Functional Properties of Chemically Modified Egg White Proteins

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Functional properties of egg white proteins can be altered through selected chemical reactions. Acylations with acid anhydrides have received the greatest amount of attention. Oleic acid and sodium dodecyl sulfate (SDS) have also been used to affect function of egg white proteins. The charge characteristics of acylated proteins are altered through modification of the N-terminal and epsilon-amino groups. The acid anhydride used and the extent of modification have a major effect on the ionic properties of the protein. The altered ionic properties have been shown to affect the optical properties of protein sols, heat stability, foaming, performance in angel cakes, initiation of gelation, ultimate strength and freeze-thaw stability of heat-set gels. Although exact explanations of the mechanisms for the interactions of oleic acid and SDS with egg white protein are not available, increases in charge occur and result in gels with physical properties very similar to gels made from succinylated egg protein.

Chemical and enzymatic modification of food proteins, including egg proteins, continue to receive research interest. The two primary objectives of that research are (a) to learn more about the function of proteins in foods by introducing known modifications of proteins and observing subsequent function or (b) to improve function by modifications. To date, improvement in function through modification is essentially the result of empirical experiments. Research related to the first objective is leading to descriptive data that is beginning to describe specific characteristics of proteins in solution relative to measurable functional attributes. A great deal more research will be required before structural and/or chemical properties of proteins can be used to predict function in foods.

The state of the science and directions for research are outlined in the proceedings of the symposium "Food Proteins, Improvement Through Chemical and Enzymatic Modification" (1). The fundamentals of chemical modification reactions are adequately described in several general texts (2–5). Gandhi et al. (6,7) reviewed the chemical modification of isolated egg proteins and the limited studies dealing with the modification of unfractionated egg white through 1968. The objective of my paper is to review egg protein modification research since that date. The focus will be the predominant chemical modifications that have been applied to egg white or egg white proteins, where relation to functional properties of egg proteins was the primary concern.

MODIFICATION REACTIONS

Acylations. Acylation reactions have many advantages for modifying egg proteins (1). They can be carried out under relatively mild conditions with several readily available acylating reagents to obtain a wide range of ionic characteristics of the modified proteins. The ability to alter protein charge has been an attractive advantage, since biophysical and functional properties of proteins are significantly influenced by their ionic condition.

Acylating reagents can be used to obtain a shift in the charge of modified protein functional groups. Under the conditions generally used, in which ≤60% of available amino groups are modified, the primary reactants are the epsilon-amino group of lysine, N-terminal amino groups and lesser amounts of available sulfhydryl groups (6,8,9). At higher levels of modification (>60%), other functional groups will be modified (tyrosyl, serinyl and threonyl hydroxyls and sulfhydryls) (6).

Amino groups of native egg white protein will generally be protonated at pH values normally observed in albumen (7.5 to 9.5) and also at the pH values required to successfully acylate proteins (>7) (1). An acylating reagent such as acetic anhydride will result in a one-unit change in charge by modifying an amino group that would normally be protonated, protein-NH_2 + $(CH_3CO)_2O$ → protein-$NHCOCH_3$ + CH_3COO^- + H^+, while a cyclic dicarboxylic anhyride such as succinic anhydride will result in a two-unit change in charge by modifying an amino group, protein-NH_2 + $(CH_2CO)_2O$ → protein-$NHCOCH_2CH_2COO^-$ + H^+.

The net effect of these reactions applied to native egg proteins is that the overall charge characteristics become more negative relative to the ionic properties of the native protein. Those alterations are easily revealed by electrophoretic and ion exchange chromatography procedures (8,10,11) or by titration (12). Figure 1 presents a diagram of a typical electrophoretogram obtained from succinylated egg white proteins. The cathodic migration of lysozyme is reversed as level of modification increases. Conalbumin and other anodically migrating proteins also show effects of modification. As would be expected, is substantial alteration of charge affected lysozyme activity and iron binding by conalbumin, as shown in Figures 2 and 3 (8). These results suggest that the native structures of these proteins necessary for their respective activities were being altered.

