# Heat Aggregation of Dry-Heated Egg White and Its Inhibiting Effect on Heat Coagulation of Fresh Egg White

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The characteristics of dialyzed and freeze-dried egg white that was dry-heated under various conditions (70−125 °C, 0 min−6 h) were studied. The dry-heated egg whites (DHEWs, 120 °C) showed the formation of insoluble aggregates (coagula) within 50 min, turbid soluble ones (50 min−2 h), and transparent soluble ones (2−6 h) according to the dry-heating times when solubilized at 10% concentration (pH 7.4) and reheated at 60 °C for 3.5 min. A transparent DHEW solution without any coagula when reheated was obtained by using a shorter dry-heating time and elevating the dry-heating temperature. It was also found that NaCl suppressed the turbidity development of DHEW solution promoted by $CaCl_2$. When 10% DHEW solutions (120 °C, >2 h) were mixed with fresh egg white at the ratio of 1:1 in volume and heated at 60 °C for 3.5 min, coagulum formations in their mixtures were inhibited. The patterns of native− and SDS−PAGEs and gel filtration suggest that soluble aggregates of ovalbumin formed in the dry-heating process inhibit the formation of ovotransferrin coagula.

**Keywords:** *Egg white; dry-heating; heat coagulation; soluble aggregate*

## INTRODUCTION

The effect on the physical properties of egg white and whole egg of heating at pasteurization temperature has been studied by many researchers (Seideman et al., 1963; Cunningham and Lineweaver, 1965, 1967; Garibaldi et al., 1968; Chang et al., 1970; Cunningham, 1974; Ball et al., 1987). The sensitivity of egg white proteins to heat denaturation and heat aggregation has also been a subject of interest (Matsuda et al., 1981; Watanabe et al., 1985). The pasteurization of egg white is usually carried out for a few minutes at a temperature near 60 °C because of the high heat sensitivity of egg white proteins. However, functional properties such as whipping were also reported to begin deteriorating at temperatures as low as 54 °C (Cunningham and Lineweaver, 1965). Thus, the establishment of a method for the heat pasteurization of egg white without any loss of functional properties has been sought.

It has been reported that when spray-dried egg white was heated in a controlled dry state at 80 °C for various periods of time, its functionalities (gelling, emulsifying, foaming properties, and water-holding capacity) were improved significantly with an increase in heating time without any loss in solubility (Kato et al., 1989, 1990a,b; Mine, 1996). Moreover, the increase in solubility was shown to be due to the deamidation of the denatured proteins (Mine, 1997).

In a previous study (Xu et al., 1997), we showed that when solutions of spray-dried (60−70 °C) and then heat-treated egg white (55−65 °C, 3 days in a dry state to reduce microbial numbers) were reheated at 60 °C for various times, the insoluble materials in their solutions almost failed to form. Such a phenomenon was also found to be mainly attributable to changes in proteins occurring in the process of heating in a dry state. Thus, it is of interest to know the effect of heating in a dry state on the basic food-functional properties of egg white proteins such as aggregation and/or coagulation.

Our objective was to determine the heat aggregation of dialyzed and freeze-dried egg white that was dry-heated under various conditions and its inhibiting effect on heat coagulation of fresh egg white. This may increase further applications of egg white in food processing to make possible heat treatments for egg white (including pasteurization) without coagula production.

## MATERIALS AND METHODS

**Preparation of Samples.** Egg white was prepared from 1- or 2-day-old eggs (produced by a strain of White Leghorn layers) blended in a Waring blender without foaming sufficient to provide a homogeneous mixture, dialyzed against distilled water at 4 °C, and freeze-dried after the pH had been adjusted to 7.4. The freeze-dried egg white (FDEW; moisture content = 5.4%) in a sealed vial was dry-heated in the oven at temperatures from 70 to 125 °C for various durations, and the samples prepared were designated DHEWs.

**Heat Aggregation and/or Coagulation of DHEW.** The various DHEWs were solubilized to obtain a concentration of 10% (w/w) with water at pH 7.4 and centrifuged at 2000*g* for 20 min to remove insoluble proteins. Each sample (4.5 mL) of the prepared DHEW solutions in stoppered test tubes (6 mm i.d. × 45 mm) was reheated (60 °C, 3.5 min) in a water bath under set conditions, cooled immediately in tap water, and centrifuged at 2000*g* for 20 min. Soluble protein content in the resultant supernatant was determined by the measurement of dry weight in a vacuum. Turbidities of their supernatants diluted (40 times) with 20 mM phosphate buffer (pH 7.4) were measured at 540 nm. The soluble protein content and turbidity of their supernatants were regarded as indices of aggregation and/or coagulation of DHEW.

