Correlation of the Protein Structure and Gelling Properties in Dried Egg White Products

Akihiro Handa,* Kenji Hayashi, Hiroyuki Shidara, and Namio Kuroda

Research Institute, Q.P. Corporation, 5-13-1 Sumiyoshi-Cho, Fuchu-Shi, 183-0034 Tokyo, Japan

The relationship between protein structure and aggregation, as well as heat-induced gelling properties, of seven dried egg white (DEW) products was investigated. Strong correlations were found between average molecular weight and hydrophobicity plus surface SH groups of DEW-soluble protein aggregate (SPA). This suggests that hydrophobic interactions and disulfide bond formation between protein molecules were involved in the aggregation. The average molecular weight of DEW products with alkaline pHs was relatively higher than those with neutral pHs and the same degree of protein unfolding, probably because of more disulfide bond formation between protein molecules. In addition, strong correlations were found between hydrophobicity, surface SH groups plus average molecular weight of DEW−SPA, and physical properties of the gels from DEW products. These data indicated that controlling the aggregation of DEW proteins in the dry state is crucial to controlling the gelling properties of DEW.

Keywords: *Egg white; gelation; protein gels; water holding capacity; protein aggregation*

INTRODUCTION

Egg white (EW) is an important ingredient in food processing because of its variety of functional properties such as gel formation, water-holding property, foaming capacity, and emulsifying ability (*1*). Above all, heat-induced gelation and water-holding properties are widely applied in surimi, meat, and noodle products (*2*−*4*).

Commercial dried egg white (DEW) is produced by spray-drying or pan-drying EW after desugarization to prevent browning and loss of solubility by the Maillard reaction during pasteurization and storage (*5*). Dry-heating (storage in a hot room under controlled temperature and relative humidity conditions) is one of the most promising approaches for improving the gelling properties of DEW (*6*). The partially unfolded conformation formed by dry-heating may contribute to the improved gelling properties of DEW (*7*). Commercial DEW products, typically with 4−8% moisture content, are generally dry-heated at 60−80 °C for 3−30 days for pasteurization or improving the gelling properties.

The development of laser light scattering techniques has enabled advances in polymer science for characterizing the aggregation behavior and network structure in polymer gels. Laser scattering is an ideal means for determining the actual molecular weight and whether any aggregates have formed (*8*). Kato et al. (*9*) determined the apparent molecular weights of soluble ovalbumin aggregates (formed by heating 0.1% ovalbumin solution) under various conditions using the low-angle laser light scattering technique in combination with high-performance liquid chromatography (HPLC). Mine (*10*) examined the heat-induced aggregation behavior of ovalbmin and succinylated ovalbumin using a multiangle laser scatter coupled with size-exclusive HPLC, and concluded that an elastic gel consists of a more expanded protein polymer network when compared with that of a less elastic gel.

Although there are many kinds of DEW products in the world, with different gelling properties, few studies have been conducted to compare the protein structure and the gelling properties of DEW products or to elucidate the gelation mechanism of EW proteins. The objective of this study was to investigate the relationship between protein structure and aggregation, as well as gelling properties, of DEW products.

MATERIALS AND METHODS

Sample. Seven DEW products manufactured by Q. P. Corp. (Tokyo, Japan) with different gelling properties were used for this study (sample 1, Type W; sample 2, Type K; sample 3, WDP-1; sample 4, Type K no. 5; sample 5, Type K no. 10; sample 6, Type M no. 4; sample 7, Type M). The moisture content on a wet basis was determined by drying samples to a constant weight at 110 °C (*11*). A sample solution with 12.5% 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 Company, Ltd., Osaka, Japan) for preparation of heat-induced gel. The pH values of the DEW products were defined by measuring the pH values of the sample solutions. The DEW protein solutions (approximately 0.1% protein in 50 mM phosphate buffer, pH 7.0, for average molecular weight and hydrophobicity measurements, or in 86 mM Tris−glycine buffer, pH 8.0, for SH group measurements) were centrifuged (10000*g*, 30 min) then passed through a Millex-GP filter (0.45-μm, Millipore Corporation, Bedford, MA) to remove the small amount of insoluble proteins. Protein concentration was determined according to the method of Lowry et al. (*12*). Ovalbumin (grade V, minimum 98%, Sigma Chemical Co., St. Louis, MO) was used as the standard.

Average Molecular Weight of DEW−SPA. Average molecular weight of DEW−SPA (soluble protein aggregate) was measured by the method of Mine (*10*). The DEW protein solutions were applied to a multiangle laser light scattering (MALLS) experiment on a DAWN DSP-F laser photometer (Wyatt Technology, Santa Barbara, CA) using a 632.8 nm laser

* To whom correspondence should be addressed. Phone: 81-42-361-0025. Fax: 81-42-361-6271. E-mail: akihiro_handa@kewpie.co.jp.

