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IN SELECTED FOOD SYSTEMS

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EGG ALBUMEN PROTEINS INTERACTIONS IN SELECTED FOOD SYSTEMS

By

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ABSTRACT

EGG ALBUMEN PROTEINS INTERACTIONS
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The present study was aimed at the investigation of albumen proteins functional properties and specific protein-protein interactions in two food systems. Foamability was evaluated in an angel food cake system and coagulability in a custard model system. Ovomucin, lysozyme, globulins, ovomucoid, conalbumin, and ovalbumin were isolated from egg white. They were further tested singly and in combinations in these food systems. Various combinations of three levels of each protein according to a mixtures experimental design constituted the treatments for the cake system. The effects of the proteins interactions in this system were analyzed through response surface methodology to define protein levels for optimization of cake parameters. Ultrastructural examinations of selected foams in transmission and scanning electron microscopes and of coagulums in a scanning electron microscope were performed.

Foamability studies of the individual protein solutions showed that globulins solution had good foaming properties

and produced a cake with high volume and excellent textural characteristics. Ovalbumin solution did not exhibit good air incorporation ability and produce a coarse textured cake of relatively large volume. Conalbumin, lysozyme, ovomucin, and ovomucoid had little or no foaming capacity.

In interaction studies it was found that ovomucin, lysozyme, and globulins were the proteins primarily involved in foaminess and volume of angel food cakes . Association of ovomucin with globulins significantly favored foam formation. However, cake volume correlated negatively with foaminess. Lysozyme depressed foaming capacity by complexing with ovomucin. This resulted in considerable cake volume improvement.

Surface response analysis of foaming index and cake volume defined ovomucin, lysozyme, and globulins levels range for production of a target cake. These were 0.2-1.0%, 0.0-1.8%, and 12.2-14.8% for ovomucin, lysozyme, and globulins, respectively.

Studies of gelation properties of the albumen proteins showed lysozyme, globulins and combinations of lysozyme with globulins produced the firmest gels. Ovomucin and ovomucoid showed no heat gelation abilities. The following order of heat stability of the proteins was found: conalbumin < ovalbumin < globulins < lysozyme. Denaturation transition

temperatures of these proteins were 57.3, 71.5, 72.0, and 81.5°C, respectively. It was observed that the primary effects of protein-protein interactions in this food system were a denaturing action exerted by the least heat stable proteins over the more stable ones.

Transmission electron microscopic examinations of foams revealed the existence of a layer of partially cross-linked polypeptides at the film surface and scanning electron microscopy studies showed that ovomucin concentrated at the film surface preventing the formation of a cohesive membranous layer. The depressive effect of ovomucin on cake volume seemed to conform with formation of less stable foams and lack of heat coagulative properties of the protein film. Lysozyme, by complexing with ovomucin, altered the viscous nature of this protein.

Scanning electron microscopic investigations of coagulums showed a characteristic association of the polypeptides in grape-like clusters of variable sizes. Globulins polypeptides aggregated in membrane-like arrangements and exhibited excellent binding abilities. Smaller cluster sizes seemed to correlate with firmer gels.

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To Craig, for his presence.

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INTRODUCTION

Increased interest in improving the egg processing technology has led to an extensive research concerning egg proteins functionality. Of the processes employed by the egg industry, drying, particularly spray-drying, has been known to adversely affect the whipping ability of dehydrated albumen and whole egg (MacDonnell et al., 1950; Bergquist and Stewart, 1952; Joslin and Proctor, 1954). It also accelerates the flavor deteriorative changes of yolk-containing products (Bergquist, 1977). The incorporation of sugar or salts to liquid yolk is required to prevent changes in viscosity upon freezing (Cotterill, 1977). The microbiological problem of egg products was brought under control with the application of heat, such as pasteurization or other heat treatments. However, accompanying functional changes have required the use of whipping aids (Miller and Winter, 1950; Clinger et al., 1950; Forsythe, 1964).

The changes observed in dried egg white are apparently caused by alteration of one or more of the proteins. A combination of drying per se and physical treatment may be involved in promoting these changes (MacDonnell et al., 1950). Heat could also have an effect provided a temperature high enough is reached in the product during

drying.

The degree of alteration in functional properties is generally measured through performance tests in food products. In studies on the proteins primarily responsible for foaming in an angel food cake, globulins, including lysozyme, were rated as excellent foamers while ovomucin was classed as a foam stabilizer (MacDonnel et al., 1955). By conducting physical tests it was concluded that globulins, ovomucin, and conalbumin have high foaming power, whereas ovalbumin, lysozyme, and ovomucoid do not foam as easily (Nakamura, 1963).

In studies of coagulation ability of egg products several factors such as salts, sugar, acid, alkali, and temperature have been listed as the major parameters influencing the heat induced gelling of eggs (Baldwin, 1977). The different heat sensitivity of albumen proteins (Cunningham and Lineweaver, 1965) suggests some proteins may play major roles in defining the gelling ability of egg white.

While several workers have studied the functional aspects of the proteins in egg white by conducting simple performance and physical tests (Nakamura, 1964; Nakamura and Sato, 1964; Snider and Cotterill, 1972; Cunningham, 1976; Egelandsdal, 1980), these studies could yield misleading information as to how the proteins would behave in the more complex food systems.

The effects of interactive forces among ingredients, particularly protein-protein interactions, on foaming and

coagulation abilities need to be evaluated in relation to performance in food systems. The findings associated with such studies may lead to the development of improved egg products or lead to new applications. Protein functionality data of extrapolative value for predicting performances of novel proteins in food systems could reduce production costs (Kinsella, 1976). Furthermore, such data may serve in comparative studies of proteins functions and molecular conformation. Therefore, in view of these considerations, the present study focused on the determination of possible interactions among the albumen proteins, as well as the characterization of functional properties of each protein.

