatures of the thermal analyzer were then increased to 120 °C at 1.5 °C/min, kept at 120 °C for 1 h, increased again from 120 to 150 °C at 1.5 °C/min, and finally kept at that temperature for 15 min. The moisture content in FDEW kept at 150 °C for 15 min was regarded as zero, and the moisture content (M , %) of FDEW was calculated as follows: $M = 100 \times (W - W_i) / W$, where W and W_i were the weights of FDEW during heating and after heating at 150 °C for 15 min, respectively. The weight and temperature were computed automatically.

Inhibiting Effect of DHEW on Heat Coagulation of FDEW. Each DHEW and FDEW solution was prepared by

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solubilizing it with distilled water, centrifuging at 2000*g* for 20 min to remove insoluble proteins, and adjusting their concentrations to 10% (w/w). Every DHEW was solubilized without any coagula, and FDEW was solubilized at the level of ~95%. Then 4.5 mL each of the mixtures of 10% DHEW and FDEW solutions at the ratios of 0–4 of FDEW to 1 of DHEW in stoppered test tubes (6 mm i.d. × 45 mm) was heated (60 °C, 5 min) in a water bath under set conditions, cooled immediately in tap water, and centrifuged at 2000*g* for 20 min. The soluble protein content in the supernatant was determined by the measurement of dry weight in a vacuum, and the turbidity of the supernatant diluted (20 times) with 20 mM phosphate buffer (pH 7.4) was measured by reading absorbance at 540 nm. The turbidity and soluble protein content were regarded as indices of aggregation (soluble aggregate) and coagulation (insoluble aggregate) of FDEW proteins.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE). SDS–PAGE in the presence and absence of 2-mercaptoethanol (2-ME) was carried out according to the method of Laemmli (1970) using 12.5% polyacrylamide gel, as described in the previous paper (Xu et al., 1998). The gel sheets were stained with a solution of 0.2% Coomassie Brilliant Blue R-250 in water/methanol/acetic acid (5:5:1, v/v/v) and destained by 7% acetic acid overnight.

Circular Dichroism (CD). CD spectra were determined as follows: 10% (w/w) FDEW and DHEW solutions were diluted 100-fold with 20 mM sodium phosphate buffer (pH 7.4) containing 0.1 mM EDTA and filtered through a membrane filter (0.45 μm). The protein solution thus obtained was used for CD analysis. CD was measured on a Jasco J-600 spectropolarimeter (Tokyo, Japan) in the far-ultraviolet region (190–250 nm) with a 1-mm path length cell at 25 °C. The accuracy of the data was improved by averaging eight scans integrated with the data processor (Model DP-501), and CD spectra were expressed in terms of mean residue ellipticity [θ], which was calculated on the basis of average residue weight of 111.

Measurement of Surface Hydrophobicity. Ten percent (w/w) DHEW solutions before and after heating were diluted 1000-fold with 20 mM phosphate buffer (pH 7.4) containing 0.1 mM EDTA. To 1 mL of diluted solution was added 30 μL of 0.5 mg/mL 1-anilinonaphthalene-8-sulfonate (ANS; Aldrich Chemical Co., St. Louis, MO) solutions in the same phosphate buffer as the fluorescence probe, and the mixture was incubated at 25 °C for 1 h. The mixture was excited at 370 nm, and the fluorescence intensity (FI) was measured at 470 nm in a Hitachi F-3000 spectrophotofluorometer (Tokyo, Japan) at room temperature (25 °C). Emission spectra were corrected for background fluorescence caused by ANS in reactions lacking protein. Mean values of three determinations are shown.

Measurement of Sulfhydryl Content. Measurement of sulfhydryl group was performed by using Ellman's reagent (Beveridge et al., 1974). Ten percent (w/w) FDEW and DHEW solutions were diluted 20-fold with 20 mM phosphate buffer (pH 7.4) containing 0.1 mM EDTA for surface sulfhydryl content and 80-fold with 0.5 M phosphate buffer (pH 7.4) containing 1 mM EDTA, 8 M urea, and 0.5% SDS for total sulfhydryl content. To each 1 mL of these solutions was added 10 μL of 5 mM 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) solution in 20 mM phosphate buffer (pH 7.4) containing 0.1 mM EDTA. The color absorbance was read at 412 nm on a Shimadzu UV-2000 spectrophotometer (Tokyo, Japan). The sulfhydryl contents were calculated using a molar extinction coefficient of 13600 M⁻¹ cm⁻¹ (Monahan et al., 1995). The protein content of the solubilized protein fraction was determined according to the Lowry procedure (Lowry et al., 1951). Mean values of three determinations are shown.