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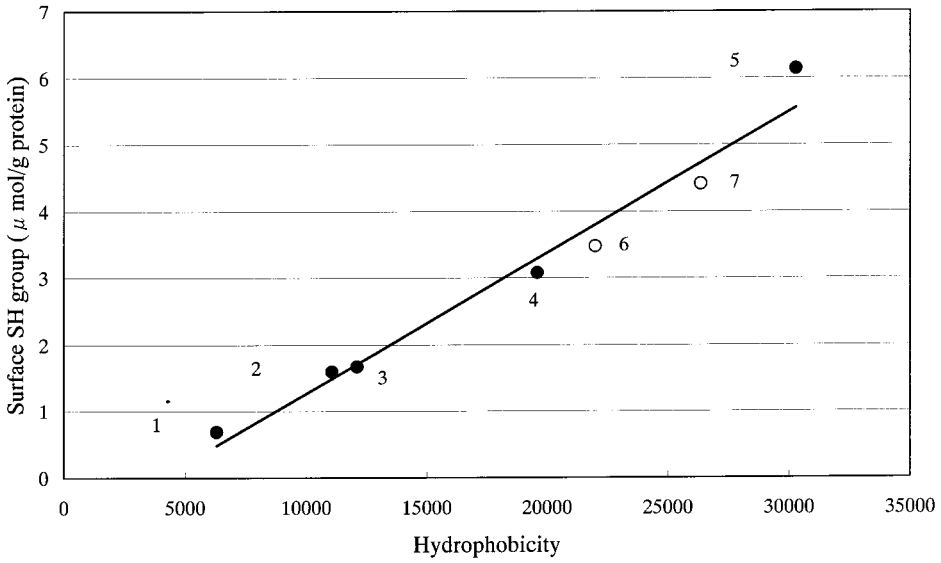

Figure 1. Hydrophobicity vs surface SH group contents of egg white proteins: dried egg white products with (●) neutral pHs ($r = 0.992$ for 5 samples) and (○) alkaline pHs ($r = 0.985$ for 7 samples).

wavelength. The MALLS system was equipped with an HPLC system consisting of Shodex KW-804 and KW-803 columns (0.75 × 30 cm, Showa Denko K. K., Tokyo, Japan). A specific refraction index increment (d*n*/d*c*) of 0.186 was obtained using a Wyatt OPTILAB DSP interferometric differential refractometer. The Zimm equation was used to determine molecular weight.

Hydrophobicity. Hydrophobicity of DEW−SPA was measured using 1-anilino-8-naphthalene-sulfate (ANS) by the method of Hayakawa and Nakai (*13*) with slight modifications. A 10-μL portion of LANS solution (8 mM in 50 mM phosphate buffer, pH 7.0) was added to 2 mL of DEW protein solutions (0.02−0.1%). Fluorescence intensity (FI) was measured with a fluorescence spectrophotometer (U-2000, HITACHI, Tokyo, Japan) at excitation wavelengths of 390 nm and emission at 470 nm. The initial slope of the FI versus protein concentration (%) plot was used as an index of the protein hydrophobicity.

Surface and Total SH Groups. The concentration of SH groups of DEW−SPA was determined using Ellman's reagent (*14*) in the absence (for surface SH groups) and in the presence (for total SH groups) of 0.25% SDS.

Preparation of Heat-Induced Gel. Gels (diameter, 36 mm and height, 30 mm) were prepared by heating DEW solutions with 12.5% solid content at 80 °C for 40 min in cylindrical casings using a water bath (LT-480, ADVANTEC, Tokyo, Japan). The pH of the DEW solutions was adjusted to 7.0 with 2 N HCl or 2 N NaOH because heat-induced gelling properties of EW are greatly affected by pH (*15*). After heating, the gels were immediately cooled in ice water and equilibrated to ambient temperature (24 ± 1 °C).

Properties of the Gels. Breaking strength and breaking strain of the gels were measured at ambient temperature (24 ± 1 °C) using a rheometer (NRM-2010J-CW, Fudo, Tokyo, Japan) equipped with a spherical plunger (diameter, 8 mm) at a crosshead speed of 60 mm/min. Breaking strength and breaking strain values were the means of measurements on four sampling units. Water-holding capacity (WHC) of the gels was calculated from $(W_0 - W_1) \times 100/W_0$, where W_0 was the initial gel weight and W_1 the weight of the gel after being left on five layers of filter paper (No. 2, diameter, 110 mm; ADVANTEC TOYO, Tokyo, Japan) at ambient temperature (24 ± 1 °C) for 60 min. WHC values were the means of measurements on two sampling units.

Statistical Analysis. All experiments were replicated three times. Mean values of three replicates were plotted in all figures. Correlation coefficients were calculated using data of each replicate.

Table 1. Moisture Content and pH of Seven Dried Egg White Products

	sample no.						
	1	2	3	5	5	6	7
moisture content (%)	6.34	7.18	7.56	6.81	7.09	7.90	7.43
pH	6.35	7.01	7.29	6.54	7.49	8.78	10.01

RESULTS AND DISCUSSION

Protein Unfolding. There was a strong correlation between hydrophobicity and surface SH groups of DEW products (Figure 1), indicating that both could reflect the degree of protein unfolding. Several hydrophobic areas exist in a polypeptide chain of ovalbumin, and most are buried in the interior of the molecule in the native state (*16*). EW protein has a substantial cystine content (2.17 g/100 g dry wt) (*17*). Ovalbumin is the only fraction that contains the free SH groups (*18*), most of which exist in the interior of the molecule in the native state as do the hydrophobic areas (*19*). Other EW protein fractions, such as ovotransferrin, ovomucoid, and lysozyme, contain S−S bridges (*18*). Kato et al. (*6*) reported that dry-heating DEW partially unfolds and increases the hydrophobicity of DEW proteins in proportion to heating temperature and time. In addition, circular dichroism and differential scanning calorimetry spectrum revealed that the increased degree of DEW protein denaturation found during dry-heating was accelerated in alkaline pH region (*20*). The differences in the degree of protein unfolding in DEW products used in this study are most likely attributed to the differences in the dry-heating temperature and time, pH, and moisture content of DEW products.

