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Improvement of Gel Properties of Dried Egg White by Modification with Galactomannan through the Maillard Reaction

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The effects of Maillard reaction on gel properties of dried egg white (DEW) with galactomannan (GM) were investigated. Maillard-reacted DEW (MDEW) was prepared by dry-heating a mixture with a weight ratio of 1:4 of GM to DEW at 60 °C and 65% relative humidity. The modification of amino groups and polymerization of DEW proteins dry-heated with GM proceeded with increasing the dry-heating time. The covalent attachment of GM to DEW was confirmed from SDS−PAGE analysis. Gel strength and water-holding capacity of MDEW gels were higher than those of DEW dry-heated without GM (control DEW) and reached maximum after 3 days of dry-heating. The appearance of MDEW gels became transparent with increasing the dry-heating time, but control DEW gels were still turbid. MDEW dry-heated for 3 days was almost soluble even after heating of its solution at 90 °C, whereas control DEW proteins precipitated. The modification of DEW with GM through the Maillard reaction was an effective method to make a firm and transparent gel from DEW at broader range of pH and NaCl concentration of the medium.

KEYWORDS: Dried egg white; galactomannan; gel properties; transparent gel; Maillard reaction; protein stabilization

INTRODUCTION

Egg white (EW) is extensively utilized as a functional food material in food processing. Heat-induced gelation is one of its important functional properties with respect to EW usage in food systems (*1*). Gel formation is strongly affected by various factors, including pH, ionic strength, and salts (*2*−*4*). EW normally forms a turbid gel on heating. Therefore, the nature of the EW gel may limit greater utilization of the protein as gelling ingredients. Preparation of a transparent gel from EW would be favorable to further applications of egg white protein as a functional food material.

Glycosylation of proteins with reducing sugar through Maillard reaction is an effective way to improve the functional properties (*5*−*7*). Preparation of protein−polysaccharide complexes with excellent emulsifying properties has been accomplished by the attachment of polysaccharides to proteins through the Maillard reaction (*8*−*11*). Handa and Kuroda (*12*) reported recently that Maillard products of DEW containing glucose enhanced the gel properties, such as gel strength and water-holding capacity. Accordingly, gel properties of DEW may be altered by modification with a polysaccharide through a Maillard reaction. The mannnase hydrolysate of guar gum (galactomannan; GM) has been developed as a soluble dietary fiber, and it has a thickening, but no direct gelling, property (*13*). Thus, GM was applied as a polysaccharide to modify DEW. We found that a transparent and firm gel can be made from DEW modified with GM through the Maillard reaction in controlled dry-heating conditions. In this paper, we report the changes of DEW that occurred in the presence of GM through the Maillard reaction and the characteristics of heat-induced gels from the Maillard-reacted DEW (MDEW). The possible mechanism by which MDEW forms transparent and firm gel is discussed.

MATERIALS AND METHODS

Modification of DEW with GM. Dried egg white (DEW), spray-dried at an exhaust temperature of 60−70 °C after decarbohydrate treatment by a glucose oxidase−catalase enzyme system, was provided by Q. P. Corp. (Tokyo, Japan). A galactomannan (GM) preparation (MW 15 000−20 000) from the mannase hydrolysate of guar gum was supplied by Taiyo Chemicals Co. (Yokkaichi, Japan). Oligomeric saccharides contained in the GM preparation were removed by dialysis in a dialysis tube (Spectra/Por 1, molecular cutoff 6−8 kDa, Spectrum Laboratory) for 24 h against distilled water and then freeze-dried. DEW was dissolved in distilled water at a concentration of 4% (w/v) with various concentrations of GM. The GM−DEW solution was adjusted to pH 7.0 with 1 N NaOH and then freeze-dried. The dried GM−DEW mixture was placed in a Petri dish, covered with perforated aluminum foil, and then dry-heated at 60 °C in a sealed glass desiccator maintained at a relative humidity (RH) of 65% (*14*) using a saturated aqueous KI solution throughout the dry-heating periods. The GM−DEW mixture was dry-heated for 0, 1, 2, 3, 5, or 7 days in a separate desiccator to prevent the influence of repeated opening and closing of the sealed desiccator. After treatment in the dry-heating system, the obtained MDEW products were stored in a brown bottle with a self-sealing cap at −20 °C until use, and the products were used in the

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following experiments without separation of unreacted DEW and unbinding GM. For the control samples, the same treatment was also applied to DEW without GM.