Effect of NaCl on Heat Aggregation and/or Coagulation of DHEW. Ten percent (w/w) FDEW and DHEW solutions (dry-heating conditions: 120 °C, up to 2 h) containing 0–0.65 M NaCl were heated (60 °C, 5 min), and the turbidities were determined as described above.

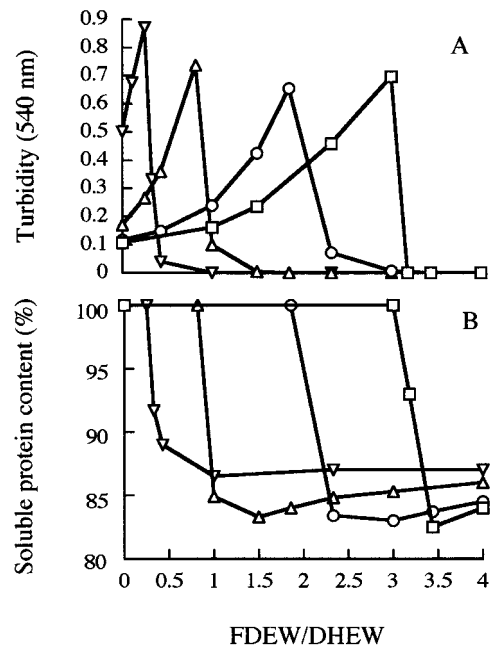


Figure 1. Turbidities (A, 20 times diluted, 540 nm) and soluble protein contents (B, determined by dry weight) in supernatant obtained by centrifugation (2000*g*) from various mixtures of 10% FDEW and DHEW solutions [FDEW/DHEW = 0:1 to 4:1; dry-heating conditions: 120 °C for 1 h (▽), 2 h (△), 4 h (○), and 6 h (□) after heating (60 °C, 5 min)].

Effect of Diluents on Turbidity of DHEW Solution Heated. Ten percent (w/w) DHEW solutions (dry-heating conditions: 120 °C, 1 and 6 h) were heated at 60 °C for 5–15 min and diluted (40 times) with 20 mM sodium phosphate buffer (pH 7.4) (diluent A), diluent A plus 0.6 M NaCl plus 0.5% SDS plus 1.5 M urea (diluent B), diluent A plus 0.01 M 2-ME (diluent C), and diluent B plus 0.01 M 2-ME (diluent D). Their turbidities were measured by reading the absorbance at 540 nm.

RESULTS

The changes in moisture content of FDEW during dry-heating under the conditions described in Materials and Methods were examined. The moisture content in FDEW was 4.5% before dry-heating and decreased abruptly to 0.3% with the increase in dry-heating temperature up to 120 °C. The dry-heating of FDEW proteins in this study was carried out mainly with a low moisture content of 0.3%.

In the heating (60 °C, 5 min) of a single system of 10% FDEW solution (pH 7.4), the coagula occurred at the level of 12.5% to the amounts of protein solubilized before heating, and the residual supernatant was clear (data not shown).

Figure 1 shows the turbidities (dilution = 20 times) and soluble protein contents (determined by dry weight) in supernatant obtained by centrifugation (2000*g*, 20 min) from various mixtures of 10% (w/w) FDEW and DHEW solutions [FDEW/DHEW = 0:1 to 4:1; dry-heating conditions: 120 °C, 1–6 h] heated at 60 °C for 5 min. In the single system (FDEW/DHEW = 0) of 10% DHEW solution (pH 7.4), the turbidities of DHEW solutions decreased from 0.5 (120 °C, 1 h) to 0.1 (120 °C, 6 h) by heating at 60 °C for 5 min without the formation of coagulum in any solution. Thus, a more transparent DHEW solution without any coagula when heated was obtained with the increase in dry-heating times. In heating the mixtures of FDEW and DHEW,