Properties of DEW. The pH and moisture contents of the DEW products on a wet basis are shown in Table 1. In the egg industry, the pH of DEW products is often adjusted by adding acids, such as citric acid and lactic acid, to liquid EW before drying. Average molecular weight of DEW−SPA ranged from 50700 to 187000 (Figure 2). The molecular weights of ovalbumin (which constitutes 54% of EW protein) and ovotransferrin (which constitutes 12% of EW protein) are 45000 and 76000, respectively (*21*). Considering these data, little protein aggregation occurred in DEW sample 1. On the

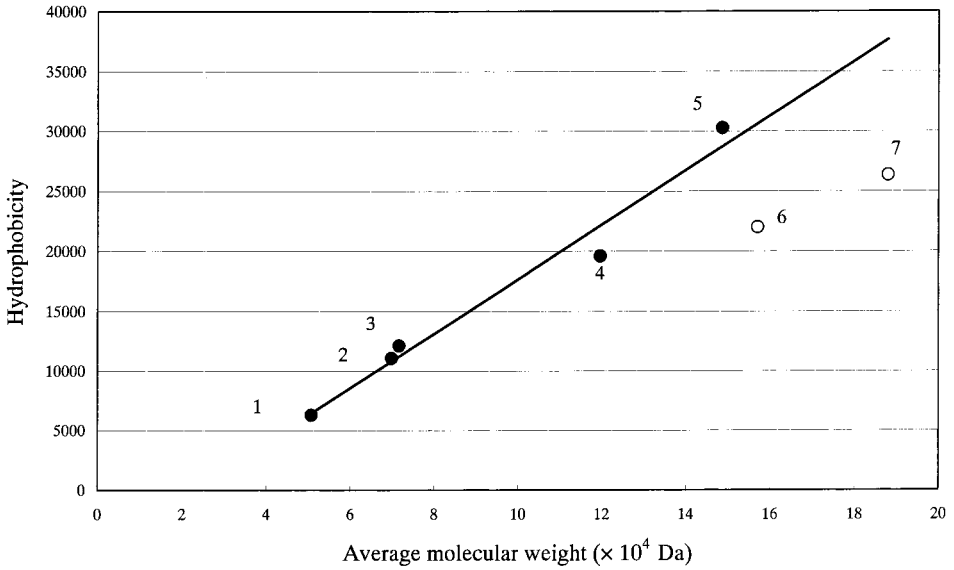

Figure 2. Average molecular weight vs hydrophobicity of egg white proteins: dried egg white products with (●) neutral pHs (r = 0.986 for 5 samples) and (○) alkaline pHs (r = 0.922 for 7 samples).

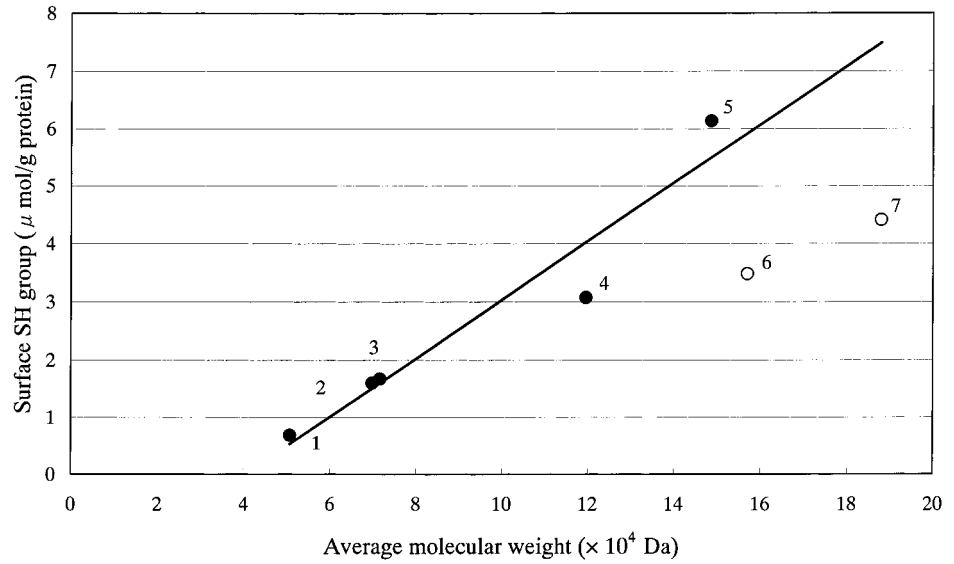

Figure 3. Average molecular weight vs surface SH group contents of egg white proteins: dried egg white products with (●) neutral pHs (r = 0.962 for 5 samples) and (○) alkaline pHs (r = 0.850 for 7 samples).

other hand, protein aggregation apparently occurred in DEW samples 2−7. Mine (*20*) reported that DEW proteins aggregated by dry-heating DEW at 75 °C for 3 days.