Measurement of Free Amino Group Contents and pH. Control and MDEW samples were dissolved in 0.1 M sodium borate buffer (pH 9.2) to give 0.5% (w/v) DEW concentration, and contents of the free amino groups in those DEW samples were measured by the trinitrobenzenesulfonate (TNBS) method (*15*). The pH of DEW samples was measured after solution in distilled water to give 10% (w/v) DEW concentration.

Measurement of Gel Properties. Control and MDEW samples were dissolved in 20 mM NaCl solution to give 10% (w/v) DEW concentration and adjusted to pH 6.5 with 0.1 M HCl or NaOH. The sample solutions were put into glass tubes (6.0 mm in diameter) previously treated with Sigmacote (Sigma Chemical Co.). The contents of each tube were deaerated by placing the tube in a Sharp sonicator (model UT-205, Tokyo) under vacuum for 1 min. The tubes were heat-sealed and heated for 30 min in a water bath at 90 °C for gelling. After the tubes were heated, they were removed from the water bath and held overnight at 4 °C before testing. After tempering at room temperature, the protein gel was taken out of each tube without disrupting the gel surface. Each gel was cut into uniformly flat 5.0-mm thick sections and compressed to 40% of its original height by a tensile tester (Tensilon UTM-II, Toyo Baldwin Co., Tokyo) as previously described (*16*). The force required to compress the gel to 40% was expressed as gel strength. Water-holding capacity (WHC) of the gel was measured by centrifugation as reported previously (*17*). After the gel was centrifuged at 2000*g* for 15 min at 20 °C using a 2-mL centrifugal tube with a 0.45-μm filter (Advantec, Tokyo), the amount of water dropped from the gel was then measured. WHC of the gel was given by [1 − (weight of water separated)/(weight of initial gel)] × 100.

Gel Electrophoresis. SDS−polyacrylamide gel electrophoresis (SDS−PAGE) was performed by using a thin slab gel electrophoresis apparatus (Advantec EP-080, Tokyo) with a slab gel made from 5% stacking gel and 12.5% separating gel, according to the method of Laemmli (*18*). Samples (20 μL containing 0.2% DEW) were prepared in Tris−glycine buffer (pH 8.3) containing 1% SDS with 1% 2-mercaptoethanol (2-ME). Electrophoresis was performed at constant current of 10 mA for 3 h in 50 mM Tris−glycine buffer containing 0.1% SDS. After electrophoresis, gel sheets were stained with Coomassie brilliant blue (CBB) for protein analysis and with periodic acid−Schiff (PAS) reagent for carbohydrate determination (*19*).

Heat Stability. Control and MDEW samples were dissolved in 50 mM sodium phosphate buffer (pH 7.4) to give 1.0% (w/v) DEW concentration and then heated at 60−90 °C for 10 min. The heated sample was diluted 5× with 0.2 M McIlvaine buffer (pH 4.2) to facilitate precipitation of heat-induced coagulum, and then the coagulum was removed by centrifugation at 4000*g* for 20 min at 4 °C. The absorbance of the supernatant fluid at 280 nm was measured to estimate protein contents of the solution. The heat stability was expressed as the percent of soluble protein in the total protein. The heat stability described in this study does not mean the conformational stability of protein, but refers to the solubility after heat treatment because the soluble protein remaining in the heated solution may be denatured.

Experimental Design and Statistical Analysis. DEW powders used in this study were commercial products from two different lots provided by Q. P. Corp. (Tokyo, Japan) in January 2001. Those powders had been stored at 4 °C in a sealed container prior to use. No differences were observed on the electrophoretic patterns and protein band intensities of those DEW powders, and gels from those samples presented very similar gel characteristics at pH 6.5 and protein concentration of 10% (w/v) (data not shown). All experiments were carried out with samples from two different lots. Each data value represents the means of at least four determinations, and the error bars indicate standard deviation.