Studies of the nutritional properties of acylated proteins high in lysine also indicate structural changes that limit the utilization of acylated proteins (9,13–15). The limitations may result from poor digestibility of modified proteins or unavailability of modified lysine. Multiple enzyme in vitro studies with acetylated and succinylated egg white suggested that the acylated egg proteins were about as digestible as native protein, but chicks fed the acylated protein as their only protein source did not grow as well (Table 1) as chicks fed native protein (9). Adding lysine to the diets containing acylated protein restored growth. Studies of egg protein biophysical properties and specific reactivity of selected egg white proteins and nutritional studies all indicate and confirm that acy-

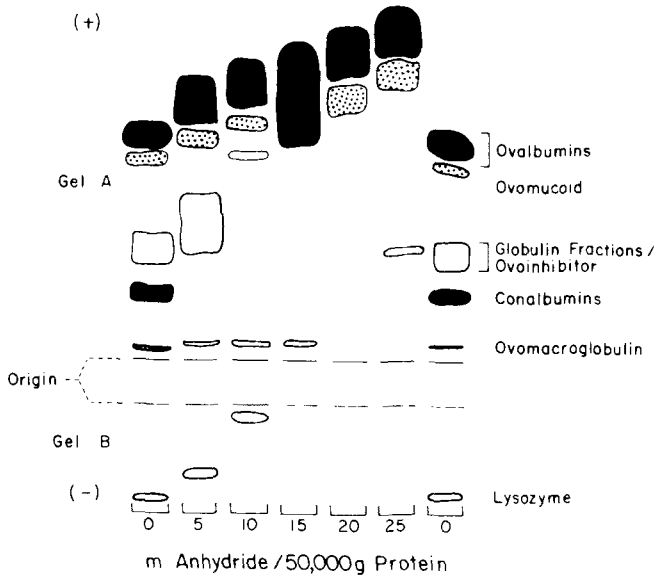

FIG. 1. Illustration of two electrophoretograms of succinylated egg white with electrophoresis conducted under identical conditions after reversing the polarity of the cell. Differences in intensity of shading represent differences in intensity of staining of the protein fractions on the original electrophoretogram (8).

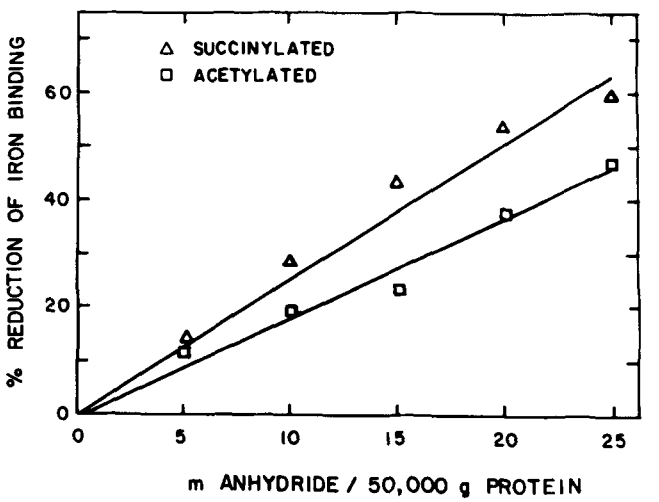

FIG. 2. Effect of acylation on reduction of iron-binding ability of egg white (8).

lation modifications of egg proteins substantially affect the proteins and would be expected to affect the function of those proteins in foods.