Relationship between Average Molecular Weight and Protein Structure. There were strong correlations between average molecular weight and hydrophobicity (Figure 2) plus surface SH groups (Figure 3) of DEW−SPA, indicating that the larger DEW−SPA consists of more unfolded proteins and has more reactive sites on its surface. Furthermore, there was a strong inverse correlation between average molecular weight and total SH groups (Figure 4) of DEW−SPA. These results suggest that unfolding of DEW proteins was essential to protein aggregation, and that the hydrophobic interaction and disulfide formation are deeply involved in the aggregation. In agreement, Kato et al. (*6*) reported that the electrophoretic patterns of DEW revealed the formation of hydrophobic and disulfide protein−protein interaction when DEW was dry-heated at 80 °C for 5 days.

The average molecular weight of DEW−SPA of DEW products with alkaline pHs (samples 6 and 7) was relatively higher than that of DEW products with neutral pHs and the same degree of protein unfolding (Figures 2 and 3). In other words, DEW−SPA of DEW products with neutral pHs were more unfolded than those with alkaline pHs (samples 6 and 7) and the same average molecular weight. In addition, more disulfide bonds were formed in DEW−SPA of DEW products with alkaline pHs (samples 6 and 7) than in those with neutral pHs and the same average molecular weight (Figure 4) and the same amount of surface SH groups (degree of protein unfolding) (Figure 5). Mine (*20*) reported that SH−SS interchange reactions in DEW proteins occurred more rapidly and, as a result, a higher molecular polymer of partially unfolded DEW proteins was formed in the alkaline pHs than in neutral pHs under the same dry-heating conditions.

Samples 2 and 3 had similar pHs and moisture contents (Table 1), and the average molecular weight, hydrophobicity, and surface SH groups of their DEW−

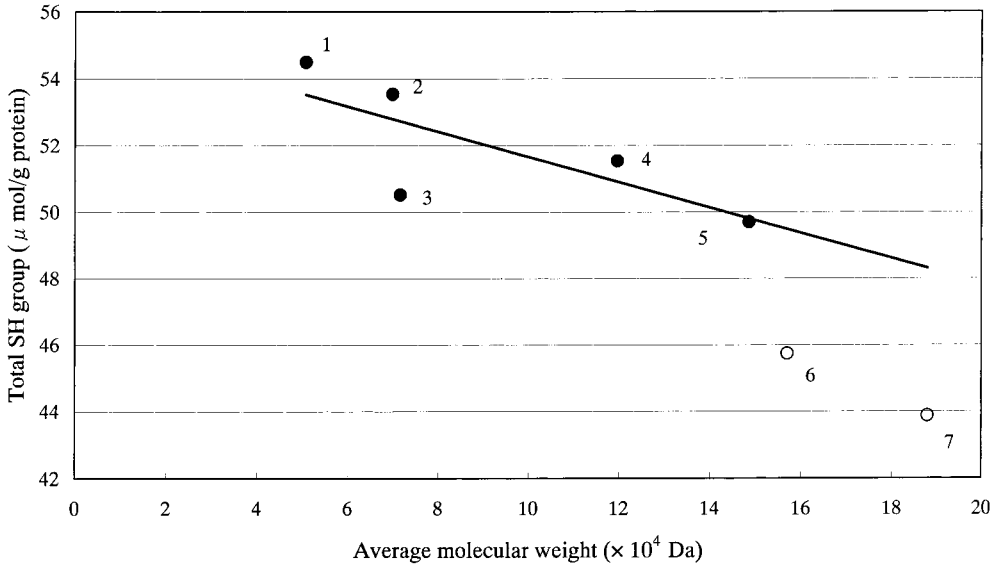

Figure 4. Average molecular weight vs total SH group contents of egg white proteins: dried egg white products with (●) neutral pHs (r = 0.903 for 5 samples) and (○) alkaline pHs (r = 0.764 for 7 samples).

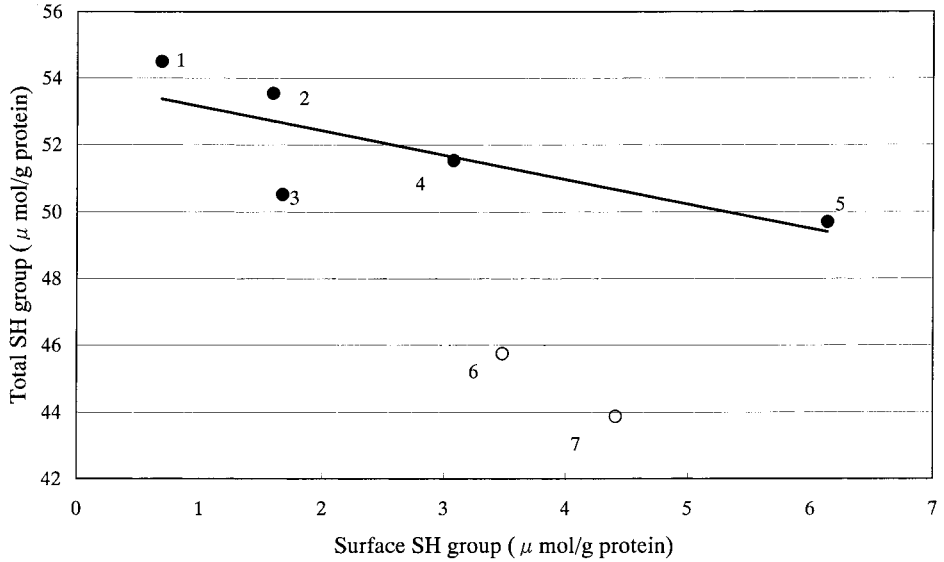

Figure 5. Surface SH group contents vs total SH group contents of egg white proteins: dried egg white products with (●) neutral pHs (r = 0.771 for 5 samples) and (○) alkaline pHs (r = 0.628 for 7 samples).