Two food systems with constant protein content were used: an angel food cake to investigate foamability and a custard model system to study coagulability. Six albumen proteins were isolated and their foaming and gelling capacities were characterized at a physicochemical and ultrastructural level.

The second objective of this study was to develop a statistically planned design from mixtures experiments to simplify and reduce the number of treatments. Multiple regression analysis was used to derive prediction equations for the analysis of response surfaces and optimization of cake parameters.

REVIEW OF LITERATURE

Literature pertaining to egg albumen proteins' characteristics and functional properties are summarized in this review. Fundamentals of protein structure and functionality in general are also reviewed to provide a better understanding of protein's functions in food systems.

Egg Composition

Eggs are a complex system of proteins and lipids dispersed in an aqueous phase with many other constituents. Whole eggs are composed of 8-11% shell, 56-61% albumen, and 27-32% yolk. The chemical composition of albumen, yolk and whole egg is displayed in Table 1 (Powrie, 1977). The major constituent of the total solids in albumen is proteins, whereas yolk solids are mostly proteins and lipids. Carbohydrates are present in the free form and in combination with proteins. In albumen, 44% of the carbohydrates are present in the free form and 56% are found in glycoproteins, while, in yolk, 70% are in the free form and 30% are present as protein-bound carbohydrates.

Albumen Proteins

Egg white has been reported to contain as many as 40

Table 1. Composition of Albumen, Yolk, and Whole Egg (Powrie, 1977).

Egg Component	Protein	Lipid	Carbohydrate	Ash
		%		
Albumen	9.7-10.6	0.03	0.4-0.9	0.5-0.6
Yolk	15.7-16.6	31.8-35.5	0.2-1.0	1.1
Whole Egg	12.8-13.4	10.5-11.8	0.3-1.0	0.8-1.0

different proteins (Vadehra and Nath, 1973), most of which have not been isolated. The physicochemical characteristics of the major albumen proteins are summarized in Table 2.

Ovalbumin

Ovalbumin is a phosphoglycoprotein composed of three forms, A_1, A_2 and A_3, which differ in their phosphate content. A_1 has two phosphate groups per molecule; A_2 has one; and A_3 has none. The relative proportion of these components is approximately 85:12:3. The protein is easily denatured by exposure to new surfaces, action of various denaturating agents, and heat, but is resistant to pasteurization conditions (Powrie, 1977). During storage of eggs ovalbumin is converted to a more heat stable form, S-ovalbumin, possibly through rearrangement of the molecule to a more thermodynamically stable form (Smith and Back, 1965).

Table 2. Physicochemical Characteristics of Egg-White Proteins (Powrie, 1977).

Protein	Relative Amount in Albumen, %	Molecular Weight	pI	Sulfhydryl Groups	Disulfide Groups	Characteristics
Ovalbumin	54	45,000	4.6	4	2(1)[b]	Phosphoglycoprotein.
Conalbumin	13	80,000	6.6(6.05)[a]	0	15[c]	Binds metal ions, anti-microbial.
Ovomucoid	11	28,000	3.9-4.3	0	8	Inhibits trypsin.
Lysozyme	3.5	14,600	10.7	0	4	Lyses bacteria cell wall.
G_2 Globulin	4.0?	30,000-45,000	5.5	?	?	
G_3 Globulin	4.0?	?	5.8	?	?	
Ovomucin	1.5	?	4.5-5.0[a]	?	?	Sialoprotein, inhibits virus hemagglutination.
Flavoprotein	0.8	35,000	4.1	?	?	Binds riboflavin.
Ovoglycoprotein	0.5?	24,000	3.9	?	?	
Ovomacroglobulin	0.5	760,000-900,000	4.5-4.7	?	?	
Ovoinhibitor	0.1	44,000	5.2	?	?	Inhibits several proteases.
Avidin	0.05	53,000	9.5	?	?	Binds biotin.

a-Osuga and Feeney (1974); b-Smith and Back (1970); c-Wenn and Williams (1968).

Conalbumin

Conalbumin, also known as ovotransferrin, is able to bind metal ions forming protein-metal complexes resistant to denaturation by heat, pressure, proteolytic enzymes, and denaturating agents (Fraenkel-Conrat and Fenney, 1950; Azari and Feeney, 1958, 1961). The protein complexes with two moles of metal ions per molecule with the relative binding stabilities: $Fe^{3+} > Cr^{3+}$, $Cu^{2+} > Mn^{2+}$, Co^{2+}, $Cd^{2+} > Zn^{2+} > Ni^{2+}$ (Tan and Woodworth, 1969), and also with Al^{3+} (Cunningham and Lineweaver, 1965). The iron binding ability of the protein imparts antimicrobial activity to egg white. In the metal free form, the protein is extremely susceptible to heat denaturation.

Ovomucoid

Ovomucoid is a glycoprotein of very high resistance to heat denaturation in acidic solutions and it shows a remarkable inhibitory activity against trypsin (Rhodes et al., 1960). The protein has an unusually high carbohydrate content (20-25%) which is present as three oligosaccharide units, each attached to the polypeptide through a glycosidic bond to asparagine (Powrie, 1977).