Anionic detergents and fatty acids. Anionic detergents and fatty acids have also been used to a lesser extent to modify egg proteins (16–18). Most early research involved adding these reagents at levels below the critical micelle concentration (cmc) (16,17). The thermal aggregation temperatures of ovalbumin and conalbumin were increased by the addition of sodium dodecyl sulfate (SDS), 2-decylcitric acid and lauric acid. The increase in aggregation temperature was believed to be the result of increasing net charge

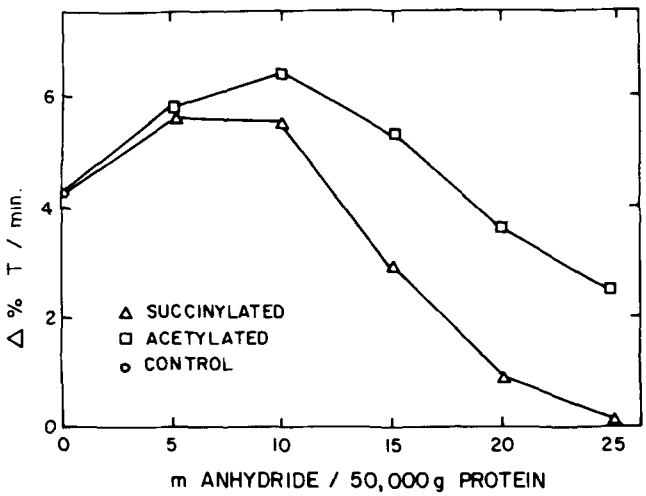

FIG. 3. Lysozyme activity of acylated egg white (8).

TABLE 1.

Feed Consumption and Weight Gain in Bioassay

	Diets[a]					
	Untreated egg white		Acetylated egg white		Succinylated egg white	
% Protein	Feed consumption	Wt gain	Feed consumption	Wt gain	Feed consumption	Wt gain
10	1259	72[a]	1075	50[b]	1059	47[b]
20	1328	126[a]	1146	89[b]	1126	80[b]
30	1250	132[a]	1074	106[b]	1046	95[b]

From King et al. (9).
[a]Values within a horizontal row with different letters are significantly different ($p < 0.01$).

on the protein (16). It was suggested that at concentrations below the cmc, the hydrophobic portions of the detergents or fatty acids associated with hydrophobic pockets on the surface of the protein left the anionic portion at the surface, resulting in an increase in surface charge and some change in structure (16). In contrast to the effects of SDS at low concentrations, addition of a cationic detergent, cetylpyridinium chloride (CPC), above the cmc stabilized ovalbumin to heat without denaturing the protein (19). The CPC-modified ovalbumin yielded circular dichroism and optical rotation data indicating that thermal unfolding was completely reversible on cooling.

Later research has found that egg proteins are also modified by additions of oleic acid and SDS at levels above the cmc (18). The nature of the interaction of oleic acid with egg proteins has not been fully elucidated. Research has confirmed that the ionic properties of the proteins have been altered and that thermal properties such as temperature for initiation of gelation and strength of gels have been altered (20). The nature of the association of oleic acid with egg

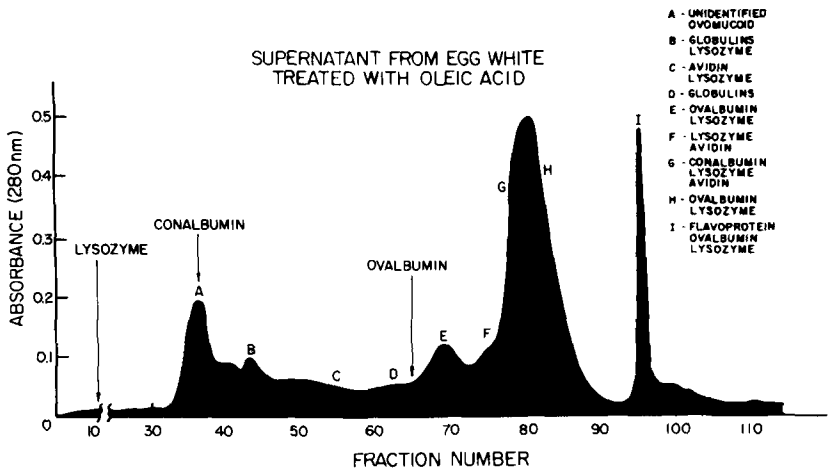

FIG. 4. DEAE-cellulose chromatograms of supernatant obtained from the 20-mol level of oleic acid. SDS-PAGE was performed to identify proteins in fractions A—I. Arrows indicate the usual elution sequence for lysozyme, conalbumin and ovalbumin in untreated egg white (18).