SPA were almost the same (Figures 2 and 3), whereas their total SH groups were quite different (Figures 4 and 5). Factors other than the pH of DEW products, such as dry-heating conditions and mineral composition of DEW products, may also affect the aggregation behavior of DEW proteins during dry-heating.

Relationship between Protein Structure and Gelling Properties. Strong correlations were found between average molecular weight of DEW−SPA and physical properties of the heat-induced gels from DEW products (Figures 6, 7, and 8), indicating that DEW−SPA plays a crucial role in the gelling properties of DEW products. Also, strong correlations were found between hydrophobicity (Figures 9, 10, and 11) plus surface SH groups (data not shown) of DEW−SPA and physical properties of the heat-induced gels from DEW products, and this relationship was found to be independent of pH. Interestingly, correlation coefficients between hydrophobicity (Figures 9−11) plus surface SH groups (data not shown) and physical properties of the gels for 7 samples were larger than those between average molecular weight of DEW−SPA and physical properties of the gels (Figures 6−8). This demonstrates that the amount of reactive sites on the surface of DEW−SPA had a closer relationship to physical properties of the gels than to the average molecular weight of DEW−SPA, and that the reactive sites contribute to the strong, elastic, and high water-binding protein network formation by heating of DEW solutions. In agreement, Margoshes (*22*) reported that surface SH groups of DEW correlated with the strength of heat-induced DEW gels. Heat-induced gelling properties of DEW were reportedly improved by dry-heating DEW (*6*), and the alkaline pHs of DEW accelerated the effect of dry-heating (*20*). However, the physical properties of the gels from DEW products with neutral pHs were relatively better than those with alkaline pHs and the same average molecular weight (Figures 6−8), probably due to more reactive sites on the DEW−SPA surface.

The breaking strain of sample 2 was larger than that of sample 3 (Figure 8), whereas WHC of sample 2 was smaller than that of sample 3 (Figure 9), although

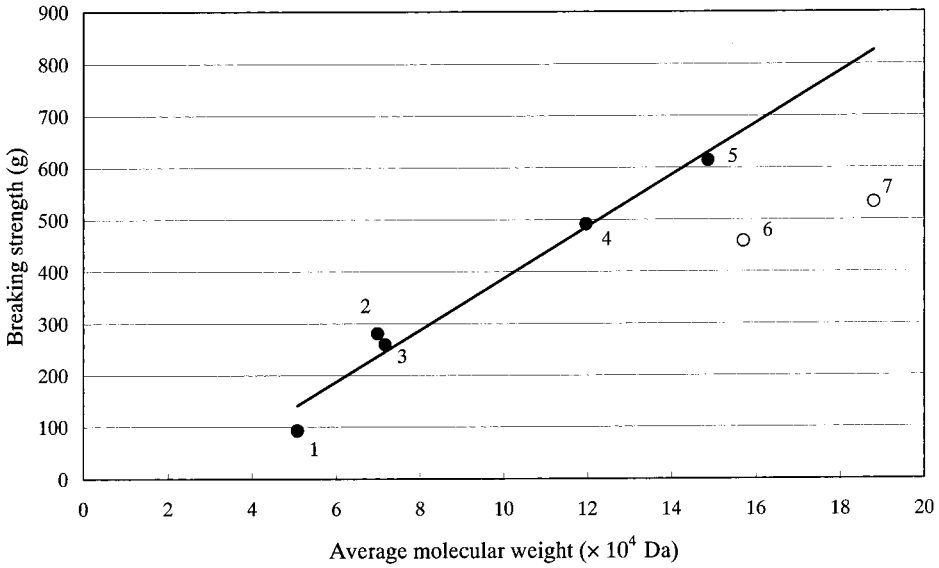

Figure 6. Average molecular weight of egg white proteins vs breaking strength of egg white gels: dried egg white products with (●) neutral pHs (r = 0.986 for 5 samples) and (○) alkaline pHs (r = 0.888 for 7 samples).