Lysozyme and Globulins

Lysozyme was identified as the protein in egg white

with a lytic action on micrococcus cells by Fleming (1922). This enzyme cleaves the β - (1,4) - glycosidic linkages between N-acetylneuraminic acid and N-acetylglucosamine in bacterial cell walls (Osuga and Feeney, 1977). Lysozyme has been reported to be more resistant to heat inactivation in phosphate buffer than in egg white (Cunningham and Lineweaver, 1965). This basic protein binds with several other egg white proteins possibly through electrostatic interactions. Hawthorne (1950) demonstrated that addition of lysozyme to ovomucin induced drastic changes in the jelly-like protein to a stringy, compact mass. Other investigators have reported the interaction of lysozyme with conalbumin (Ehrenpreis and Warner, 1956) and with ovalbumin (Nakai and Kason, 1974).

Longsworth et al. (1940) noted two other globulins fractions, G_2 and G_3, distinct from lysozyme. Feeney et al. (1963) isolated G_2 globulins and partially characterized this protein fraction. They found negligible amounts of sulfhydryl and nucleic acids. Baker (1968) reported that G_2 and G_3 globulins have isoelectric points of 5.5 and 4.8, respectively.

Ovomucin

Ovomucin imparts the gel-like character to thick egg white. This extremely large polydispersed glycoprotein is filamentous and fiber-like in nature (Robinson and Monsey,

1975). Sugihara et al. (1955) reported the virus anti-hemagglutinin activity of ovomucin and also pointed out the chalaziferous layers of eggs are formed by twisted ovomucin fibers.

Ovomucin appears to be involved in the natural thinning of thick egg white which occurs during aging of eggs, but contradictory hypotheses have been proposed. Hawthorne (1950) suggested that the interaction of ovomucin with lysozyme causes thinning. On the other hand, Cotterill and Winter (1955), and Rhodes and Feeney (1957) proposed that the dissociation, rather than its formation, was responsible for thinning. Others have claimed that reduction or hydrolysis of disulfide bonds in ovomucin (Mac Donnell et al., 1951; Tomimatsu and Donovan, 1972) and loss of the O-glycosidically linked carbohydrate units from the protein (Kato et al., 1979) were associated with deterioration of the gel structure. Variable molecular weights ranging from 7.6×10^6 to as high as 240×10^6 daltons have been reported for the protein (Lanni et al., 1949; Robinson and Monsey, 1975; Tomimatsu and Donovan, 1972).

Other Proteins

Ovomacroglobulin has been identified as component 18 by Lush (1961). Later, Miller and Feeney (1966) isolated and characterized this protein. They observed that the

protein contains low levels of sulfhydryl and sialic acid groups.

Flavoprotein is the protein in egg white combined with riboflavin (Rhodes et al., 1958, 1959). The protein has from seven to eight phosphate moieties, but no sulfhydryl groups.

Avidin has been identified as the protein which binds biotin (Eakin et al., 1941). Other minor albumen proteins include a ficin and papain inhibitor (Fossum and Whitaker, 1968), ovoglycoprotein (Ketterer, 1962), and ovoinhibitor (Matsushima, 1958).

Protein Structure

Study of a food protein at the molecular level can be aimed at understanding its behavior in food systems. The native structure of a protein is directed by its amino acid sequence and the surrounding microenvironment (water and cofactors such as metal ions). The sequence of amino acids will govern the folding of the main polypeptide chain whereas water will force the arrangement of the apolar side chains into the interior of the protein. Ultimately, this extremely complex three-dimensional configuration dictates the protein properties including functionality in food systems.

The reactive groups in specific amino acid residues which may be involved in the various types of bonds that

maintain the protein structure are displayed in Table 3. Many of these amino acid residues also participate in associations with water molecules. Table 4 displays the types of bonds present in proteins and the heat of formation (energy) involved in these interactions, whereas Fig. 1 illustrates the manner by which these bonds stabilize the protein molecule.

Whitaker (1977) has summarized the various structural levels which are present in proteins. The primary sequence of the protein is formed by condensation between the carboxyl group of one amino acid with the amino group of another amino acid. This covalent peptide bond has a heat of formation of about 100 Kcal/mol which is broken only upon hydrolysis with strong acids or bases and heat, or with enzymes.

The secondary structure consists of α-helices, β-folds, and β-turns. The helical regions are stabilized by multiple hydrogen bonds, an average of 3.6 bonds per turn, with a heat of formation of about 1-5 Kcal/mol. Water competing for binding can easily disrupt a hydrogen bond. The β-pleated sheets are primarily stabilized by hydrogen bonds, but hydrophobic and electrostatic bonds between the side chains of the amino acid residues also contribute to the structure maintenance. These β-regions are formed through inter or intrachain interactions between chain segments brought close together by β-turns.

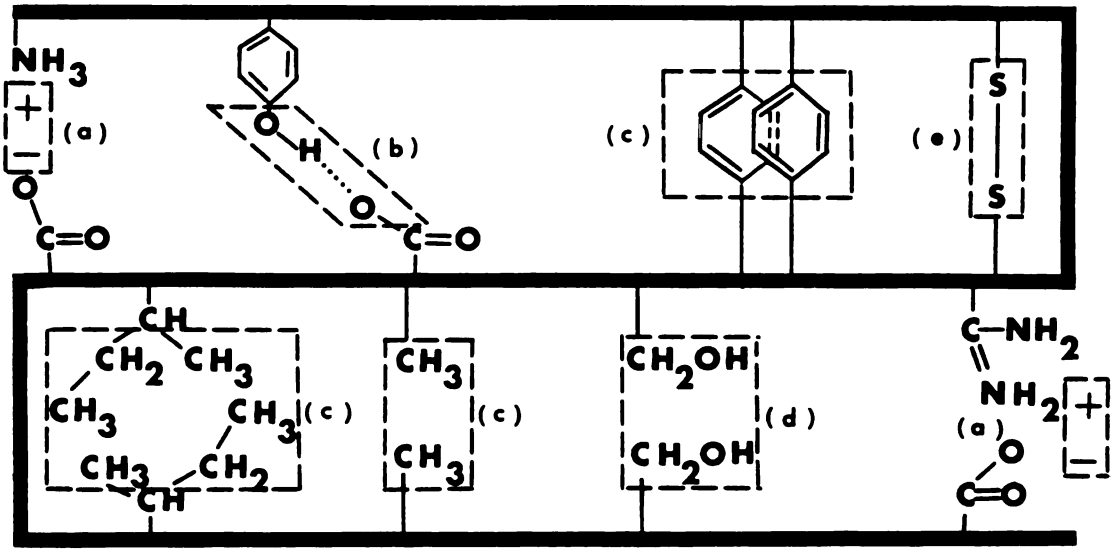
The tertiary structure is maintained by a combination