proteins may include some aspects as suggested for SDS at low concentrations (16). However, viscosity, DEAE-chromatography and electrophoretic studies suggest the possibility that mixtures of two or more different egg proteins or two or more molecules of the same protein may be surrounded by oleic acid (18), resulting in negatively charged complexes. Figure 4 presents a DEAE-chromatogram of oleic acid-modified egg white. Note that a larger fraction volume, relative to that for native ovalbumin, was required to complete the separation relative to native egg white and that lysozyme and ovalbumin were found in several of the fractions. Others have suggested similar detergent-protein complexes or micelles with protein cores (21,22).

Modification of egg white proteins with long chain anionic reagents below or above their cmc has been shown to alter the ionic properties of those proteins without chemically altering the essential amino acid lysine. Apparently two very different approaches, such as acylations or treatment with long chain anionic compounds, result in modifying the charge characteristics of proteins, which in turn influence other biophysical properties that relate to function in foods. Changes in functional performance are discussed in later sections.

Other procedures. A limited number of other chemical modifications has been applied to egg proteins. Carboxyl groups of ovalbumin were modified with 1-ethyl-3(3-dimethylaminopropyl) carbodiimide to form amides (12). The modification increases the pI and was reported to increase heat stability as the result of increased charge. Oxidizing agents potassium persulfate (7) and hydrogen peroxide (23) have been applied and their effects on chemistry and function evaluated. Persulfate oxidation resulted in a generalized degradation of egg white proteins. Lysozyme activity and iron-binding capacity were diminished as the level of oxidant was increased (7). Addition of up to 6% hydrogen peroxide resulted in destruction of the sulfur-bearing amino acids and tyrosine and substantial losses of phenylalanine and histidine (23). More recently, modification of disulfides with 2-mercaptoethanol to produce low temperature egg white gels has been reported (24). Conalbumin was identified as the primary source of disulfides reduced. Saturating the iron-binding sites on conalbumin to stabilize structure protects those disulfides from reduction. N-Ethylmaleimide is being used in my laboratory to block free sulfhydryl groups of egg white to prevent their participation in sulfhydryl-disulfide interchanges or to prevent them from oxidizing to disulfides during heating to form gels. While the potential value of thiol group modification is yet to be determined, oxidative procedures that improve the function of gluten in wheat flour do not seem to have similar potential for improving the performance of egg proteins.

FUNCTIONAL PROPERTIES

Foaming and cakes. The ability to form a stable foam capable of leavening cakes is an important functional property of egg proteins, especially albumen proteins. Acylation of egg white proteins has been reported to enhance the ability of those proteins to form foams, as measured by increased foam volume or stability (6,8,11,25). Figure 5 illustrates the effect that acetylating egg white and soybean 11S proteins has on foaming properties and digestion velocity. Acetylation opened the protein structures to attack by chymotrypsin and improved their ability to foam. The increased charge and subsequent structural changes favored foam formation. The effects of the modifications on cake volume are mixed. At low levels of gluteration with dimethylglutaric anhydride, cake volumes were improved, while poor performance was reported as the result of more extensive modification (6). Acetic anhydride sufficient to modify 50% of