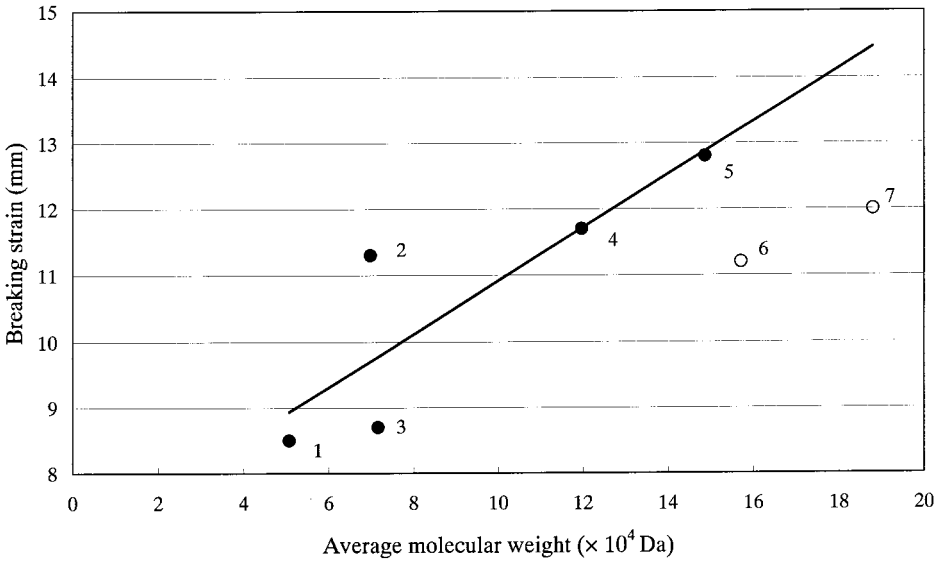

Figure 7. Average molecular weight of egg white proteins vs breaking strain of egg white gels: dried egg white products with (●) neutral pHs (r = 0.856 for 5 samples) and (○) alkaline pHs (r = 0.761 for 7 samples).

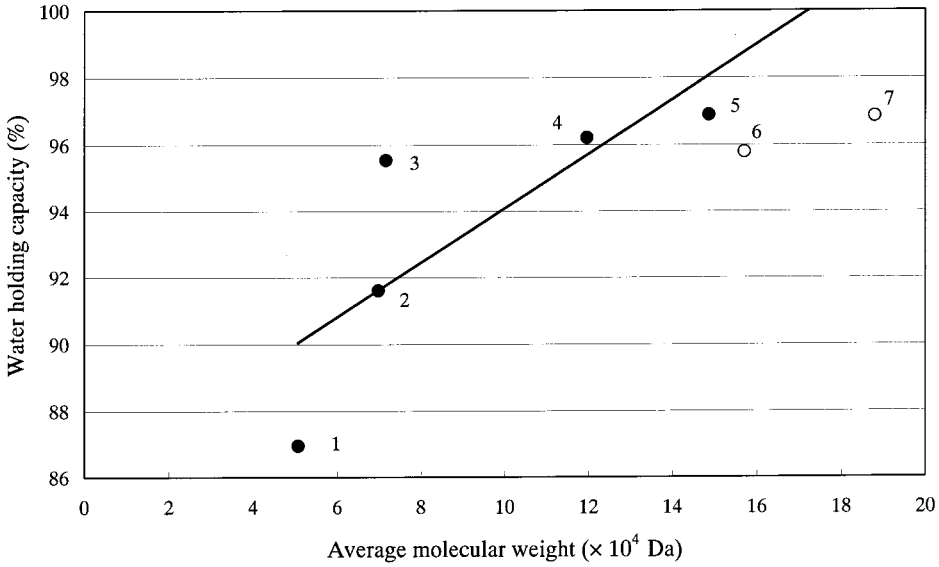

Figure 8. Average molecular weight of egg white proteins vs water holding capacity of egg white gels: dried egg white products with (●) neutral pHs (r = 0.796 for 5 samples) and (○) alkaline pHs (r = 0.766 for 7 samples).

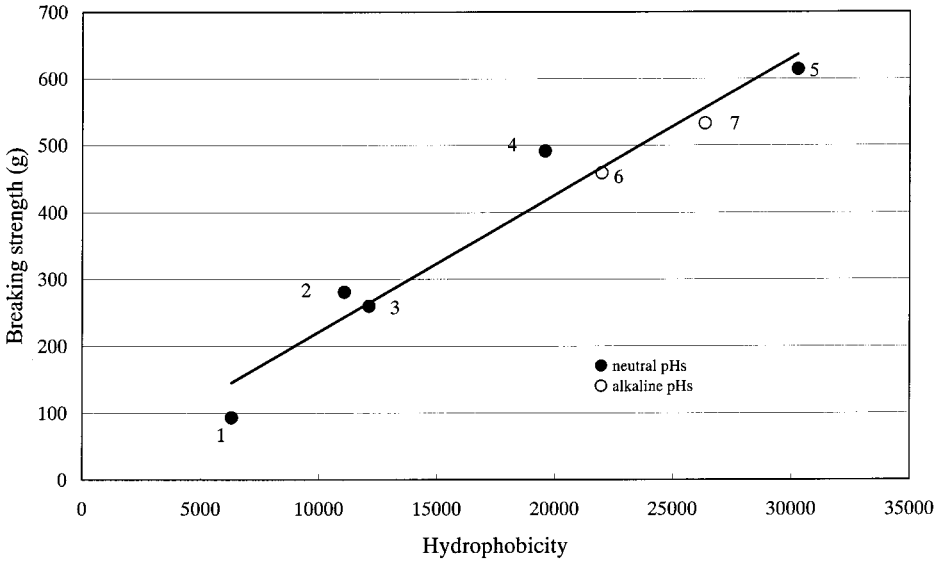

Figure 9. Hydrophobicity of egg white proteins vs breaking strength of egg white gels: dried egg white products with (●) neutral pHs ($r = 0.971$ for 5 samples) and (○) alkaline pHs ($r = 0.973$ for 7 samples).