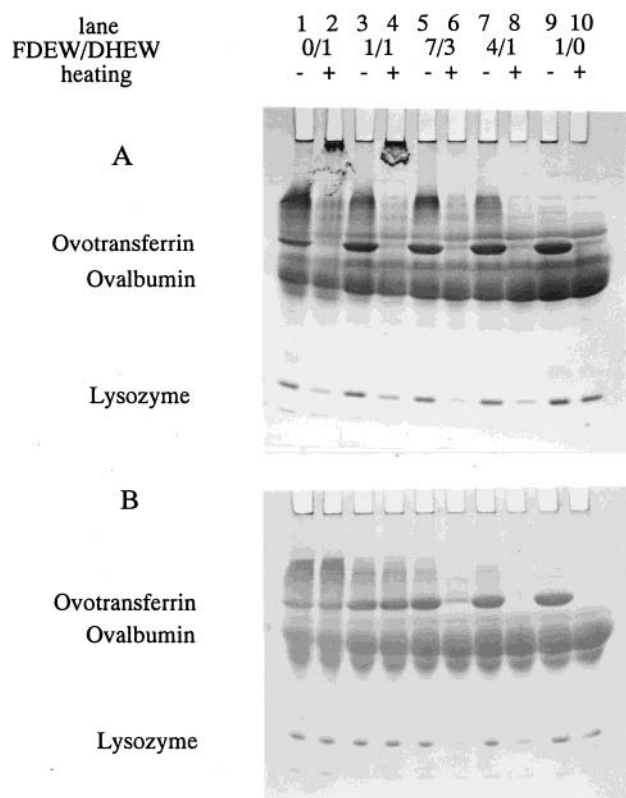


Figure 2. SDS-PAGE patterns, in the absence (A) and presence (B) of 2-ME, of supernatants from various mixtures of 10% FDEW and DHEW solutions (DHEW: 120 °C, 4 h; FDEW/DHEW = 0:1, 1:1, 7:3, 4:1, 1:0) before (-) and after (+) heating (60 °C, 5 min).

the DHEW showed the inhibiting effect on heat aggregation and coagulation of FDEW proteins. In every mixed solution of FDEW and DHEW, the turbidity first increased with the formation of soluble aggregates and then abruptly decreased on the formation of coagula. The ratio of FDEW to DHEW brought to the maximum turbidity became higher with the increase in dry-heating time.

The soluble protein content did not change until the ratios (FDEW/DHEW) reached 0.3 for DHEW treated for 1 h and 3.0 for that treated for 6 h. Thus, the DHEW treated for longer times greatly inhibited the heat aggregation and coagulation of FDEW proteins in quantities. In other words, the longer the dry-heating time, the greater the inhibiting capacity.

The SDS-PAGE patterns of supernatants from the various mixtures of 10% (w/w) FDEW and DHEW solutions (DHEW: 120 °C, 4 h; FDEW/DHEW = 0:1, 1:1, 7:3, 4:1, 1:0) before and after heating at 60 °C for 5 min are shown in Figure 2. The DHEW (lane 1) was separated into bands of ovalbumin (OVA), ovotransferrin (OT), and lysozyme (LZ) and some aggregates during electrophoreses in the absence (Figure 2A) and presence (Figure 2B) of 2-ME. As the density of OVA and OT bands decreased in comparison with the patterns of FDEW (lane 9), part of the OVA and OT appeared to form aggregates during the dry-heating process. The bands of OT and some aggregates in DHEW solution heated (lane 2) disappeared in the absence of 2-ME and were restored in the presence of 2-ME. On the other hand, heating did not greatly affect the density of the OVA band.

In the mixtures of FDEW and DHEW solutions, the aggregates existing in DHEW were also found in the

running gels in the presence and absence of 2-ME (lanes 3, 5, and 7), and those aggregates as well as OT disappeared through further polymerization on heating as seen in the patterns of Figure 2A, lanes 4 and 6. However, these components were restored in the presence of 2-ME as seen in the patterns of Figure 2B, lanes 4 and 6. On the other hand, the aggregates and OT were not restored in either the absence or presence of 2-ME (lanes 8 in Figure 2A,B), indicating the formation of coagula by heating. Thus, it can be concluded that OT bound with the soluble aggregates keeping their soluble form through a disulfide bond on heating at mixed ratios (FDEW/DHEW) lower than 7:3 and coagulated through the disulfide bond on heating at mixed ratios of more than 7:3. In other words, the DHEW treated at 120 °C for 4 h inhibited the coagulation of OT at mixed ratios lower than 7:3. This was compatible with the lowering of the soluble protein contents in Figure 1B.