RESULTS AND DISCUSSION

DEW freeze-dried with GM at a mixing ratio of 1:8, 1:4, 1:2, 1:1, and 0.5:1 (as weight ratio of GM to the protein) was dry-heated for 3 days at 60 °C and 65% RH to study the effect of the mixing ratio on the gel properties of DEW proteins after the Maillard reaction. The gel strength and visual appearance of heat-induced gels from various MDEW samples are shown in **Figure 1**. The gel from DEW dry-heated without GM for 3 days (control DEW) gave higher gel strength than that of intact DEW (0 day of dry-heating without GM), suggesting the effectiveness of dry-heating treatment of egg white proteins for the strengthening of its heat-induced gels as reported previously (*20*−*22*). MDEW with various mixing ratios formed clear gels compared with opaque gels from the control and intact DEW samples. However, the gel from MDEW with the mixing ratio of 1:0.5 was translucent and its gel strength was slightly low as compared to that of the control gel. On the other hand, the MDEW with the mixing ratio of 1:4 produced a firmer and more transparent gel compared to that with the other mixing ratios, and thus the GM−DEW mixture with the 1:4 mixing ratio of GM to protein was used for the following experiments in which the effects of dry-heating time on the protein and the gel properties were investigated.

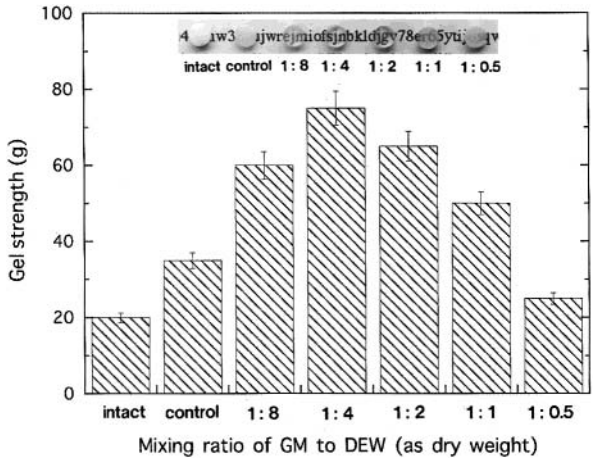

Figure 1. Strength and appearance of gels from DEW dry-heated with different levels of GM at 60 °C and 65% RH for 3 days. The intact and control samples correspond to DEW dry-heated without GM for 0 days and 3 days, respectively. Gels were produced by heating 10% (w/v) DEW solutions (pH 6.5) at 90 °C for 30 min.

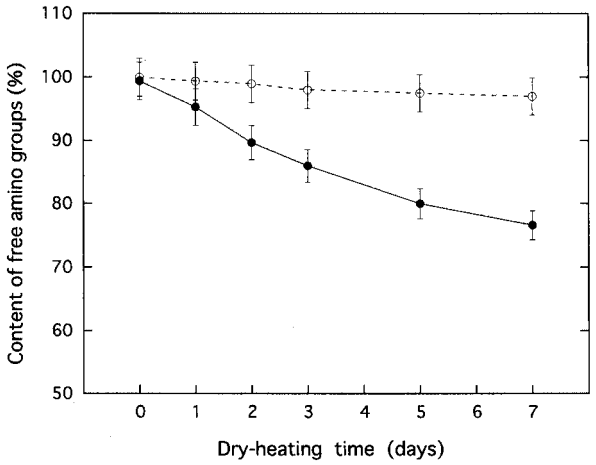

Figure 2. Changes in free amino group contents of DEW alone (○) and GM−DEW mixture (●) dry-heated at 60 °C and 65% RH for various periods of time. The mixing ratio of GM to DEW in the mixture was 1:4.

First of all, contents of free amino groups in DEW alone and the GM−DEW mixture (1:4, w/w) were measured throughout 1−7 days of dry-heating time. As shown in **Figure 2**, contents of free amino groups in the mixture decreased gradually with