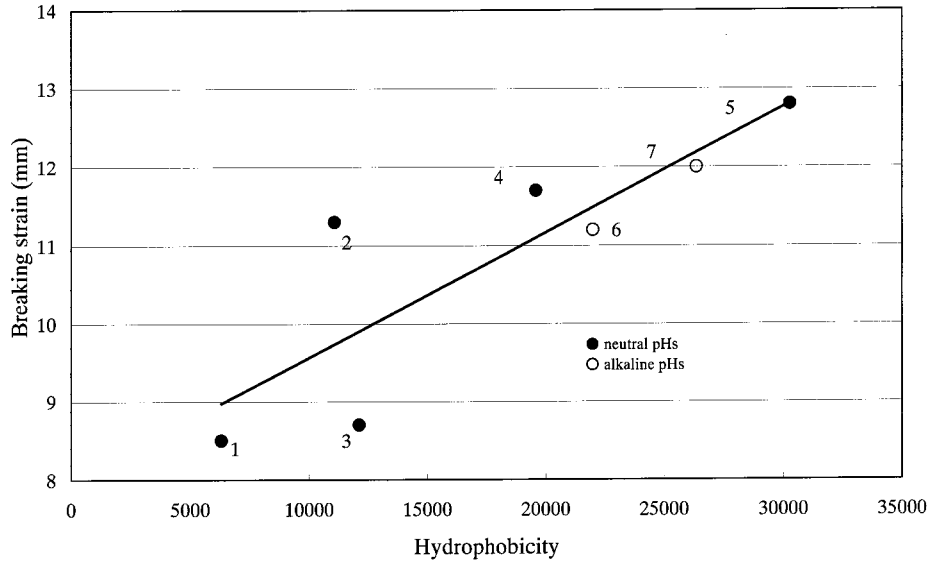

Figure 10. Hydrophobicity of egg white proteins vs breaking strain of egg white gels: dried egg white products with (●) neutral pHs ($r = 0.836$ for 5 samples) and (○) alkaline pHs ($r = 0.848$ for 7 samples).

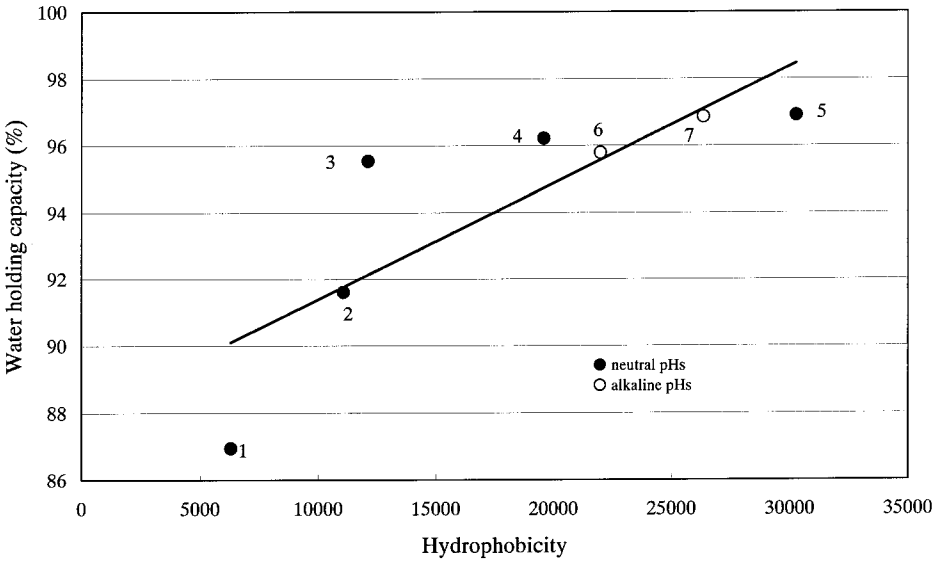

Figure 11. Hydrophobicity of egg white proteins vs water holding capacity of egg white gels: dried egg white products with (●) neutral pHs ($r = 0.789$ for 5 samples) and (○) alkaline pHs ($r = 0.824$ for 7 samples).