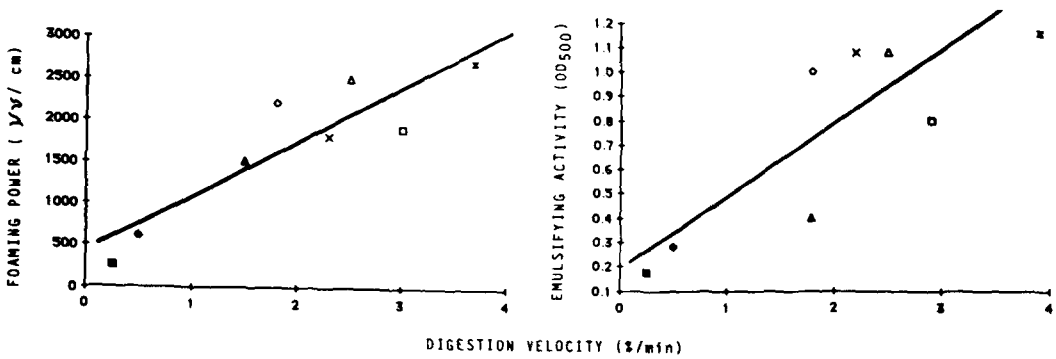

FIG. 5. Relationship between foaming or emulsifying properties and digestion velocity of ◆, ovalbumin; ■, lysozyme; ▲, conalbumin; V, soybean 11S globulin; and the same proteins acetylated: ◇, □, △, *, respectively (25).

the available amino groups resulted in decreased cake volume (8). There was no difference in the volume of cakes made with succinylated egg compared to untreated egg white (Table 2). In both instances of reduced performance of acylated eggs in cakes, the relationship of heat stability of the modified egg to subsequent performance in cakes was discussed. Higher levels of gluteration resulted in extensive increase in charge, which was believed to delay heat setting of the protein film responsible for cake structure (6). In contrast, acetylation was found to decrease heat stability of the proteins, which was believed to result in premature setting of the protein, thereby limiting volume increase during baking (8). Mild oxidative treatments with potassium persulfate resulted in some improvement of foam formation and leavening of angel cakes (7). As the severity of the treatment increased, the foaming and leavening ability of oxidized egg white decreased. The loss of leavening ability of persulfate-oxidized egg white was also linked to decreases in heat stability (7).

Anionic detergents, fatty acids and related compounds at levels above the cmc generally result in poor foaming and cake performance. There are, however, several commercial applications of low levels of anionic detergents, organic esters, bile salts and salts of fatty acids to improve foaming and cake performance of commercially processed egg products (26). Oleic acid (0.02–0.03%) has been reported to improve texture of angel cakes (27).

Emulsification. Emulsification properties of egg proteins appear to parallel their foaming properties in response to modification. Oxidation of egg white proteins with potassium persulfate did not significantly affect their emulsification ability (7). Gluterating egg white proteins increased their emulsification capacity at pH 4 with less effect at pH 6 or 8 (Table 3). Figure 5 emphasizes the parallel response of emulsification and foaming properties of egg proteins to acylation. As described earlier, acetylation alters charge on the protein, which in turn alters structural properties important to the function of egg

TABLE 2.

Functional Performance of Acetylated and Succinylated Egg White[a]

	Foam performance		Angel cake performance	
	Foam volume (ml)	Drip volume (ml)	Beating time[b] (sec)	Volume ratios[c]
Control	838.2±12.8[c]	18.3±2.8[a]	12.8±1.0[a]	4.52±0.13[a]
Acetic anhydride				
10 mole	978.8±68.8[b]	16.6±5.2[a]	12.3±1.7[a]	4.54±0.11[a]
20 mole	1,001.6±95.2[b]	15.1±10.7[a]	11.1±1.0[a]	4.09±0.10[b]
Succinic anhydride				
10 mole	1,065.3±18.2[b]	8.9±2.0[a]	12.1±0.6[a]	4.50±0.10[a]
20 mole	1,170.1±27.3[a]	14.8±1.7[a]	12.5±0.6	4.58±0.15[a]

From Ball and Winn (8).
[a]Means within a column with different superscripts are significantly different ($p < 0.05$).
[b]Beating time defined as that required to form a medium peak.
[c]Volume ration is the volume of the cake divided by the weight of cake.

TABLE 3.

Emulsifying Ability of Glutarinated Egg White

	g oil/mg protein nitrogen		
Mol DMGA/mol EWP[a]	pH 4.0	pH 6.0	pH 8.0
0	13.5	16.2	10.0
3	14.7	14.3	10.6
6	13.9	12.1	10.2
15	16.1	14.1	9.5
30	21.2	12.5	8.3

From Gandhi et al. (6).
[a]Mole of 3,3-dimethylglutaric anhydride/50,000 g egg white protein.

white proteins as emulsifiers (25).