**Inhibition of DHEW against Heat Coagulation of Fresh Egg White.** For a comparison to FDEW without dry heating, each of the various 10% DHEW solutions (dry-heating condition: 120 °C for 0 min−6 h) described above was mixed with fresh homogenized egg white [protein concentration and pH were adjusted to 10% (w/w) and 7.4, FEW] at the ratio of

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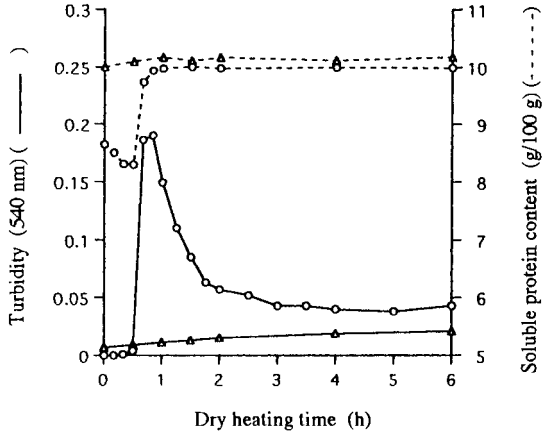


**Figure 1.** Soluble protein content (determined in dry weight) and turbidity (40 times diluted supernatant, 540 nm) of DHEW solutions (dry-heating conditions: 120 °C, 0−6 h) before (△) and after (○) reheating (60 °C, 3.5 min).

1:1 in volume, heated at 60 °C for 3.5 min, and centrifuged in a manner similar to that described above. The soluble protein content and turbidity of these supernatants were also determined as indices of the inhibition of DHEW against heat coagulation of FEW.

**Effects of $CaCl_2$ and pH at Dry-Heating on DHEW Turbidity and of NaCl on Its Turbidity Development.** FDEW was solubilized with distilled water at the concentration of 10%, $CaCl_2$ to a level of 0−7 mM was added after the pH of the solution was adjusted to 7.0−8.9, and the mixture was freeze-dried again. The FDEWs prepared were dry-heated at 120 °C for 1.5 h. The obtained DHEW containing $CaCl_2$ was solubilized at a concentration of 10%, the pH of its solutions was adjusted to 7.4, and the solutions were reheated at 60 °C for 3.5 min with and without addition of NaCl (0.4%, w/w) to see the effect on $CaCl_2$-induced turbidity development. On the other hand, to the DHEW prepared without $CaCl_2$ was added $CaCl_2$ up to 4 mM prior to reheating at 60 °C for 3.5 min. The turbidities were determined as described above.

**Polyacrylamide Gel Electrophoresis (PAGE).** The native−PAGE method was used to separate the egg white proteins. Gel sheets of 7.5% and PAGE buffer of Tris−glycine were prepared as described by Davis (1964). Electrophoresis was performed with a constant current of 20 mA for 1.5 h using a discontinuous buffer system. 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. Sodium dodecyl sulfate (SDS)−PAGE in the presence and absence of 2-mercaptoethanol (2-ME) was carried out according to the method of Laemmli (1970) as described in the previous paper (Xu et al., 1997).

**Gel Filtration.** The gel filtration experiment was carried out with a Sephadex G-150 column (2.0 × 44 cm). The samples (1.5 mL of sample diluted 10 times with 20 mM phosphate buffer, pH 7.4) were applied and eluted with the same buffer at a flow rate of 0.6 mL/min. The 3.6 mL fractions were collected and monitored at 280 nm. Fractions were dialyzed against deionized water, concentrated, and applied to SDS−PAGE as above.