Coagula from various mixtures of 10% (w/w) FDEW and DHEW solutions (DHEW: 120 °C, 4 h; FDEW/DHEW = 1:0, 4:1, 7:3) heated as described above were separated (centrifugation at 2000g, 20 min) to examine the components in the coagula and the binding forces between them. One part of the separated coagula was freeze-dried as it was, and the other was resuspended in 20 mM phosphate buffer (pH 7.4). The insoluble materials were then separated (centrifugation at 2000g, 20 min) and freeze-dried. The freeze-dried samples were then dissolved separately in electrophoretic solvent for SDS-PAGE in the presence of 2-ME. The SDS-PAGE profiles are shown in Figure 3. The coagula were found to consist of OT, OVA, and LZ in that order of quantity. The patterns of the three mixed ratios were not basically different. However, the higher the mixed ratio of FDEW/DHEW, the greater the density of OT. The insoluble materials in all of the coagula were mainly OT with a small proportion of LZ, and a large part of OVA in the coagula was solubilized with phosphate buffer. Thus, it was found that the binding forces between OVA and OT and between OVA and LZ in coagula did not mainly depend on a disulfide bond or specific noncovalent interactions. The OVA solubilized with phosphate buffer should be regarded as simply involved in the coagula.

It was considered that an analysis of the characteristics of proteins in DHEW was important for elucidating the inhibiting effect of DHEW on the heat aggregation and coagulation of FDEW proteins as described above. Therefore, we examined the changes in FDEW proteins that occurred in the processes of dry-heating and the DHEW proteins under heating in the solution state (10%, pH 7.4) at 60 °C for 5 min.

The effect of heating in the dry-state on the secondary structure of FDEW proteins was examined by the determination of CD spectra. Figure 4 shows the CD spectra of FDEW and DHEWs (dry-heating conditions: 120 °C, 1 and 6 h). As seen in the patterns, even dry-heating for 6 h to FDEW hardly affected the CD spectra. In other words, dry-heating did not greatly affect the secondary structure of egg white proteins.

As shown in Figure 5, the surface hydrophobicities of DHEW increased ~3-fold with increased dry-heating time up to 6 h in the dry-heating process and ~2-fold in the heating process. The increasing in the surface hydrophobicity indicated more or less the alterations in the tertiary structure of FDEW proteins, although hardly any changes in the secondary structure occurred in the dry-heating process (Figure 4).

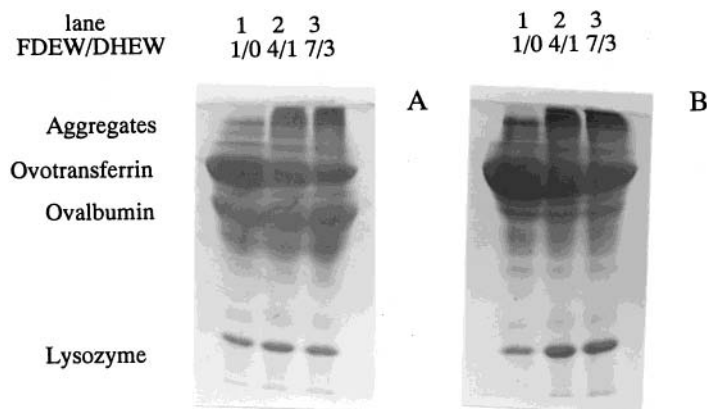


Figure 3. SDS-PAGE patterns of coagula (A) from various mixtures of 10% (w/w) FDEW and DHEW solutions (DHEW: 120 °C, 4 h; FDEW/DHEW = 1:0, 4:1, 7:3) after heating (60 °C, 5 min) and insoluble materials (B) in 20 mM phosphate buffer (pH 7.4) from the coagula.