Gelation. There are several interesting subtopics related to the gelation phenomena. They include the stability of proteins to thermal treatments, optical and rheological properties of gels and the freeze-thaw stability of gels.

As discussed earlier, modification reactions that increase surface charge on proteins tend also to improve their stability to heat treatments by increasing charge repulsion, thereby delaying thermally driven aggregation (6,8,11,16,17,19). Stabilizing the structure of proteins by providing for internal cross-linking has also been suggested as a possible mechanism for the action of low levels of SDS (16) and for the action of the cationic detergent cetylpyridium chloride (19). An iodine-mediated oxidation resulting in an internal cross-linking of lysozyme at tryptophan 108 and glutamic acid 35 caused increased resistance to heat denaturation and attack by chymotrypsin (28).

Some of the modification procedures discussed earlier result in reduced thermal stability of egg white proteins (7,8,23,24). Acetylation sufficient to block 50% of the available amino groups reduced heat stability of egg white relative to succinylated and native egg white (Fig. 6). Others working with a mixture of conalbumin and ovalbumin did not find a decrease in heat resistance due to acetylation (11). Later studies of the mechanical properties of acetylated egg white gels demonstrated that they were weaker than gels made from succinylated egg white, suggesting that acetylated egg white proteins respond to heat treatments differently (29). Using an instrumental approach that allowed the detection of the transition of a sol to a gel (Fig. 7), it was determined that oleic acid–modified egg white began to gel at 68 C, while native and succinylated egg white initiated gelation at 71 C and 76 C, respectively (20). The higher temperature for development of rigidity by succinylated egg white reflects the effect of increased charge (8,12). The increased charge characteristics brought about by oleic acid modification did not raise the temperature for initiation of gelation. The final rigidities for the oleated and succinylated gels were higher than the value for native egg white.

Oxidative destruction of the sulfur-bearing amino acids with hydrogen peroxide (23) or the reduction of disulfides with 2-mercaptoethanol (24) causes egg

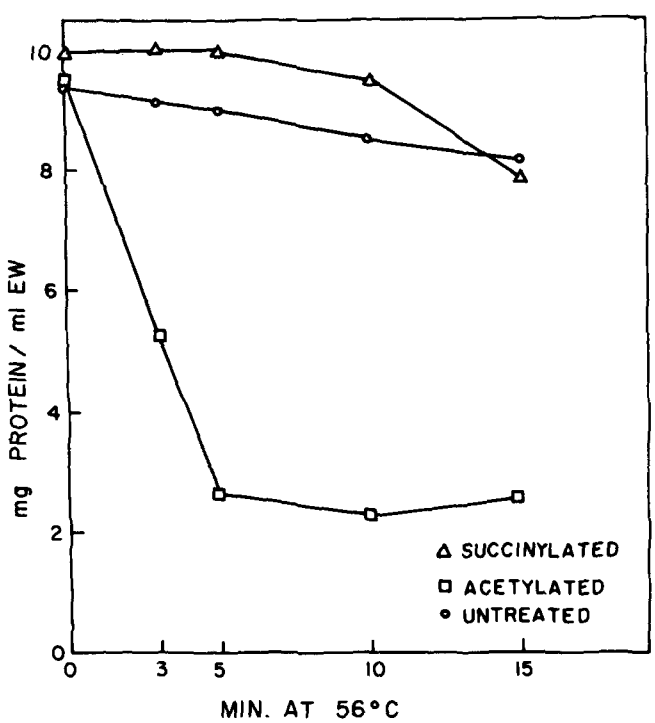

FIG. 6. Averages across all treatment levels for protein remaining soluble as a function of heating time at 56 C (8).