## RESULTS

The soluble protein content (determined in dry weight) and turbidity (diluted 40 times) of various DHEW solutions (dry-heating condition: 120 °C, 0 min−6 h) reheated at 60 °C for 3.5 min were examined to discriminate between aggregation and coagulation (Figure 1). From the results of DHEW solutions [10% (w/w), pH 7.4] before reheating, which showed almost the same solubility and turbidity without the dependence on dry-heating time, it was found that dry-heating at 120 °C for FDEW did not apparently affect the solubility of its treated materials, DHEWs. However, the reheating treatments to DHEW solutions induced changes in soluble protein content and turbidity depending on the dry-heating time, as seen in Figure 1. The soluble protein content in the FDEW (dry-heating time = 0 min) reheated solution was lower by ∼13% than that in the unreheated FDEW solution. Furthermore, the soluble protein content in DHEW reheated solutions decreased slightly with the time of dry-heating up to 30 min, increased abruptly to ∼10% (solubility = 100%) in dry-heating time up to 1 h, and then held at ∼10% in subsequent dry-heating time up to 6 h. On the other hand, the turbidity of DHEW (dry-heating up to 30 min) reheated solutions was lower than that of unreheated ones, and that of DHEW reheated solutions increased upon increasing dry-heating to >30 min, reaching a maximum at ∼1 h, greatly decreasing up to 2 h, and thereafter remaining approximately constant. Dry-heating with increasing time up to ∼30 min resulted in the lowest soluble protein content, that is, the highest formation of insoluble aggregates (coagula) when DHEWs were solubilized and reheated. However, when the DHEW solutions (dry-heated for >1 h) were reheated in the same manner, they showed hardly any formation of coagulum and development of turbidity. Thus, the dry-heating for FDEW apparently induced the formation of both insoluble materials (coagulation) and soluble ones (aggregation resulting in a turbid or transparent solution) in the reheat treatments. Thus, we observed the formation of aggregates having high solubility in the samples dry-heated for >2 h at 120 °C and heated under pasteurization conditions.

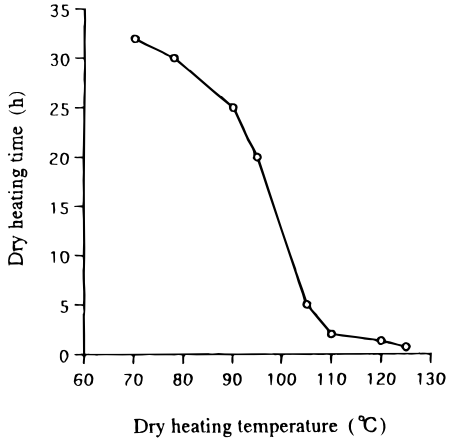


**Figure 2.** Relationship between temperatures (70−125 °C) and times (15 min−32 h) in dry-heating conditions for FDEW to reach a value of 0.1 in turbidity at 540 nm. Turbidities were measured on the 40 times diluted supernatants from DHEW solutions (10% w/w, pH 7.4) reheated at 60 °C for 3.5 min.

The relationships between temperature (70−125 °C) and time (10 min−35 h) in dry-heating of FDEW to obtain the value of 0.1 in turbidity without formation of any coagula were examined in their various 10% DHEW solutions reheated at 60 °C for 3.5 min (Figure 2). The value of 0.1 was arbitrarily selected because the transparency of samples in the systems used in this study was observed to be less than around 0.1. The values decreased from 32 h at 70 °C to 15 min at 125 °C, indicating a gradual reduction in the duration of dry-heating with the elevation of dry-heating temperatures to 90 °C, a rapid reduction to 105 °C, and then a slow reduction to 125 °C. To prepare the transparent solution which showed a low turbidity value of 0.1 with increase in dry-heating temperature, the dry-heating periods were shortened.