Table 3. Reactive Groups of Proteins (Olcott and Fraenkel-Conrat, 1947).

Group	Structure	Origin
Amino	$-NH_2$	Lysine, peptide chain end
Guanidyl	$-NHCNH_2$ (=NH)	Arginine
Imidazole	$-CH=CH$ (N, NH, CH)	Histidine
Amide	$-COONH_2$	Glutamine, Asparagine
Aliphatic hydroxyl	$-OH$	Serine, Threonine
Indole	$-C$ (=) HC — NH	Tryptophan
Thimethyl	$-SCH_3$	Methionine
Disulfide	$-S-S-$	Cystine
Phenol	$-C_6H_4-OH$	Tyrosine
Sulfhydryl	$-SH$	Cysteine
Carboxyl	$-COOH$	Glutamic acid, Aspartic Acid, peptide end chain

Table 4. Types of Chemical Bonds in Proteins (Wehrli and Pomeranz, 1969).

Bond	Mechanism	Energy (Kcal/mol)
Covalent	2 atoms bound by a common electron pair	30-100
Ionic (Electrostatic)	Attraction between opposite charges	10-100
Hydrogen	Affinity of hydrogen for electronegative atom (i.e. oxygen)	2-5
Van der Waals	Long range interaction between nonpolar groups	up to 0.5



From Anfinsen (1959)

Fig. 1. Schematic diagram of bonds within and between polypeptide chains. (a) electrostatic interaction; (b) hydrogen bonding; (c) hydrophobic interaction; (d) dipole-dipole interaction; (e) disulfide bond.

of covalent, electrostatic, hydrogen, and hydrophobic bonds and by Van der Waals attractive forces. Of these bonds, the covalent disulfide bond is the strongest with an energy of 50 Kcal/mol.

The quaternary structure of the protein is formed by the association of two or more polypeptide chains. These are held together by the same types of bonds which stabilize the tertiary structure.

Albumen Proteins

The conformation of the majority of the proteins present in egg white has not been elucidated. However, several studies have documented the amino acid composition of a number of them. The amino acid composition of ovomucin, lysozyme, ovomucoid, conalbumin and ovalbumin, reported by several investigators, is summarized in Table 5.

Ovalbumin is a monomeric and nearly spherical globular protein (Osuga and Feeney, 1977). Approximately 50% of the amino acid residues in ovalbumin are hydrophobic, and the protein contains four sulfhydryl groups and one disulfide (Table 5). The thiol groups have variable reactivity towards several chemicals. Nitroprusside, ferricyanide and porphyridin do not react with native ovalbumin (Mirsky and Anson, 1936; Greenstein, 1938), whereas iodoacetate, iodoacetamide, p-chloromercuribenzoate (Anson, 1940; Marshall

Table 5. Amino Acid Composition of Egg Albumen Proteins.

Amino Acid	Ovomucin[a] (moles/ 10^5g)	Lysozyme[b] (residues/ mol)	Ovomucoid[c] (residues/ mol)	Ovotransferrin[d] (residues/mol)	Ovalbumin[e] (residues/ mol)
Acidic					
Aspartic Acid	84.3	21	30.1	82.0	31
Glutamic Acid	95.3	5	13.8	77.4	50
Basic					
Lysine	46.8	6	12.8	64.9	20
Histidine	18.5	1	4.4	13.3	8
Arginine	30.4	11	6.0	35.6	19
Aliphatic					
Glycine	53.5	12	14.7	56.0	18
Alanine	51.4	12	10.8	54.8	34
Valine	52.7	6	14.9	54.5	30
Isoleucine	32.5	6	2.9	28.0	24
Leucine	69.1	8	10.8	54.7	32
Aromatic					
Tyroxine	24.9	3	5.7	22.3	9
Phenylalanine	31.2	3	5.1	28.5	20
Tryptophan	10.9	6	---	12.7	3
Hydroxyl					
Threonine	75.4	7	13.8	36.6	15
Serine	90.2	10	11.5	45.7	36
Sulfur					
Cystine(half)	50.2	8	16.3	29.1	6[f]
Methionine	17.7	2	1.8	13.1	15
Imino					
Proline	67.9	2	7.2	28.9	16

a - Robinson and Monsey (1972).

b - Jolles et al. (1963).

c - Davis et al. (1971).

d - Wenn and Williams (1968).

e - Fothergill and Fothergill (1970).

f - 4 Sulfhydryls, 1 Disulfide.

and Neuberger, 1972 ; MacDonnell et al. , 1951) will react with some, but not all, of the sulfhydryl groups. This masked nature of the protein also restricts the reactivity of other groups such as phenolic hydroxyl (Crammer and Neuberger, 1943), lysine residue (Steven and Tristram, 1958), and carboxyl groups (Atassi and Rosenthal, 1969). Upon denaturation, the phenolic hydroxyl in tyrosine residues ionize (Crammer and Neuberger, 1943).