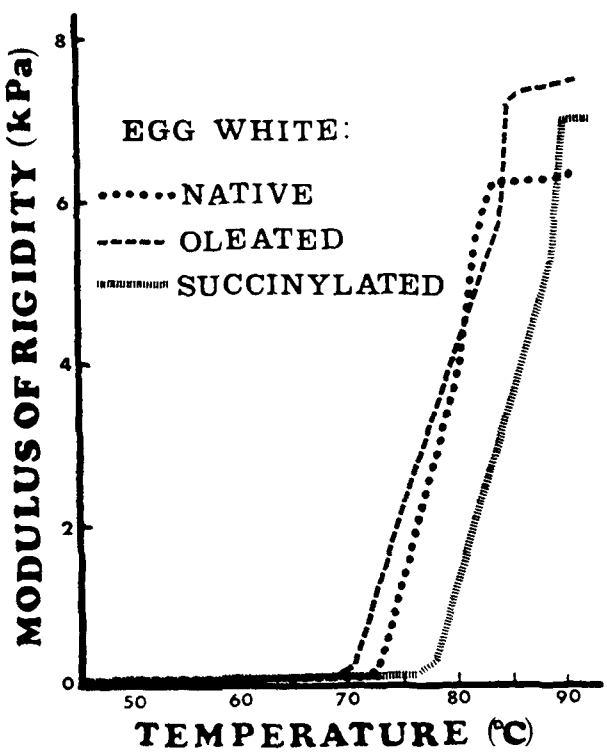

FIG. 7. Shear rigidity thermograms for native and modified egg white. Heating rate, 0.5 C/min (20).

white to gel at low temperatures, 25 C and 35 C, respectively, after standing for up to 24 hr. While the initial effects of the two described procedures are very different, the overall effect of each treatment is the loss of native structure (denaturation) and subsequent interactions (aggregation) with time resulting in a gel structure. The properties of those types of gels are not yet fully understood.

Gels made from chemically modified egg white at pH values above 7 tend to be translucent. Table 4 presents the optical densities of gels prepared from various egg white preparations. It is generally known that native egg white exhibits greater transparency as pH is increased in the alkaline range. At a pH of 11, egg white will set to a transparent gel at room temperature (30). Increased charge on the proteins is believed to affect the protein-protein interactions that form the gel network, which may eventually determine the optical properties of the gel. Scanning electron photomicrographs of gels (Fig. 8) made with modified and native egg show major differences in their fine structure (20). Translucent gels, made from succinylated or oleated egg white, exhibited structures that featured a more dense protein matrix with larger openings, which were believed to have contained water prior to fixing the samples. The gel structure, differences in gel contents and properties of the soluble intracellular components determine how light interacts with the gels. A partial digestion of egg white with pepsin at pH 4 recently has been reported to produce translucent gels after heating at pH 7 (31).

The mechanical failure properties reported in Table 5 show that succinylated and oleic acid–modified egg white produced very strong (high shear stress) and highly deformable (high strain) gels (29). As noted above, the temperature for initiation of gelation was different for these two materials, and there are major differences in the modification reactions, yet the mechanical failure properties (29), optical properties and fine structure of the gels are very similar (20). The mechanical-failure characteristics of native and acetylated egg were not significantly different. These results along with others reported above suggest that the extent of denaturation resulting from modification prior to heating, such as results from treatment with oleic acid, or the extent of thermal unfolding prior to aggregation, such as results from succinylation, are important in determining the mechanical properties of egg white gels.

TABLE 4.

Absorbance at 550 nm After Two Min of Heating Egg White at 60 C

	pH	
Egg white sample[a]	7.5	9.5
Native	∞	0.32
Oleated	∞	0.11
Succinylated[b]		0.05

[a]20 moles reagent/50,000 g egg white protein.
[b]pH 8.5.