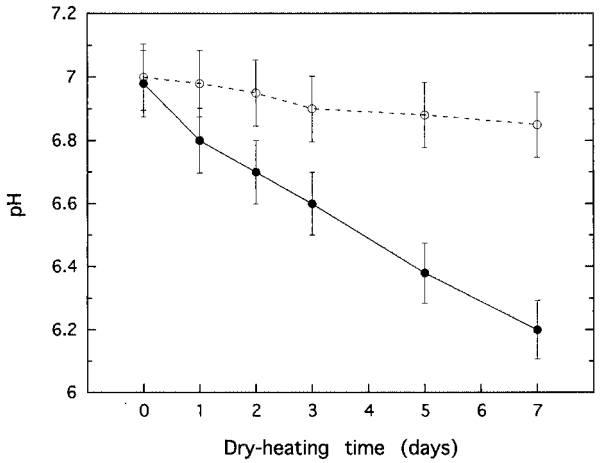

Figure 3. Changes in pH of DEW alone (○) and GM–DEW mixture (●) dry-heated at 60 °C and 65% RH for various periods of time. The mixing ratio of GM to DEW in the mixture was 1:4. The pH was measured at 10% (w/v) DEW solution in distilled water.

increasing dry-heating time, while there were no significant changes in its contents in the sample of DEW alone even after 7 days. Any browning and loss of protein solubility of DEW alone and the mixture were not observed throughout the dry-heating for 7 days (data not shown). The abolishment of browning in MDEW samples may be due to the lower reactivity of GM induced from steric hindrances of polysaccharide in comparison with monomeric sugars, because brown color development was not observed as in a protein−glucose system (*23*−*25*).

The changes in pH of DEW alone and the GM−DEW mixture (1:4, w/w) were examined as the function of dry-heating time, as shown in **Figure 3**. The DEW alone and the mixture prior to freeze-drying were adjusted to pH 7.0 in the present study to slow a Maillard reaction, which progresses more rapidly at alkaline pH (*26*). The pH values of DEW alone lowered slightly to near pH 6.8 within 7 days, while those of the mixture decreased gradually with increasing dry-heating time and reached pH 6.2 after 7 days. It has been reported that a Maillard reaction can also cause a pH reduction due to the production of acidic side products (*27*). The decreased pH of the mixture may be possibly induced through the Maillard reaction, as also suggested from a decrease of available lysine contents in the mixture as shown in **Figure 2**. Zhang et al. (*28*) and some other investigators (*29*, *30*) have documented that the thermal deamidation of some proteins, including egg white proteins, occurred in low-water environments (such as dry-heating) and that the deamidation led to a pH reduction due to an increase in negative charge of the proteins. In the case of DEW alone, therefore, protein deamidation may be mainly responsible for the lowering of pH. The significant decreases of pH and free amino group contents in GM−DEW mixture during dry-heating at 60 °C and 65% RH suggested that a Maillard reaction would proceed in the mixture.

SDS−PAGE patterns of DEW alone and the GM−DEW mixture (1:4, w/w) under reducing condition were compared for different dry-heating times (**Figure 4**). The intact DEW (0 day of dry-heating without GM, Lane 0 in **Figure 4A**) mainly consisted of ovalbumin (OA), ovotransferrin (OT), lysozyme (LZ), and ovomucoid (OM), and some aggregates based on comparison with standard proteins. The protein profiles of DEW alone remained unchanged throughout dry-heating for 7 days (**Figure 4A**). In SDS−PAGE patterns of the mixture (Figure 4C), the OT band rapidly disappeared with the dry-heating time,