Fothergill and Fothergill (1970) reported that the C-terminal sequence of ovalbumin is Cys-Val-Ser-Pro. These authors also pointed out that one end of the disulfide was located in this C-terminal peptide.

A single polypeptide chain forms conalbumin (Bezkorovainy et al., 1968; Greene and Feeney, 1968). The primary sequence of conalbumin has not been completed, but indications do not support the presence of two identical half structures (Bezkorovainy and Zschocke, 1974). Williams (1975A, 1975B) isolated a 35,000 daltons C-terminal fraction which contained all of the carbohydrate present in the protein.

The amino acid composition of conalbumin (Table 5) indicates the presence of approximately 15 disulfide bonds and about 55% of reactive residues. These include 159 potentially negatively charged groups, about 65 amino groups, 22 phenolic groups, and 35 guanidine groups.

Ovomucoid has a single polypeptide chain with helical regions (Ikeda et al., 1968) and eight disulfide groups

in the chain. Kato et al. (1974) reported on the presence of three similar domains in ovomucoid. Two fragments of molecular weights of 15,000 and 10,000 daltons were isolated from CNBr-treated ovomucoid (Beeley, 1972). Andrews (1965) suggested that ovomucoid is probably an expanded molecule, rather than compact. Donovan (1967) also concluded from hydrodynamic parameters that ovomucoid is neither compact nor highly asymmetric, but is highly hydrated due to the presence of carbohydrate moieties.

Approximately 60% of the amino acid residues in ovomucoid are potentially reactive (Table 5). Of these residues, about 44% are acidic.

Ovomucin is an extremely large protein probably composed of extended glycoprotein chains held together by disulfide links (Gottschalk et al., 1972). The protein appears to be in a flexible, extended-β or random-coil conformation containing little or no α-helix (Donovan et al., 1970). Tomimatsu and Donovan (1972) indicated that ovomucin is composed of short chains cross-linked in a branching arrangement and retains a random-coil conformation upon alkaline hydrolysis. Robinson and Monsey (1975) observed that ovomucin is an aggregate (molecular weight 720,000) composed of chains of globular units, each with an approximate molecular weight of 103,000 daltons.

Ovomucin has a relatively high content of disulfide groups. About 180 residues are acidic, 96 basic and 260 are apolar (hydrophobic) residues (Table 5).

Lysozyme is the only protein in egg white with a completely elucidated conformation. The amino acid sequence of this protein was characterized by Canfield (1963) and its conformation was determined with x-ray analysis by Blake et al. (1966A). The molecule has an ellipsoidal shape with a marked cleft on one side, and dimensions of about 45 x 30 x 30 Å. The main chain has a relatively small proportion of α-helix, three runs, each about 10 residues long, and long stretches with irregular conformation. Several of these stretches have an extended β-pleated sheet arrangement.

The charged side chains, acidic and basic, and the terminal groups are distributed over the surface of the molecule. The remaining polar chains also appear to be on the surface whereas the majority of the non-polar (hydrophobic) chains are in the interior of the molecule. A number of hydrophobic groups are located on the surface of the cleft where the active site of the enzyme is situated.

Lysozyme appears to exist as a dimer between pH 5 to 9 (Sophianopoulos and VanHolde, 1964). The contact between the molecules does not obstruct the access to the active site (Blake et al., 1966B).

The proteins classed as globulins are poorly characterized. The fraction identified as G_2 globulins has 0.8% tryptophan, 3.35% tyrosine, and traces of sulfhydryl and nucleic acids (Feeney et al., 1963).

Ovomacroglobulin has, in 10^5 g of protein, about

143 acidic residues, 80 basic amino acids, 11.5 half cystine and approximately 235 hydrophobic groups (Miller and Feeney, 1966). Donovan et al. (1969) reported that the protein was nearly spherical and had little α-helix.

Protein Denaturation

Protein denaturation is a familiar phenomenon though it means different things to different researchers. In all interpretations the most accepted concept is that of any modification involving the secondary, tertiary or quaternary structure, without the rupture of any primary covalent bonds. Native proteins can show subtle or major changes in conformation during denaturation, usually through disulfide bond scission (Kinsella, 1976). These changes increase the protein interfacial area and often result in a slight increase of bound water. However, the effects of protein unfolding (denaturation) followed by protein aggregation, result in an overall reduced solubility (Fennema, 1977).

The extent and rate of denaturation may vary with protein sources and causative factors such as heating, freezing, radiation, extreme dilution, sonication, and exposure to air-water or oil-water interfaces (Kinsella, 1976). Several denaturating chemical agents act by disrupting specific secondary forces which maintain the protein structure. Urea, guanidine hydrochloride and some

surfactants break hydrophobic interactions (Gordon and Warren, 1968). Nonetheless, some anionic detergents may stabilize proteins against denaturation (Hegg and Lofquist, 1974).

Perutz (1974) suggested that denaturation by acid or alkali involves the combined effects of total surface change of the protein and the ionization of specific groups on particular amino acids. These ionized groups attract water molecules which, in turn, disrupt hydrophobic associations causing unfolding.

The partial or total denaturation of protein molecules is obviously an important parameter of significant effects on protein functionality. Solubility is a critical factor in the functional behavior of proteins in food proteins. However, denaturation is required in the preparation of many foods and in texturization to expose masked reactive groups of the protein for aggregation or a certain degree of association to occur.

Protein Bonding

The importance of the major types of bonds in maintaining the three-dimensional configuration of proteins was discussed in the Protein Structure section. The same types of bonds are also involved in the many phenomena or processes that characterize the behavior of proteins in food systems. Of these bonds, the disulfide (S-S) linkage

has the strongest stabilizing effect in protein conformation and is relatively common in many proteins.