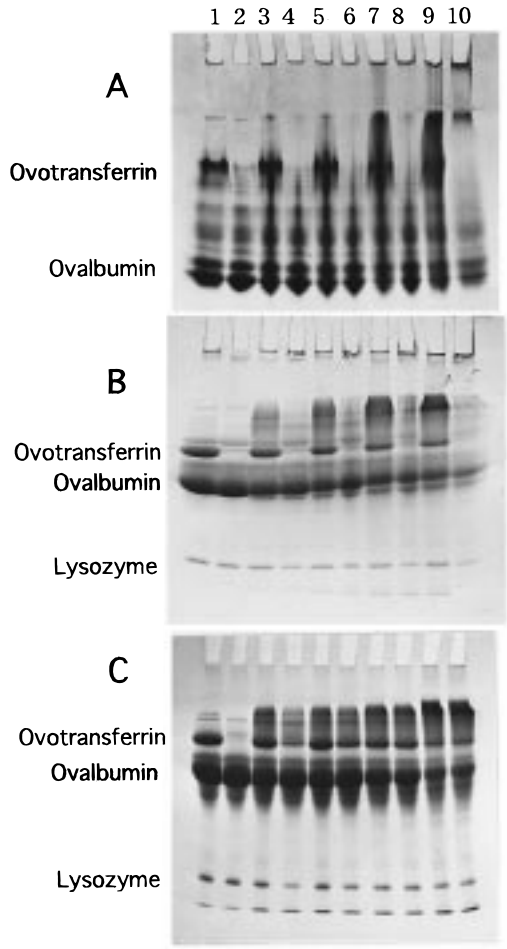


**Figure 3.** Patterns of native− and SDS−PAGEs on supernatants from various DHEW solutions (dry-heating conditions: 120 °C, 0 min−4 h) before (lanes 1, 3, 5, 7, and 9) and after (lanes 2, 4, 6, 8, and 10) reheating at 60 °C for 3.5 min: (A) native−PAGE; (B) SDS−PAGE in the absence of 2-ME; (C) SDS−PAGE in the presence of 2-ME; (lanes 1 and 2) 120 °C, 0 min; (lanes 3 and 4) 120 °C, 30 min; (lanes 5 and 6) 120 °C, 1 h; (lanes 7 and 8) 120 °C, 2 h; (lanes 9 and 10) 120 °C, 4 h.

The patterns of supernatant obtained by centrifugation (2000*g*, 20 min) of DHEW solutions (dry heating condition: 120 °C, 0 min−4 h) on native−PAGE (Figure 3A) and SDS−PAGE in the absence of (Figure 3B) and presence (Figure 3C) of 2-ME are shown in Figure 3. The proteins in FDEW solution (dry-heating time = 0 min, lane 1 in Figure 3A−C) were clearly separated into major bands of ovalbumin (OVA) and ovotransferrin (OT) during electrophoreses. Lysozyme (LZ) was also found in the SDS−PAGE (Figure 3B,C). Moreover, the intensities of bands of OVA, LZ, and especially OT decreased gradually with longer dry-heating time (lanes 1, 3, 5, 7, and 9 in Figure 3A−C). This indicates the formation of some soluble aggregates between molecules such as OVA−OVA, OT−OT, and OVA−OT, since the solubilities of DHEW were almost 100% (soluble protein content = 10%; Figure 1). The aggregation phenomenon in such samples was further illustrated by increasing the amounts of aggregates in the stacking gel and at the beginning of the separating gel by increasing the dry-heating times. Hence, the longer the dry-heating time, the greater the amount of soluble aggregate. The decrease in stained bands corresponding to OVA monomer in the SDS−PAGE patterns (Figure 3B,C) suggested that the soluble aggregates were formed through

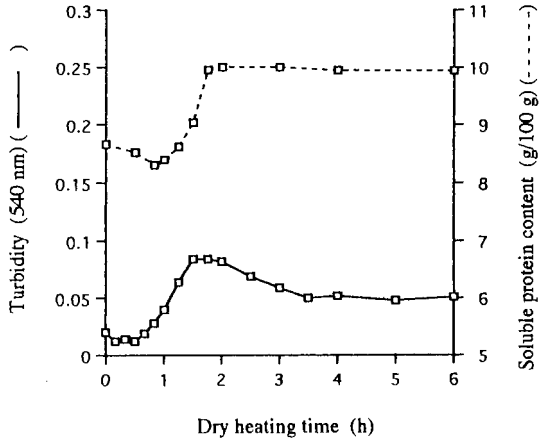


**Figure 4.** Inhibition of DHEW on the coagulation of FEW as shown by the soluble protein content and turbidity of the supernatants from the mixtures of various DHEW solutions (dry-heating conditions: 120 °C, 0 min−6 h) and FEW (1:1) heated at 60 °C for 3.5 min.

intermolecular disulfide linkages and hydrophobic interactions which were not easily dissociated into monomer types even by SDS and 2-ME.

In the patterns of native−PAGE on DHEW solutions followed by reheating at 60 °C for 3.5 min (lanes 2, 4, 6, 8, and 10 in Figure 3A), the bands of OT disappeared in all of the samples, and the intensities of OVA bands gradually decreased as the dry-heating time was increased. Moreover, soluble aggregates that could not migrate into the stacking gel and remained at the beginning of the separating gel increased with the increase in dry-heating time, and their SDS−PAGE patterns without 2-ME also showed the disappearance of OT (lanes 2, 4, 6, 8, and 10 in Figure 3B). However, their OT bands appeared in the patterns with 2-ME only in the samples dry-heated for >1 h (lanes 6, 8, and 10 in Figure 3C). Thus, OT in DHEW changed from an insoluble to a soluble form according to dry-heating times, and disulfide linkages between OT molecules were found to relate to the formation of coagula caused by the reheating. With the increase in dry-heating times, the soluble aggregates that were not easily dissociated into monomer types increased through disulfide linkages and hydrophobic interactions by the reheat treatments. The absence of almost any changes in bands corresponding to OVA before and after reheating also indicated that in the reheating treatment at 60 °C and 3.5 min OVA might not denature and aggregate further.