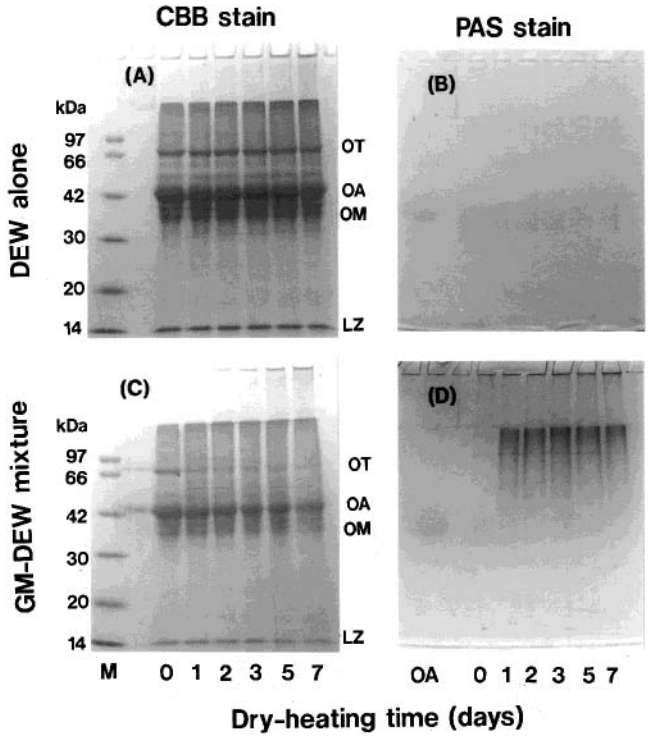

Figure 4. SDS–PAGE patterns under reducing condition of DEW alone (A, B) and GM–DEW mixture (C, D) dry-heated at 60 °C and 65% RH for various periods of time. The mixing ratio of GM to DEW in the mixture was 1:4. A and C show CBB stain for protein, and B and D are PAS stain for carbohydrate. Lane M and lane OA indicate marker proteins (phosphorylase, 97 kDa; bovine serum albumin, 66 kDa; aldolase, 42 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20 kDa; lysozyme, 14 kDa) and ovalbumin, respectively. OT, OA, OM, and LZ are ovotrnsferrin, ovalbumin, ovomucoid, and lysozyme, respectively.

and subsequently the OA and LZ bands decreased gradually, and proportions of aggregates that could migrate slowly into the SDS-gel increased during the dry-heating. Those aggregates corresponded to the broader band that was strongly detected with a PAS stain for carbohydrate (**Figure 4D**), while OA and OM, main glycoproteins in EW protein, were also observed as a faint band by the stain (**Figure 4B** and **D**). The result strongly indicated that the covalent attachment of GM to the constituent proteins of DEW was performed through the Maillard reaction under the conditions of 60 °C and 65% RH. The reactivity of GM to each protein in DEW may be dependent on the conformational stability of protein to heat, because denaturation temperatures of OT, globulins, OA, and LZ at pH 7 were 57.3, 72.0, 76.5, and 81.5 °C, respectively, as reported previously (*31*).

The gel properties of DEW alone and the GM−DEW mixture (1:4, w/w) dry-heated for various periods of time were compared by measuring the gel strength, visual appearance, and WHC. In addition, the gel properties of the mixture (1:4 of GM to DEW, w/w) without the Maillard reaction were also examined to evaluate the effect of added GM to the dry-heated DEW proteins after heating for gelling. The gel from intact DEW (corresponded to 0 day of dry-heating without GM) was turbid, had a soft texture, and exhibited considerable syneresis. As shown in **Figure 5**, the strength of gel from GM−DEW mixture with the Maillard reaction increased greatly from day 1 of the dry-heating and thereafter increased gradually, and then slowed after 5 days, and appearance of the gel changed from opaque to transparent as the dry-heating time was increased. On gel

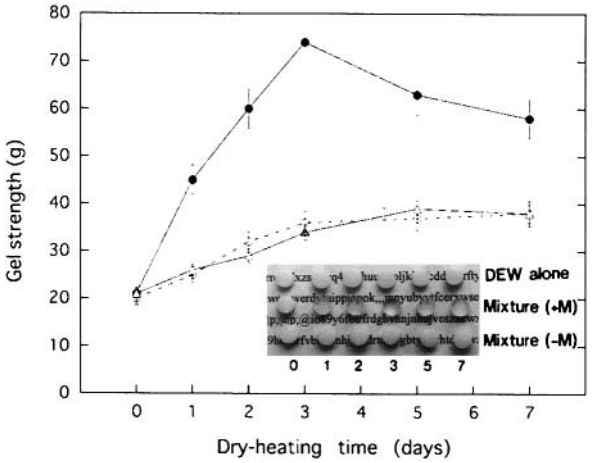

Figure 5. Strength and appearance of gels from DEW alone (○) and GM–DEW mixture (●) dry-heated at 60 °C and 65% RH for various periods of time, in addition to the mixture (△) of DEW with added GM without Maillard reaction. The mixing ratio of GM to DEW in those mixtures was 1:4. Gels were produced by heating 10% (w/v) DEW solutions (pH 6.5) at 90 °C for 30 min. Mixture (+M) and (–M) mean the mixture of GM and DEW with or without Maillard reaction, respectively.