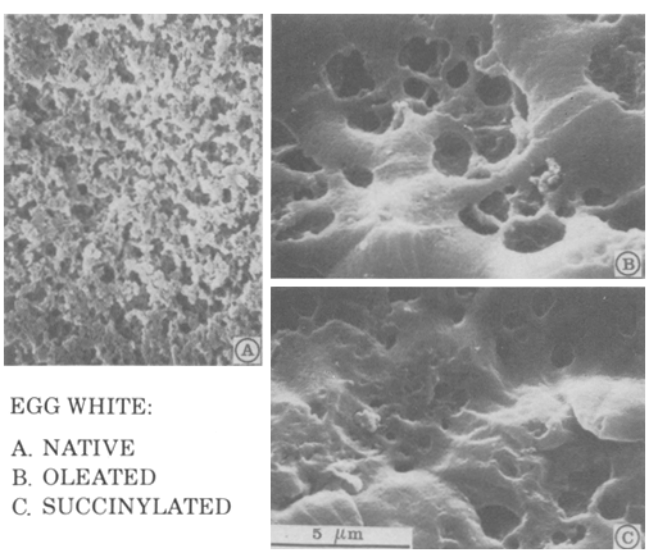

FIG. 8. SEM micrographs of cryofractured surfaces of heat-induced gels from native and modified egg white. Magnification, 5000X (20).

TABLE 5.

Comparison of Mean Values of Torsional Failure Parameters for Gel Materials[a]

Egg white	Shear stress (kPa)	True shear strain	Shear modulus (kPa)
Native	12.96[a]	1.090[a]	10.07[b]
Acetylated, 24 m[b]	11.54[a]	1.048[a]	12.53[c]
Oleic acid treated, 25 m	19.75[b]	2.784[b]	5.62[a]
Succinylated 25 m	19.36[b]	2.548[b]	6.01[a]

From Montejano et al. (29).
[a]Means within a column with different superscripts are significantly different ($p < .05$).
[b]Moles anhydride/50,000 g egg protein.

The mechanical-failure characteristics of acetylated egg are interesting. Although acetylation increased charge on the protein, it did not improve its thermal stability (8) or gel strength. In subsequent research with a different rheological device, a capillary extrusion apparatus, increases in apparent viscosity of egg white gels were related to increases in pH but not to succinylation (32). Yield force and rigidity moduli also increased with pH but not with succinylation level. The lack of detection of rheological changes expected as the result of succinylation may indicate that capillary extrusion methods are not detecting the same physical factors as torsion failure testing. It was noted that the high deformability of succinylated and oleic acid–modified egg prevented accurate use of compression failure testing procedures (29).

TABLE 6.

Water Retention Index[a] (WRI) of Modified Egg White

Reagent	Moles of reagent/50,000 g egg white protein	WRI	
		Cooked, unfrozen	Cooked, frozen/thawed
Control		0.29±0.06	0.10±0.03
Acetic anhydride	10	0.40±0.02	0.22±0.01
	20	0.46±0.02	0.32±0.01
Succinic anhydride	10	0.44±0.04	0.31±0.02
	20	0.50±0.02	0.53±0.02
Oleic acid	20	0.92±0.08	0.84±0.17
	25	1.00±0.01	1.00±0.01
	50	1.00±0.01	1.00±0.01
Methyl oleate	25	0.33±0.06	0.14±0.03
Sodium dodecyl sulfate	5	0.42±0.09	0.30±0.08
	15	0.69±0.10	0.73±0.19

From King et al. (18).
[a]Egg sample pressed between two sheets of Whatman No. 1 paper; WRI = area of egg film/total wetted area. WRI of 1 represents excellent water retention properties.

The freeze-thaw stability of cooked egg white gels is improved by increasing pH, succinylation or treatment with oleic acid (33,18). Table 6 presents a summary of water retention indices determined in my laboratory. It is interesting to note that modification treatments that favor the formation of strong, elastic and translucent gels also result in the best freeze-thaw stability. The improvement in freeze-thaw stability of egg white gels is also observed when modified egg white is blended with yolk prior to cooking (33).

The research published to date confirms that the functional properties of egg white proteins can be altered through chemical and enzymatic methods. Utilization of this information is, however, relatively limited. Perhaps the greater value of the current research is in adding to our catalog of information relating specific conditions or properties of proteins to functional properties in a food system. The ultimate goal will be to relate specific biophysical properties of proteins to well-defined functional attributes. Additional research at the molecular level in conjunction with research to define the details of function of proteins in food systems will be required.

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