Next, the inhibition of DHEW on the formations of coagulation in FEW heated at 60 °C for 3.5 min was examined. The various DHEW solutions (dry-heating conditions: 120 °C, 0 min−6 h; concentration = 10%, w/w; pH 7.4) were mixed with FEW and heated, and their soluble protein contents and turbidities were determined (Figure 4). Coagula occurred at ∼13% in the mixture of FDEW (dry-heating time = 0 min) and FEW. They increased slightly with dry-heating times up to 1 h in the DHEW−FEW mixtures and were then gradually converted into soluble forms. The soluble protein content in their mixtures reached approximately 10% (solubility = 100%) when dry-heated for >2 h. On the other hand, the turbidity increased with increasing soluble protein content in dry-heating up to ∼2 h and then gradually decreased with the increase in dry-heating times. Thus, the DHEW (dry-heating condition: 120 °C, >2 h) inhibited the formation of coagula

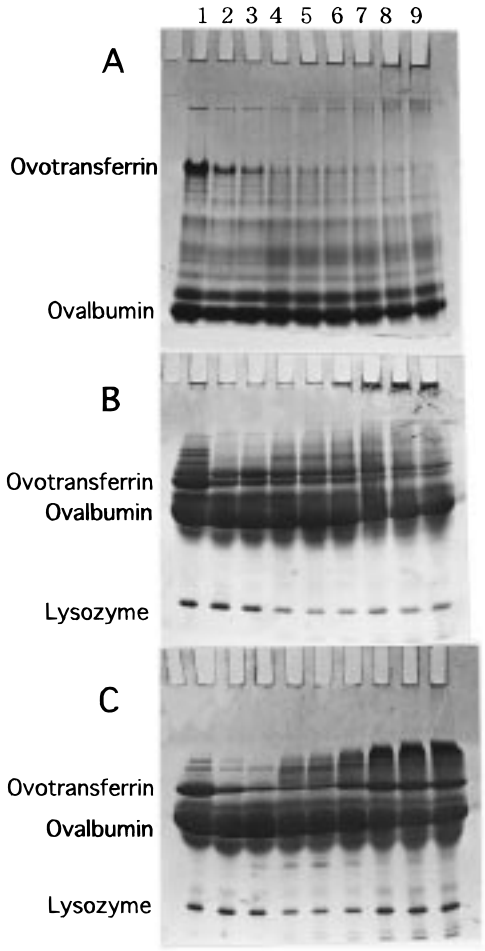


**Figure 5.** Patterns of native− and SDS−PAGEs of the supernatants from mixtures of DHEW solutions and FEW (1:1) heated at 60 °C for 3.5 min: (A) native−PAGE; (B) SDS−PAGE in the absence of 2-ME; (C) SDS−PAGE in the presence of 2-ME; (lane 1) unheated mixture of DHEW solution and FEW; (lane 2) heated FEW; (lanes 3−9) heated mixture of DHEW solution and FEW (3, 120 °C, 0 min; 4, 120 °C, 30 min; 5, 120 °C, 1.0 h; 6, 120 °C, 1.5 h; 7, 120 °C, 2.0 h; 8, 120 °C, 4 h; 9, 120 °C, 6 h).

and the development of turbidity in FEW heated under pasteurization conditions.

The results analyzed by native− and SDS−PAGEs on the supernatants from the mixtures of DHEW solutions (dry-heating condition: 120 °C, 0 min−6 h) and FEW that were heated at 60 °C for 3.5 min are shown in Figure 5. The patterns of native−PAGE (Figure 5A) indicated that the bands of OT disappeared on the formation of soluble aggregates and/or coagula in all samples used. In the patterns of SDS−PAGE in the absence of 2-ME, the aggregated OT could not be greatly dissociated (Figure 5B), indicating the formation of disulfide linkages between OT molecules. When compared with the same samples in the presence of 2-ME, OT was partially dissociated in the samples dry-heated for >1.5 h with increasing dry-heating times (Figure 5C). The intensities of the OVA band in Figure 5 before and after heating showed almost no change, indicating that OVA in FEW mixed with DHEW might not denature and aggregate further in the heating treatment, comparable to the manner of DHEW mentioned above (Figure 3).