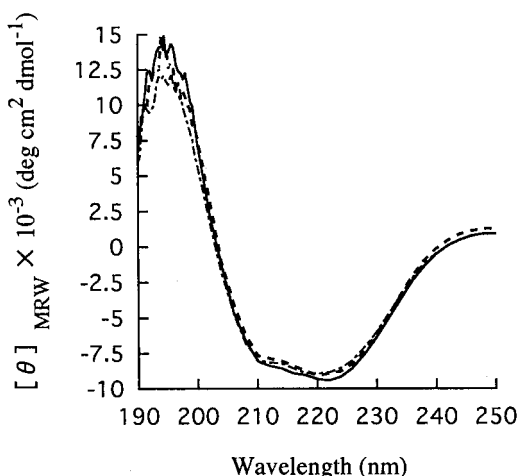


Figure 4. CD spectra of FDEW and DHEWs (dry-heated at 120 °C, 2 and 6 h) solutions: (—) FDEW; (---) DHEW (120 °C, 1 h); (—) DHEW (120 °C, 6 h).

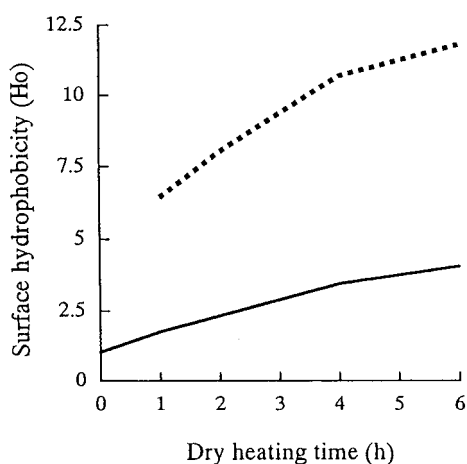


Figure 5. Surface hydrophobicity of DHEW (120 °C, up to 6 h) before and after heating (60 °C, 5 min): (—) before heating; (---) after heating. $H_o = (F - F_0)/C$; F , fluorescence reading of the protein-ANS conjugate solution; F_0 , fluorescence reading of the ANS solution; C , protein concentration (g/mL).

As shown in Figure 6, in the dry-heating process the surface sulfhydryl contents in the DHEW increased with the increase in dry-heating time, although their values increased at lower levels from 0.04×10^{-5} M/g of protein in FDEW (dry-heating time = 0 h) to 0.26×10^{-5} M/g of protein in DHEW (dry-heating time = 6 h), whereas the total sulfhydryl contents in the DHEW decreased

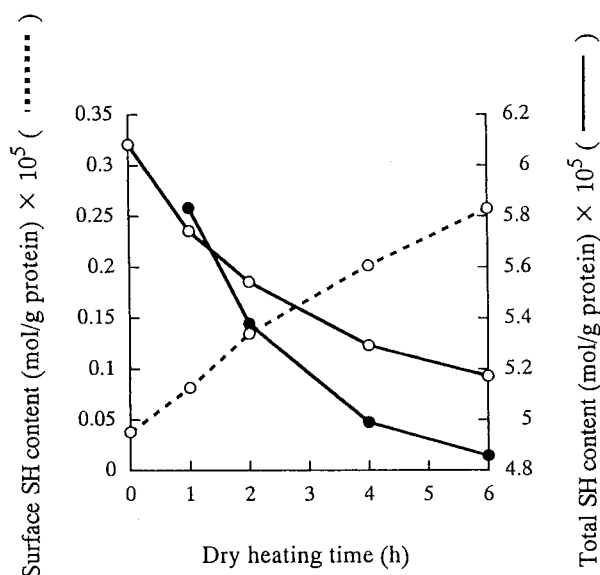


Figure 6. Surface sulfhydryl content in DHEW (120 °C, up to 6 h) and total sulfhydryl content in the DHEWs before (○) and after (●) heating (60 °C, 5 min).

with the increase in times from 6.08×10^{-5} to 5.15×10^{-5} M/g of protein before heating and from 5.84×10^{-5} (dry-heating time = 1 h) to 4.84×10^{-5} M/g of protein after heating. The surface sulfhydryl contents in the FDEW (dry-heating time = 0 h) could not be determined correctly due to the development of turbidity after heating. Those results suggested that sulfhydryl groups in DHEW proteins were partially and gradually exposed to the surface of the proteins, and sulfhydryl-disulfide exchange and sulfhydryl-sulfhydryl oxidation reactions were accelerated by dry-heating according to dry-heating time and the heating. Thus, the disulfide bond was also found to be responsible for the formation of aggregates in the dry-heating process and the heating.