The sulfhydryl and disulfide are potentially the most reactive groups in proteins, though their availability may be hindered by the characteristic conformation of the protein. Nonetheless, a great deal of evidence indicates the sulfhydryl-disulfide interchange phenomena play a major role in proteins functionality. They are involved in aggregation of polypeptide chains to form a coagulum (Jensen et al., 1950), in the polymerization of actin and in the ATP-ase activity of myosin (Poglazov, 1966), in the pre- and post-rigor reactions important in muscle tenderization (Gawronski et al., 1967), and in the formation of the gluten structure of bread dough (Wehrli and Pomeranz, 1969).

One of the first indications of the participation of sulfhydryl groups in aggregation of denatured proteins stems from observations on the nature of coagulums formed by heated bovine plasma albumin (Jensen et al., 1950). Later, a chain-type mechanism for the exchange reaction under protein denaturation conditions was proposed by Jensen (1959). According to this investigator, the sulhydryl group initiates a chain reaction with the disulfide groups as follows:

$$RSSR + R'S^- \rightleftharpoons RSSR' + RS^-$$

$$RSSR' + R''S^- \rightleftharpoons R'SSR'' + RS^-$$

An important consideration in the proposed mechanism is that once a few sulfhydryl groups participate in the reaction, the polymerization or aggregation process may ensue without any further decrease in measurable sulfhydryl groups since, for every disulfide linkage formed, another mercaptide group is generated (Buttkus, 1974).

Evidences of other types of chemical reactions in protein aggregation has been clearly shown in studies of gelatin, a protein with no sulfhydryl or disulfide groups (Harrington and Rao, 1970). Hydrogen bonds are primarily responsible for the gel structure and some hydrophobic and electrostatic interactions may be involved in cross-bonding. The gel structure can be easily disrupted by heating or by addition of 2M KCl solutions at pH 5 to 7.

In extensively denatured proteins several types of bonds may occur. Buttkus (1974), in studies of aggregates of egg white and myosins, indicated that a combination of disulfide, hydrophobic, hydrogen and other types of bonds may be involved in protein-protein interactions.

Functional Properties of Proteins

Functional properties of proteins are defined as physicochemical properties that influence some performance aspect, especially in aqueous dispersion, affecting the characteristics of the product favorably (Hermansson and Akesson, 1975).

There are several functional characteristics or physicochemical properties desired in protein-containing products. Some of the broad categories are summarized in Table 6. These properties, singly or in combination, contribute to structural, foaming, binding, emulsifying, thickening, and gelling qualities in foods as well as to color, odor, flavor, and mouth-feel. The functional properties discussed in this review are specific properties associated with foamability and coagulability.

Viscosity

Proteins absorb water and may swell in this process, thereby imparting thickening and an increase in viscosity of dispersions, slurries, or pastes (Kinsella, 1976). Highly soluble, non-swelling proteins have low viscosity, whereas soluble proteins with swelling capacity exert high viscosities. This property is dependent on molecular size, shape and charge of the protein and presence of prosthetic groups, carbohydrates in particular (Kato and Sato, 1972).

Surfactant Properties

The lowering of water surface tension by proteins is primarily caused by absorption and orientation of the molecules at the surface (Peter and Bell, 1930) and this function reflects the protein composition and conformation

Table 6. General Classes of Functional Properties of Proteins Important in Food Applications. (Kinsella, 1976).

General Property	Specific Functional Term
Organoleptic	Color, flavor, odor, texture, mouth-feel, etc.
Hydration	Solubility, dispersibility, water absorption, swelling, gelling, water holding capacity, synerisis, viscosity, etc.
Surface	Emulsification, foaming, lipid binding, flavor binding, stabilization, etc.
Structural	Elasticity, cohesion, chewiness, etc.
Textural	Aggregation, gelation, fiber formation, extrudability, dough formation, etc.
Other	Compatibility with additives, enzymatic, inertness, modification properties.

(Adam, 1941). The tendency is to have the hydrophobic groups arranged on the surface of the liquid whereas the hydrophylic groups are extended into the aqueous phase. Reduction of surface tension facilitates deformation of the liquid phase and, therefore, formation of new surfaces.

Foaming Properties

The ability of air incorporation exhibited by protein solutions depends on the formation of a fully extended, semi-rigid, and coherent protein film absorbed on the

air-solution interface (Thuman et al., 1949). Low surface tension appears to be necessary for readiness of foaming or for initiation of a foam, whereas high viscosity imparts foam stability characteristics (Peter and Bell, 1930).

These concepts have been accepted and further speculations on the mechanisms of foam formation essentially describe the same phenomena with minor variations. In a description of food foams, Kinsella (1976) stated that food foams are dispersed air droplets in a liquid containing a surfactant. The surfactant lowers the surface tension of the liquid and facilitates deformation of the liquid. Proteins with excellent foaming power have the ability to surround air droplets and undergo a certain degree of denaturation followed by limited intermolecular attractions. Such protein-protein interactions enhance the cohesive nature of the film capsule, thereby imparting stability and elasticity to the membrane.

Factors that influence foaming properties of proteins are all related to the effects they have on the inherent physicochemical characteristics of the protein. These factors include protein source, method of preparation, solubility, concentration, pH, temperature, salts, sugars, and lipids (Kinsella, 1976).

The overall net charge on the molecule is affected by pH and it has been suggested that when there is a minimum net charge on the protein complex, which occurs at the isoelectric point, and at very high and low pH values

(Thuman et al., 1949), the rate of spreading (unfolding) of the protein molecule at the air-water interface is maximum (Adam, 1941). In the alkaline range, cations improve spreading and on the acid side of the isoelectric point, anions are effective. Di and tri valent ions with least hydration capacity are the most effective and assistance to spreading may be due to salt formation between the ions in solution and the protein in the film.