The coagula formed by the heat treatment of DHEW solutions (dry-heating condition: 120 °C, 0 and 30 min) and each mixture of DHEW solutions (dry-heating condition: 120 °C, 0, 30, 60 and 90 min) and FEW described above were separated (precipitated by centrifugation: 2000*g*, 20 min). The insoluble materials with 20 mM phosphate buffer (pH 7.4) were freeze-dried, dissolved separately in electrophoretic solvent for SDS−PAGE in the presence of 2-ME, and subjected to SDS electrophoresis (Figure 6). The coagula were found to consist of OT as the main component with OVA and LZ as minor ones. Almost all of the OT in DHEW and the mixture of DHEW−FEW would probably coagulate by dry-heating for <30 min and heat treatments of 60 °C and 3.5 min, as seen in comparison with the patterns in Figures 3 and 6.

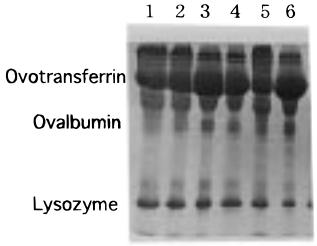


**Figure 6.** SDS−PAGE patterns of coagula (precipitates at 2000*g*, 20 min) from DHEW solutions (dry-heating conditions: 120 °C, 0 and 30 min) and mixtures of DHEW solutions (dry-heating conditions: 120 °C, 0−1.5 h) and FEW heated at 60 °C for 3.5 min. Each separated coagulum was washed with 20 mM phosphate buffer (pH 7.4), freeze-dried, dissolved with electrophoretic solvent, and electrophoresed. Coagula from reheated DHEW solutions [dry-heating conditions: (lane 1) 120 °C, 0 min; (lane 2) 120 °C, 30 min)]. Coagula from mixtures of reheated DHEW solutions [dry heating conditions: (lane 3) 120 °C, 0 min; (lane 4) 120 °C, 30 min; (lane 5) 120 °C, 1 h; (lane 6) 120 °C, 1.5 h] and FEW.

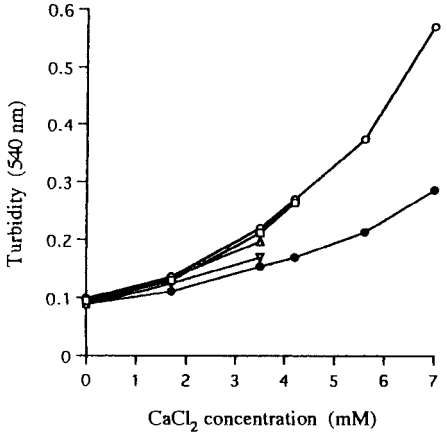


**Figure 7.** Effects of $CaCl_2$ (0−7 mM) and pH (7.0−9.0) at dry-heating on the turbidity of DHEW and of NaCl (0.4%) on its turbidity development. FDEWs (pH 7.0−8.9) containing $CaCl_2$ were dry-heated at 120 °C for 1.5 h. Their solutions (pH 7.4) prepared at 10% were reheated at 60 °C for 3.5 min with and without NaCl (0.4%). The turbidities of 40-fold diluted supernatants were then determined. (□) $CaCl_2$ at pH 7.0; (○) $CaCl_2$ at pH 7.4; (△) $CaCl_2$ at pH 8.1; (▽) $CaCl_2$ at pH 8.9; (●) $CaCl_2$ at pH 7.4 + NaCl (0.4%).

The DHEW (dry-heating condition in the presence of $CaCl_2$: 120 °C, 1.5 h) solutions (pH 7.4) were also reheated at 60 °C for 3.5 min, and their turbidity levels, which did not show the formation of any coagula, were determined (Figure 7). Turbidities increased with increasing amounts of $CaCl_2$ added, and such turbidity developments were suppressed by the addition of NaCl, especially in the region of higher concentrations of $CaCl_2$. The effect of pH (7.0−8.9) at the dry-heating of FDEW on turbidity development was a slight decrease in turbidity with increasing pH. Furthermore, when

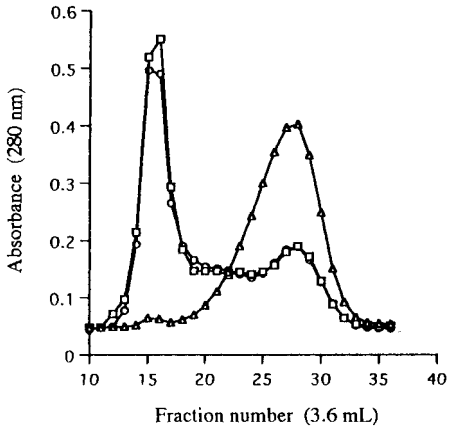


**Figure 8.** Gel filtration profiles of FDEW and DHEW solutions: (△) FDEW; (○) DHEW (dry-heating condition: 120 °C for 4 h); (□) DHEW (dry-heating condition: 120 °C for 4 h) reheated (60 °C for 3.5 min).