The 10% (w/w) DHEW solutions (dry-heating conditions: 120 °C, up to 2 h), into various amounts of NaCl (up to the concentration of 0.65 M) were added, were also heated at 60 °C for 5 min, and their turbidity was determined (Figure 7). The maximum turbidity without any coagula under the conditions used in this study was ~ 0.7 . In each DHEW solution, the turbidities decreased as more NaCl was added. Furthermore, in the DHEW solutions containing the same amounts of NaCl, lower turbidity was obtained by increasing the dry-heating time. Thus, the transparent solutions in the samples

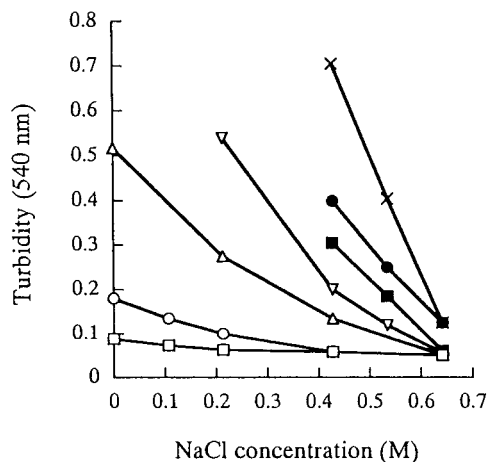


Figure 7. Effect of NaCl on the turbidity development of FDEW (x) and DHEW [120 °C, 20 min (●), 30 min (■), 40 min (▽), 50 min (△), 60 min (○), 120 min (□)] solutions after heating (60 °C, 5 min).

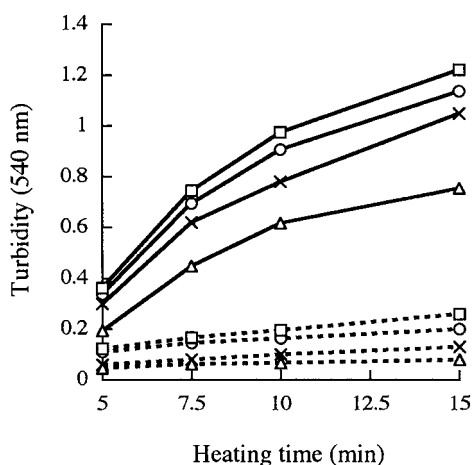


Figure 8. Effect of diluents on the turbidity of the DHEW [120 °C, 1 (—) and 6 (---) h] solutions after heating (60 °C, up to 15 min). Ten percent (w/w) DHEW solutions heated were diluted (40 times) with 20 mM sodium phosphate buffer (pH 7.4) (diluent A, □), diluent A + 0.6 M NaCl + 0.5% SDS + 1.5 M urea (diluent B, ○), diluent A + 0.01 M 2-ME (diluent C, x), and diluent B + 0.01 M 2-ME (diluent D, △), and their turbidities were measured by reading the absorbance (540 nm).

dry-heated for relatively longer times such as 1 and 2 h were prepared even in the lower NaCl concentrations, whereas those for relatively shorter times such as 20 and 30 min only in the higher NaCl concentrations.

Figure 8 shows the effect of the diluents (described under Materials and Methods; dilution = 40 times) on the turbidities of the 10% (w/w) DHEW solutions (dry-heating conditions: 120 °C, 1 and 6 h) induced by heating at 60 °C for up to 15 min. It was proved that the development of turbidity with increased heating time was suppressed to a greater extent in the 6 h-treated sample than in the 1-h-treated one in every diluent. It is indicated that the differences in turbidities among samples diluted with diluents A–C were small, whereas those between samples diluted with diluents A and B or C and D were larger. Thus, the binding forces related to the development of turbidity in DHEW solution on heating should be ascribed to the cooperative actions of disulfide bonds and noncovalent forces such as hydrophobic interactions, electrostatic attraction, and hydrogen bonds.