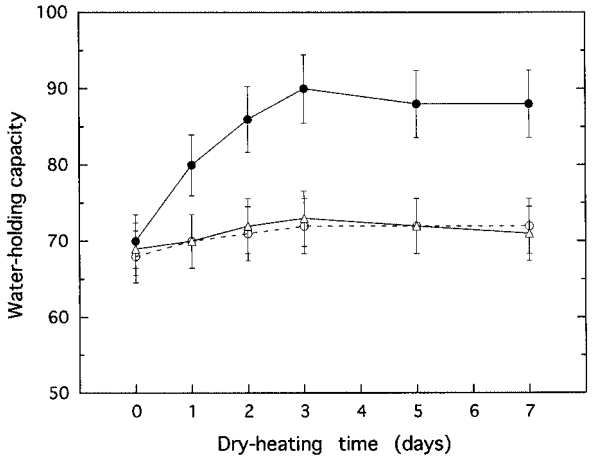

Figure 6. Water-holding capacity of gels from DEW alone (○) and GM–DEW mixture (●) dry-heated at 60 °C and 65% RH for various periods of time, in addition to the mixture (△) of DEW with added GM without Maillard reaction. The mixing ratio of GM to DEW in those mixtures was 1:4. Gels were produced by heating 10% (w/v) DEW solutions (pH 6.5) at 90 °C for 30 min.

from DEW alone, the gel strength increased slightly with increasing the dry-heating time, whereas its appearance remained turbid throughout the dry-heating for 7 days. The strength and appearance of gel made from the GM−DEW mixture without the Maillard reaction presented gel characteristics very similar to those of DEW alone. This result indicates that free GM added did not affect the denaturation and aggregation of DEW proteins on heating for gelling, and that the attachment of GM to DEW was important to alter the gel properties of the protein.

The WHC of heat-induced gels is shown in **Figure 6**. The WHC of gel from DEW alone did not show significant changes when the dry-heating time was increased. On gel made from DEW alone, and that from the mixture of DEW with added GM without the Maillard reaction, no significant differences in WHC of those gels were observed. On the other hand, WHC of gel from GM−DEW mixture with the Maillard reaction increased with increasing the dry-heating time, leveling off at 5 days, and its values increased greatly from 70% for 0 day to

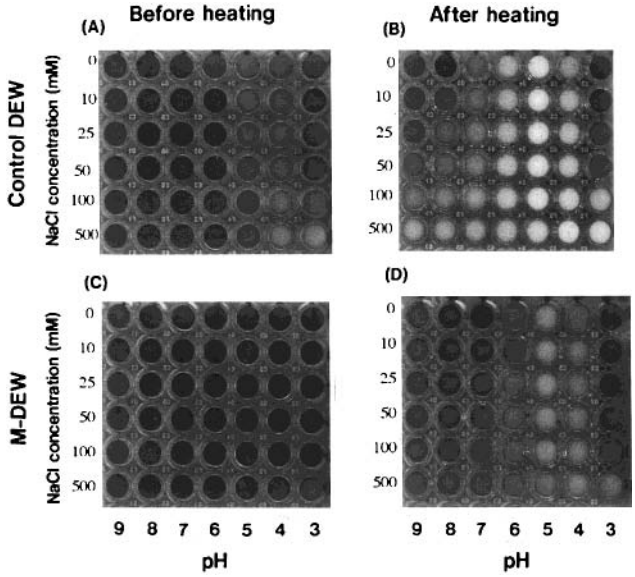

Figure 7. Effect of pH and NaCl concentration on the appearance of gels from DEW alone (A, B; control DEW) and GM–DEW mixture (C, D; MDEW) dry-heated at 60 °C and 65% RH for 3 days. The mixing ratio of GM to DEW in the mixture was 1:4. Each well contains 250 μL of 10% (w/v) DEW solutions, and the photographs show the DEW samples before (A, C) and after (B, D) heating for 30 min at 90 °C for gelling.

near 90% after 3 days. The higher WHC of MDEW gels might likewise be due to the construction of a fine gel network (*32*, *33*). It was found that such improvements (transparency, gel strength, and WHC) of DEW gel at pH 6.5 could be achieved by attachment of GM to DEW through the Maillard reaction. It was assumed that the gel properties of MDEW probably are due to the high hydration capacity of the attached polysaccharide chains.