$CaCl_2$ was added to the DHEW solutions (dry-heated without $CaCl_2$) prior to reheating at 60 °C for 3.5 min, the observed turbidity of the solutions was no different from that of the DHEW containing $CaCl_2$ at dry-heating (data not shown). This suggested that $CaCl_2$ added to FDEW did not affect the aggregate formation of proteins during dry-heating.

Gel filtration profiles of DHEW solutions (dry-heating condition: 120 °C, 4 h) before and after reheating (60 °C, 3.5 min) were also compared with those of FDEW solution (Figure 8). The pattern of FDEW indicated one broad peak (fractions 20−33) containing monomer types of OVA, OT, and ovomucoid from native− and SDS−PAGEs (data not shown). On the other hand, the patterns of DHEW with and without the following reheat treatments were almost the same, indicating that the peaks in fractions 11−19 consisted of some aggregates (in SDS−PAGE OVA and OT as main proteins), while those in fractions 20−33 consisted of some aggregates and monomers of OVA (in SDS−PAGE OVA as main protein). The peaks of fractions 25−33 in FDEW greatly decreased on the formation of aggregates during dry-heating. However, the following reheat treatment apparently did not induce further aggregations, although the native− and SDS−PAGE patterns of such samples were different from each other in the bands of soluble aggregates (Figure 3).

To analyze the proteins in coagula and their binding mechanisms, SDS−PAGE patterns of the coagula (precipitates obtained by centrifugation, 2000*g*, 20 min) formed in DHEW (dry-heating conditions: 120 °C, 0, 30, 40, and 50 min) reheated solutions were also determined without washing, in contrast to the case in Figure 6. It was found that the coagula consisted of OT, OVA, and LZ and that OT was the main component in the 0−30 min dry-heated samples, whereas OVA was the main component in the 40−50 min dry-heated ones (Figure 9A). The fact that the intensities of LZ did not change in the 0−50 min dry-heated samples suggested that LZ hardly affected the formation of OT coagula during dry-heating. We also determined SDS−PAGE patterns of soluble components extracted from coagula, which were formed in DHEW (dry-heating condition: 120 °C, 50 min) solutions when reheated (Figure 9B). The extraction was carried out with four solutions: distilled water (pH adjusted to 7.4, solution A), 20 mM phosphate buffer (pH 7.4, solution B), B + 0.6 M NaCl + 0.5% SDS + 1.5 M urea (solution C), and C + 0.01 M 2-ME (solution D). The main component extracted with solutions A−C was OVA, and those with solution D were OVA, OT, and LZ. Thus, it was suggested that the main binding forces among components in coagula were hydrophobic and disulfide bonds.

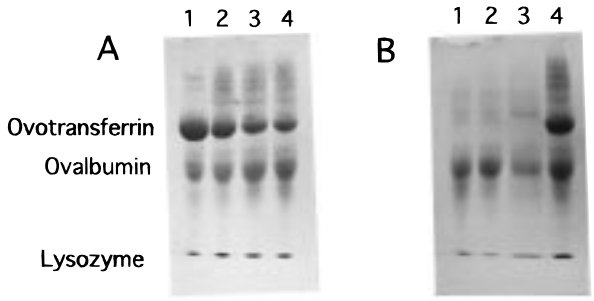


**Figure 9.** SDS−PAGE patterns of coagula from various DHEW solutions reheated (60 °C, 3.5 min): (A) coagula from DHEWs [dry-heating conditions: 120 °C, 0 min (lane 1); 30 min (lane 2); 40 min (lane 3); 50 min (lane 4)]; (B) extracts of coagula from DHEW solutions (dry-heating conditions: 120 °C, 50 min) with solution A (distilled water adjusted to pH 7.4) (lane 1), solution B (20 mM phosphate buffer, pH 7.4) (lane 2), solution C (solution B + 0.6 M NaCl + 0.5% SDS + 1.5 M urea) (lane 3), solution D (solution C + 0.01 M 2-ME) (lane 4).