The effect of salts on foaming is highly dependent on concentration. Low levels may enhance solubility whereas salting out possibly occurs at higher concentrations. Therefore, varying results have been observed on whippability of soy protein (Watts, 1937; Eldridge et al., 1963), fish protein concentrate (Hermansson et al., 1972), egg foams (Sechler et al., 1959), and wheat protein (McDonald and Pence, 1961).

Excessive whipping of protein solutions produces numerous smaller bubbles resulting in more unstable foams. This effect has been demonstrated by MacDonnell et al. (1955) who reported that reduced angel food cake volumes were obtained when these cakes were prepared with overwhipped egg white. Decrease in bubble elasticity resulting from excessive insolubilization of proteins at the air-albumen interface was pointed out as a possible causative factor by these authors.

Several proteins such as fish protein concentrate and soy isolates (Groninger and Miller, 1975; Eldridge et al.,

1963), whey protein concentrates (DeVilbiss et al., 1974), sunflower isolate (Lin et al., 1974), and oilseed proteins (Lawhorn et al., 1972) exhibit the ability to incorporate air. Therefore, these proteins have potential food uses in aerated products.

Gelation (Coagulation)

Protein gels are composed of three-dimensional matrices of partially associated polypeptides with water held in the interstices (Kinsella, 1976). Ferri (1948) described gelation as a two-stage process initiated by heat denaturation of the protein molecules into unfolded polypeptides and then association of the polypeptides forming the gel matrix. The interactions may involve hydrogen bonds, disulfide bonds, hydrophobic associations, or a combination of these (Catsimpoolas and Meyer, 1970).

Typical protein gels are coagulated egg white, soybean curd (tofu), casein curd (cheese), and gelatin jelly. Studies on gelation properties indicate most other proteins have the capacity to form gels upon heat treatment. These include soy proteins (Saio et al., 1974), fish protein (Hermansson and Akesson, 1974), leaf protein (Lu and Kinsella, 1972), sunflower, fababean, and field pea proteins (Fleming et al., 1975). Protein concentration, pH, salts, sugar, lipids, and temperature all affect firmness and characteristics of protein gels (Catsimpoolas and Meyer,

1970; Saio et al., 1974; Fleming et al., 1975; Lu and Kinsella, 1972).

Albumen Proteins Functionality

Studies concerning characterization of individual albumen proteins functionality reflect the possibility of determining which proteins are altered during processing.

MacDonnell et al. (1950) have demonstrated that shear stress forces caused by homogenization, and not pressure, are the factors responsible for damaging the whipping ability of egg white during spray drying. The high degree of surface formation, such as is found during atomization, has also been indicated as a causative factor (Bergquist and Stewart, 1952), as well as heat treatment (Clinger et al., 1951; Cunningham and Lineweaver, 1965; Hill et al., 1965; Garibaldi et al., 1968).

Apparently, one of the proteins associated with the detrimental effects of physical treatments on whippability is ovomucin. Forsythe and Bergquist (1951) studied the ovomucin fraction in blended and homogenized egg white and observed that the decrease in whipping ability and in volume of angel food cake prepared with homogenized egg white appeared to be related to a decrease in ovomucin fibers length.

In studies with pasteurized egg white, Garibaldi et al. (1968) indicated that the heat induced formation of

ovomucin-lysozyme complex followed by denaturation and aggregation was associated with the longer whipping of the pasteurized stabilized egg white. Disappearance of ovomacroglobulin was also observed with the treatment.

Addition of whipping aids to pasteurized egg white and to spray-dried albumen are required to restore the foaming ability of these products. The anionic surface-active agents, such as sodium lauryl sulfate, are effective for dried albumen while cationic and nonionic agents do not improve foaming. On the other hand, sodium lauryl sulfate has limited effectiveness for pasteurized liquid and frozen products. For these products an organic ester, triethyl citrate, is commonly used, and bile salts and fatty acids salts have been reported to be effective as well (Bergquist, 1977). Mechanisms by which these whipping aids function are not known, but their variable effectiveness suggest the alterations caused by the different processes, may involve other proteins or other underlying effects.

Foaminess

Of the several studies conducted on foaming properties of individual albumen proteins, the work of MacDonnell et al. (1955) is the only reported investigation utilizing a food system for testing. They isolated globulins, lysozyme, and ovomucin from egg white and through successive

tests in angel food cake, determined that the whipping ability of egg white depended on globulins and lysozyme, while the foam stability was associated with ovomucin. They also pointed out ovalbumin, easily coagulated with heat, as the protein responsible for supporting the structure of angel food cakes.

Nakamura (1963) studied the spread surface monolayer characteristics of several albumen proteins and observed that ovomucin, globulins, and conalbumin, which were easily spread (and denatured to a certain extent), have high foaming power, whereas lysozyme, ovomucoid, and ovalbumin resist spreading, and, therefore, exhibit low foaming characteristics. Later, Nakamura (1964) showed that changing ovalbumin conformation through denaturation with heat, alkali or acid, improved foaming power as much as three times over that of the native form. He also observed the increase in surface life time of the denatured protein monolayer.

In investigations of egg albumen under repeated whipping conditions, Cunningham (1976) reported the retention of ovomucin, globulins, lysozyme, and to a lesser extent, of conalbumin in the foam of beaten egg white. Nakamura and Sato (1964), using a similar approach, observed the dependence of egg-white foam stability on the highly viscous ovomucin (B) fraction.