The properties of heat-induced gels from EW are also sensitively affected by various factors, including pH and ionic strength (*2*, *4*, *34*). The effects of pH and NaCl concentration on the appearance of heat-induced gels from DEW alone and GM−DEW mixture dry-heated for 3 days, namely control DEW and MDEW, respectively, were examined (**Figure 7**). Protein solutions (10%, w/v) were adjusted to pHs 3−9 and NaCl concentrations from 0 to 500 mM, and then heated at 90 °C for 30 min in the wells of a 96-well microplate as reported previously (*35*). Before heating for gelling, control DEW solutions were clear, except most samples of pHs 4 and 5 were slightly turbid at all of the NaCl concentrations (0−500 mM), and samples at pH 3 and above 100 mM NaCl were turbid. After heating for gelling, control DEW samples at pHs 4−6 and all NaCl concentrations, and those at pHs 3 to 9 and above 100 mM NaCl produced turbid gels. The control DEW formed transparent gel only when pH was away from the isoelectric point (pI) of OA (pI 4.5), a major protein in EW protein (*36*), and at lower NaCl concentrations. On the other hand, MDEW gave transparent solutions in most cases before heating for gelling. This indicates that the attachment of GM to DEW gave high protein solubility even at high NaCl concentrations and around pH 4.5. After heating for gelling, most MDEW samples at pHs 4 and 5 formed turbid gel, whereas with pH 3 and above pH 7, the MDEW samples gave transparent (or slightly translucent) gel when NaCl concentration was below 100 mM. Consequently, MDEW formed transparent gels over a wide range of pHs and NaCl concentrations after heating for gelling, as compared with control DEW. Thus, the modification of DEW with GM through the Maillard reaction was an effective method

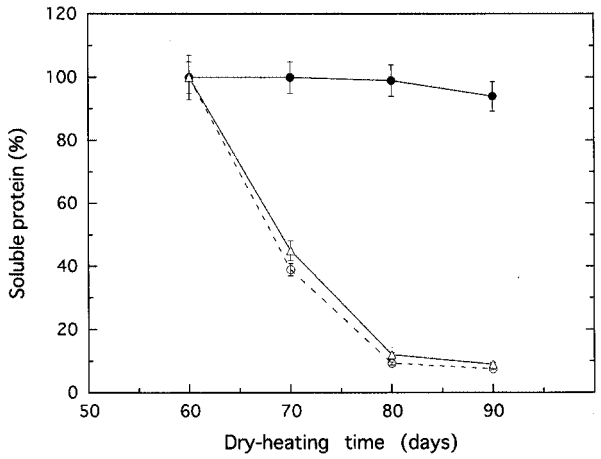

Figure 8. Heat stability of DEW alone (○) and GM–DEW mixture (●) dry-heated for 3 days at 60 °C and 65% RH, in addition to the mixture (△) of DEW with added GM without Maillard reaction. The mixing ratio of GM to DEW in those mixtures was 1:4. The protein solution was prepared in 50 mM phosphate buffer (pH 7.4) at 1% (w/v) protein concentration and then heated for 10 min at different temperatures.

to prepare transparent gel from DEW in the wide range of pHs and NaCl concentrations tested.

It has been reported that the heat stability of protein is enhanced by the conjugation with polysaccharide (*37*, *38*). The effect of heat treatment on protein solubility of DEW alone and GM−DEW mixture dry-heated for 3 days, namely control DEW and MDEW, respectively, is shown in **Figure 8**. In addition, heat stability of the mixture of DEW with added GM without the Maillard reaction was also examined in order to evaluate the effect of the added polysaccharide. The MDEW was almost soluble even after heating of its protein solution at 90 °C for 10 min, whereas more than 90% of proteins in control DEW and its mixture with added GM without the Maillard reaction precipitated at temperature higher than 80 °C. The solubility of intact DEW after heat treatment was almost the same as that of the control DEW (data not shown). This result means that the free GM added did not act to stabilize DEW proteins to heat denaturation. The increase in heat stability of MDEW proteins probably may be due to the protection against precipitation of the denatured proteins. The high stability of MDEW to heat treatment would be favorable to pasteurization for food application. It is possible that such properties of MDEW on heating would effectively act on the formation of a firmer and transparent gel by altering a type of protein network structure required for gelling. Further studies are needed to determine possible causative factors responsible for the altered gel characteristics of MDEW, together with an understanding of the heat-induced interactions among the constituent proteins of EW.

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