## DISCUSSION

This study found that high-temperature heating in a dry state of FDEW was an effective method for inhibiting the formation of insoluble aggregates (coagula) and soluble aggregates resulting in a turbid solution when the DHEW solution was heated (60 °C, 3.5 min). Moreover, it was interesting that DHEW prepared at 120 °C as described above inhibited the heat-induced coagulation of FEW when the DHEW solution was mixed with FEW and heated. Thus, heat pasteurization of egg white without the formation of any coagula and with less development of turbidity was found to be possible by the addition of DHEW.

The inhibition against the heat coagulation of egg white is important for its applications to secondary food processing. Hence, dry-heating conditions (temperature and time) for FDEW were obtained to prepare transparent solutions without any coagula when reheated at 60 °C for 3.5 min (Figure 2). The rapid decrease in dry-heating time with the elevation of dry-heating temperature above 90 °C to reach a value of 0.1 in turbidity might show that the denaturation level of dry-heated proteins and their aggregation behavior were different under dry-heating conditions, probably due to the evaporation of water involved in dry-heating. It was reported that dried egg white could be stored at elevated temperatures (50−70 °C) without significant impairment of functional properties (Banwart and Ayres, 1956) and that water content greatly affected the denaturation temperature of protein; that is, the lower the water content in protein, the higher the denaturation temperature (Kiriyama et al., 1997).

Soluble aggregates were formed during dry-heating as seen in the results of native−PAGE, gel filtration, soluble protein content, and turbidity. However, a monomer of OVA was also found to be present in samples dry-heated for >2 h. It is not entirely clear whether such a monomer was native or denatured and how the formation of coagulates was inhibited. The molten structure that is partially unfolded and more flexible than the native form may be generally formed by controlled heating in the dry state (Kato et al., 1989;

Tani et al., 1995). The fact that heat treatment in the dry state of egg white hardly affected the circular dichroism spectrum patterns as described by Kato et al. (1989) also suggested the formation of the molten structure in DHEW in this study. Thus, soluble aggregates formed during dry-heating might be polymers of partially denatured proteins. It was also found that these aggregates did not greatly polymerize further in subsequent heat treatment for reasons still unclear.

It is generally accepted that the aggregation of food proteins largely depends on pH and on both protein (concentration) and salts (concentration and variety). The fact that $CaCl_2$ was not related to the formation of soluble aggregates (development of turbidity) at heating in the dry state, but rather promoted it at reheating at 60 °C and 3.5 min, might be due to the cross-linking formative action of $Ca^{2+}$ dissociated from $CaCl_2$ in solution. It was reported that $Ca^{2+}$ binding to unfolding protein molecules caused an increase in the reactive sulfhydryl group content and hydrophobicity (Jeyarajah and Allen, 1994) and that the differences in concentrations of $CaCl_2$ resulted in different rates of aggregation (Hongsprabhas and Barbut, 1997). According to these views, it may be concluded that $Ca^{2+}$ in the reheating solution formed salt bridges between adjacent (partially denatured) OT and OVA molecules or their aggregates and that $Na^+$ inhibited the aggregate formation via $Ca^{2+}$-mediated interactions. This inhibition might depend on the replacement of $Ca^{2+}$ by $Na^+$, which is not available for intermolecular protein binding. The fact that NaCl suppressed to some extent the formation of a turbid solution might be advantageous in food processing.

It was suggested that the formation of a linear aggregate (transparent solution) or random aggregate (turbid solution) was controlled by the balance of hydrophobic interaction and electrostatic repulsion for the denaturation and aggregation of protein by heat treatments (Kitabatake et al., 1987, 1988; Matsudomi et al., 1991). The transparent solutions obtained in this study might be due to the soluble linear aggregates of OVA resulting from hydrophobic interaction with the disulfide bond.

Thus, the results reported here led to the conclusion that the soluble linear aggregates of OVA formed during dry-heating for >2 h at 120 °C inhibited the formation of OT coagula formed by the sulfhydryl−disulfide interchange reaction and hydrophobic interaction among the monomers and/or aggregates of OT in the pasterization process (in both DHEW and its mixture with FEW). Further studies are needed to better elucidate the mechanisms responsible for the inhibition of coagulation of FEW proteins in heat treatments when DHEW was mixed and to promote the secondary application of heated egg white as a food-processing material.

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