DISCUSSION

In the discussion on the mechanisms by which DHEW inhibits the heat aggregation and coagulation of FDEW, the denaturation and aggregation of DHEW proteins and the interactions between DHEW proteins and FDEW proteins should be taken into consideration.

The dry-heating at 120 °C was regarded to be heating under dehydrated conditions, as indicated by the low moisture content of 0.3%. The denaturation temperature of proteins strongly depends on moisture content. It was reported that the two major egg white proteins of OVA and OT did not denature even at temperatures >100 °C when the moisture content was <1% (Kiriya et al., 1997). Egg white proteins dry-heated at 120 °C in this study did not greatly denature, although soluble aggregates were formed when the DHEWs were solubilized with water.

The molten globular state, which is distinguishable from both the native and the completely denatured state (Hirose, 1993; Tani et al., 1995), has been demonstrated in the denaturation process of many globular proteins. DHEW proteins might be in a molten globular state with almost no secondary structure as indicated by CD patterns, although the detailed structural characteristics of FDEW proteins unfolded during dry-heating were not elucidated. Kato et al. (1989, 1990a) also reported that heating in the dry state at 80 °C for 10 days hardly affected the secondary structures of egg white proteins.

Surface hydrophobicity of samples in the processes of dry-heating and heating generally increased. Each determined value after heating was generally ~3 times that before heating. Such increases in surface hydrophobicity in the DHEW indicate the possibility that the DHEW proteins interact with FDEW proteins through hydrophobic interaction when the mixed solutions of DHEW and FDEW were heated. On the other hand, the increases in surface sulfhydryl contents and the decreases in total sulfhydryl contents in DHEW proteins show the possibility of soluble aggregate formation through a disulfide bond in the dry-heating process and the subsequent heating. In practice, the total sulfhydryl contents in the DHEWs decreased after heating, although they were at a low level.

The heat aggregation of food proteins largely depends on salt concentrations (Kitabatake et al., 1987, 1988). When the DHEW solutions were heated at various concentrations of NaCl, it was found that turbidity in every heated DHEW sample developed at lower rather than higher NaCl concentrations and that turbidity developments in solutions of DHEW according to dry-heating times were not greatly effected by the presence or absence of NaCl (Figure 7). A similar phenomenon was seen when the heat-induced aggregate of preheated egg white in the dry state showed a relatively slower response to an increase in salt concentration as reported by Kato et al. (1989, 1990a,b). The results in this study suggested that DHEW according to dry-heating times was subjected to changes in the charge balance on the protein surface, and aggregation in such DHEW as developing the turbidity was inhibited by such changes in the heating.

Examination of lowering effects of turbidity with various diluents showed that the cooperative actions of NaCl, SDS, urea, and 2-ME were important for the dissociation of aggregates responsible for turbidity development in the solution of DHEW treated for 1 h. On the other hand, in the solutions of DHEW treated

for 6 h, turbidity development was at a low level and the diluting effects were not so great.

It is generally accepted that whether a transparent or a turbid solution occurs when the protein solution is heated depends on the aggregated types of proteins; that is, a linear aggregate and a random aggregate result in a transparent and turbid solution, respectively. The formation of such aggregates is controlled by the balance between hydrophobic interaction and electrostatic repulsion for the denaturation and aggregation of protein by heat treatments (Kitabatake et al., 1987, 1988; Matsudomi et al., 1991).

From the above discussion, it was suggested that the transparent solutions obtained in this study might be due to the soluble linear aggregates of DHEW proteins, especially OVA, resulting from hydrophobic interaction and electrostatic repulsion with disulfide bond, and then the coagulum formations on OT and LZ were inhibited by the soluble polymer formations between the soluble aggregates-OT and -LZ. However, the detailed manner in which the soluble aggregates and polymers formed is unclear.

In conclusion, DHEWs (dry-heating conditions: 120 °C, 1–6 h) with increased dry-heating time inhibited the heat aggregation and coagulation of FDEW when its solutions were mixed with DHEW solutions and heated (60 °C, 5 min). In the dry-heating process, the mild conformational changes in FDEW proteins and the formation of water-soluble aggregates occurred, whereas in the heating process, the soluble aggregates in the DHEW solution interacted with OT and LZ in FDEW and suppressed the OT-OT and OT-LZ interactions that led to coagulation. Additional research will be needed to elucidate the inhibiting mechanism at the molecular level and so to expand utilization of dry-heated egg white in the food industry.