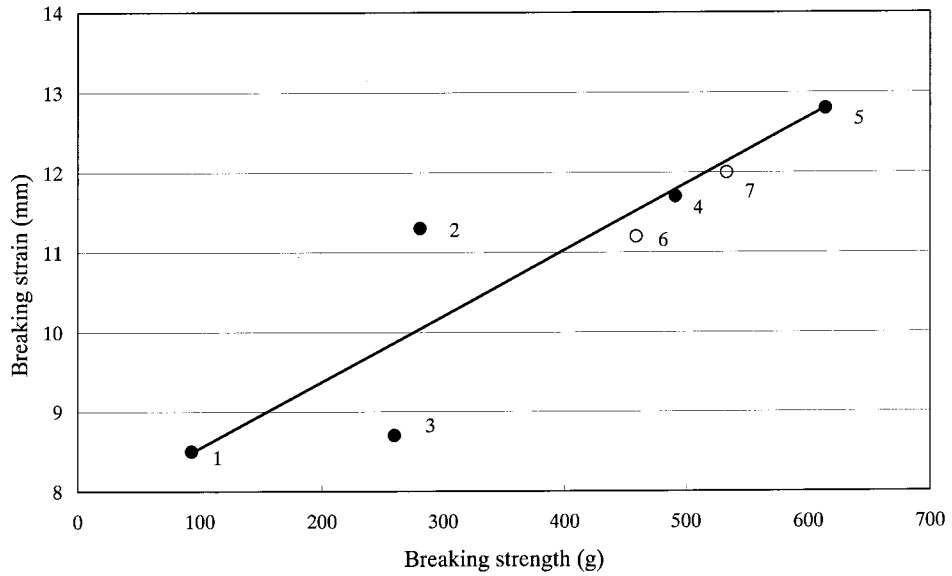

Figure 12. Breaking strength vs breaking strain of egg white gels: dried egg white products with (●) neutral pHs ($r = 0.893$ for 5 samples) and (○) alkaline pHs ($r = 0..902$ for 7 samples).

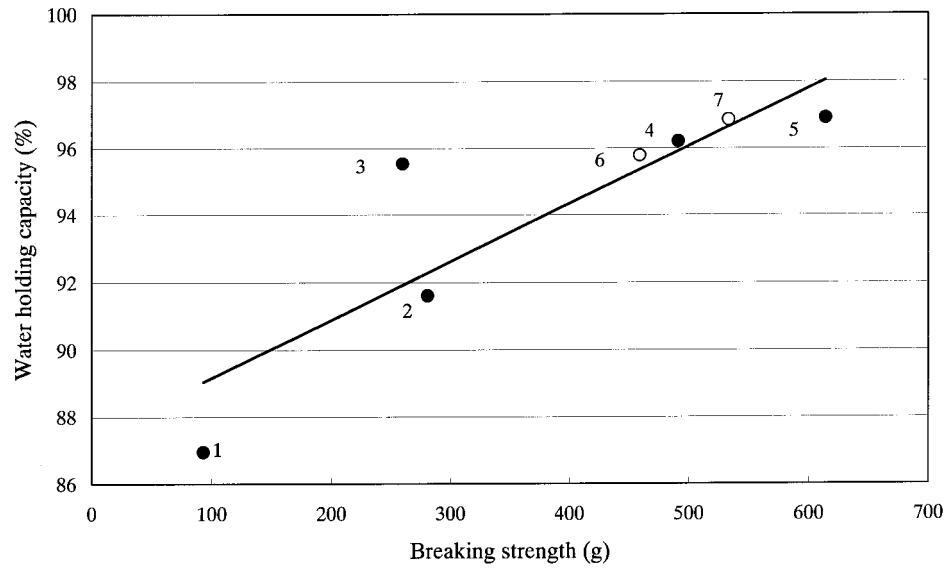

Figure 13. Breaking strength vs water holding capacity of egg white gels: dried egg white products with (●) neutral pHs ($r = 0.850$ for 5 samples) and (○) alkaline pHs ($r = 0.873$ for 7 samples).

average molecular weight and breaking strength of both samples were not considerably different (Figures 2 and 7). Again, a substantial difference was found in total SH groups, rather than in hydrophobicity and surface SH groups of both DEW−SPA (Figures 2−4). Therefore, it is assumed that differences in secondary structure, tertiary structure, or flexibility of DEW−SPA might cause the differences in breaking strain and WHC of the gels. However, further study is needed to elucidate how DEW−SPA forms affect heat-induced gelling properties of DEW products.

Relationship among Gelling Properties. Strong correlations were found between breaking strength and breaking strain plus WHC of heat-induced gels of DEW products (Figures 12 and 13). Those results demonstrate that stronger gels are more elastic and have larger WHC. As shown in Figures 4 and 5, DEW−SPA structures differed depending on the pH; also, deamidation was reported to have occurred in DEW proteins when dry-heated at mild alkaline pHs (*23*). However, the pH, interestingly, did not affect those relationships (Figures 12 and 13). Handa and Kuroda (*24*) reported that there were strong correlations among breaking strength, breaking strain, and WHC of heat-induced gels from Maillard-reacted DEW. Furukawa and Ohta (*25*) also reported that hardness and WHC of heat-induced soy protein gels correlated ($r = 0.983$) when the degree of intermolecular disulfide bonds was controlled by addition of L-cysteine. On the other hand, Karleskind et al. (*26*) found no relationship between shear stress and WHC in whey protein concentrate gels. Hermansson and Lucisano (*27*) also concluded that changes in the gel structure may affect textural characteristics and WHC differently in blood plasma gels. The sample preparation methods and the protein types most likely influence the relationship between textural properties and WHC of protein gels.

CONCLUSION

Strong correlations were found between hydrophobicity, surface SH groups, plus average molecular weight

of DEW−SPA and physical properties of the gels from commercial DEW products, regardless of dry-heating method. The relationship between average molecular weight of DEW−SPA and the degree of protein unfolding of DEW−SPA plus gelling properties of DEW was influenced by the pH of DEW products. On the other hand, the relationships between average molecular weight of DEW−SPA and gelling properties of DEW, as well as the relationship among gelling properties, were not influenced by the pH of DEW products. Kato et al. (*28*) reported that the enthalpy of denaturation of DEW was decreased with an increase of dry-heating time. Therefore, the relationship between enthalpy of denaturation and protein structure, as well as gelling properties of commercial DEW products, merit investigation.

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