LITERATURE CITED

- Bergquist, D. H. Egg dehydration. In *Egg Science and Technology*, 4th ed.; Stadelman, W. J., Cotterill, O. J., Eds.; Food Products Press: Binghamton, NY, 1995; Chapter 14, pp 335–376.
- Beveridge, T.; Toma, S. J.; Nakai, S. Determination of SH- and SS- groups in some food proteins using Ellman's reagent. *J. Food Sci.* **1974**, *39*, 49–51.
- Hirose, M. Molten globule state of food proteins. *Trends Food Sci. Technol.* **1993**, *4*, 48–51.
- Kato, A.; Ibrahim, H. R.; Watanabe, H.; Honma, K.; Kobayashi, K. New approach to improve the gelling and surface functional properties of dried egg white by heating in dry state. *J. Agric. Food Chem.* **1989**, *37*, 433–437.
- Kato, A.; Ibrahim, H. R.; Watanabe, H.; Honma, K.; Kobayashi, K. Structural and gelling properties of dry-heating egg white proteins. *J. Agric. Food Chem.* **1990a**, *38*, 32–37.
- Kato, A.; Ibrahim, H. R.; Takagi, T.; Kobayashi, K. Excellent gelation of egg white preheated in the dry state is due to the decreasing degree of aggregation. *J. Agric. Food Chem.* **1990b**, *38*, 1868–1872.
- Kiriyama, M.; Takada, A.; Urabe, Y.; Nemoto, N. Thermal properties of ovalbumin and ovalbumin gels in various solvents. *Netsu Sokutei* **1997**, *24*, 118–126.
- Kitabatake, N.; Hatta, H.; Doi, E. Heat-induced and transparent gel prepared from hen egg ovalbumin in the presence of salt by a two-step heating method. *Agric. Biol. Chem.* **1987**, *51*, 771–778.
- Kitabatake, N.; Shimizu, A.; Doi, E. Preparation of transparent egg white gel with salt by a two-step heating method. *J. Food Sci.* **1988**, *53*, 735–738.
- Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–685.
- Lowry, O. H.; Resebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.
- Matsudomi, N.; Ishimura, Y.; Kato, A. Improvement of gelling properties of ovalbumin by heating in dry state. *Agric. Biol. Chem.* **1991**, *55*, 876–881.
- Mine, Y. Effect of pH during the dry heating on the gelling properties of egg white proteins. *Food Res. Int.* **1996**, *29*, 155–161.
- Mine, Y. Effect of dry heat and mild alkaline treatment on functional properties of egg white proteins. *J. Agric. Food Chem.* **1997**, *45*, 2924–2928.
- Monahan, F. J.; German, J. B.; Kinsella, J. E. Effect of pH and temperature on protein unfolding and thiol disulfide interchange reactions during heat-induced gelation of whey proteins. *J. Agric. Food Chem.* **1995**, *43*, 46–52.
- Parkinson, L. L. The effect of storage on the proteins of frozen, liquid egg and spray-dried egg. *J. Sci. Food Agric.* **1997**, *28*, 811–816.
- Smith, C. F. Treatment of egg albumen. U.S. Patent 3,161,527, Dec 15, 1964.
- Tani, F.; Murata, M.; Higasa, T.; Goto, M.; Kitabatake, N.; Doi, E. Molten globule state of protein molecules in heat-induced transparent food gels. *J. Agric. Food Chem.* **1995**, *43*, 2325–2331.
- Xu, J. Q.; Shimoyamada, M.; Watanabe, K. Gelation of egg white proteins as affected by combined heating and freezing. *J. Food Sci.* **1997**, *62*, 963–966.
- Xu, J. Q.; Shimoyamada, M.; Watanabe, K. Heat aggregation of dry-heated egg white and its inhibiting effect on heat coagulation of fresh egg white. *J. Agric. Food Chem.* **1998**, *46*, 3027–3032.

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