Mechanisms of foam formation have been proposed. Proteins with good foaming ability are easily surface

denatured and form a well developed network through peptide linkages. These linkages appear to be dependent on the presence of a high ratio of non-polar/polar side chains in the protein (Nakamura, 1963). Meyer and Potter (1975), in a study of whipped albumen with sodium hexametaphosphate and triethyl citrate plus trisodium citrate, pointed out the correlation between stable foams and denaturation of proteins at the air-albumen interface. They further suggested sodium hexametaphosphate and trisodium citrate increase foam stability possibly through cross-linking of the proteins and triethyl citrate improves foamability through ovalbumin denaturation.

The effect of chemical modification of egg white on functional performance has been investigated. Egg white altered with 3, 3-dimethyl glutaric anhydride exhibited no heat coagulation ability, but foaming capacity was not altered appreciably (Gandhi et al., 1968). Grunden et al. (1974) treated egg white with several proteolytic enzymes and noted a considerable increase in foaming capacity with the treatments, but an adverse effect on foam stability.

Gelation

Although the gelation properties of isolated albumen proteins from the stand point of food applications have not been characterized, the effect of temperature on several proteins has been well documented. Most of these reported

studies have been undertaken for evaluation of factors influencing their biological activities.

MacDonnell et al. (1953) studied the virus antihemagglutinin activity of ovomucin and noted the extreme stability of the protein to heating at 70 and 100°C for 30 and 60 minutes, respectively, and at pH values ranging from two to eleven.

Ovomucoid is remarkably stable to heating at 100°C for 30 min., in acidic solution (Fredericq and Deustch, 1949). The antitryptic activity of this protein is destroyed with a heat treatment of 80°C for 30 min. at pH 9, but not at pH 7 and below (Lineweaver and Murray, 1947).

Lysozyme has been shown to withstand heating at 100°C in acidic solution with little loss of lytic activity (Smolelis and Hartsell, 1952). However, on the alkaline side, the protein loses enzymatic activity or becomes insoluble (coagulates), with rates depending on pH and temperature (Sandow, 1926; Beychock and Warner, 1959).

Conalbumin is very heat sensitive and denatures on heating at 61°C for 3-4 min. at pH 5.5-5.6, although not at pH 7.0 (Kline et al., 1953). Azari and Feeney (1958) showed that the iron-complex form was much more stable to heating at 64°C than the metal-free protein, and no precipitation occurred even after one hour of heating.

Several studies have reported on the effect of heat on ovalbumin. Chick and Martin (1911, 1912) indicated

that the increase in rate of heat denaturation of ovalbumin was extremely high with relatively small increments in temperature. Lewis (1926) showed that ovalbumin is most stable to heating between pH 6.5 and 7.0 and found minimum denaturation even after 30 min. at 65°C.

The gelation ability of denatured ovalbumin with urea was characterized by McKenzie et al. (1963). They noted that with faster rate of unfolding and higher concentration of polypeptides, a finer gel network was formed. At pH 3 in 7M urea there was a rapid unfolding of the polypeptide, but aggregation did not ensue.

Cunningham and Lineweaver (1965), in studies of stability of egg white to pasteurization conditions, indicated that globulins G_2 and G_3 are fairly heat stable at 60°C in egg white adjusted to pH 6.

Recently, Egelandsdal (1980) reported on the characteristics of ovalbumin gels obtained at various pH and ionic strength values. At low ionic strengths maximum gel rigidities occurred on each side of the isoelectric point of the protein. Increasing the ionic strength shifted the maxima away from the isoelectric point. The firmest gel was formed on the acid side which also correlated with a high net charge on the molecule. At very high net charges, on both alkaline and acid sides, rigidity was minimum. The author also suggested that ovalbumin gelling was primarily the effect of electrostatic attractions.

EXPERIMENTAL PROCEDURE

In order to investigate the foaming and gelling abilities of several egg albumen proteins and mixtures thereof, the experimental procedure was divided into five sections. The first section consisted of a selective fractionation of the egg white into six major proteins which constituted the variables in this study. The second sequence consisted of statistically planning the experimental design to minimize the number of observations for assessing the functional properties of the variables of interest. The third and fourth series described the testing of the proteins in an angel food cake to measure foamability and a model depicting custard gels for measurement of gelling properties, respectively. Lastly, selected samples were prepared for examination in transmission and scanning electron microscopes.

Protein Fractionation

All chemicals used in this section were ACS reagent grade. Proteins used as standards were ovalbumin (lot no. 105C-8022), lysozyme (lot no. 75C-8483), conalbumin (lot no. 46C-8125), globulins (lot no. 95C-8115), and ovomucoid (lot no. 66C-8120), all obtained through Sigma

Chemical Company (St. Louis, Missouri).

Source of Eggs

Approximately one day old eggs from Single Comb White Leghorn hens, strain H & N, were purchased at a local farm. The hens were, on an average, 11 to 12 months old and were fed a ration containing a commercial premix, corn and soy meals, and calcium salts. Eggs were brought back to the laboratory and separation of the white from the yolk proceeded immediately.

Egg Albumen Preparation

Eggs were thoroughly washed under cold running tap water, the shell broken, and the white separated from the yolk. Any chalaziferous material present in the whites was removed with a pair of tweezers. The whites were weighed and gently homogenized in a Osterizer blender, model 857-05JX at the slowest speed and lowest settings available for 30 to 45 seconds. The blender jar was filled two-thirds of its maximum capacity and a commercial food wrapping film was carefully layered on the surface of the whites and around the jar walls to eliminate air filled spaces and prevent incorporation of air during blending. Following homogenization, the albumen was treated as shown in Figure 2 to isolate the various proteins. Because of the lengthy procedure, and the considerably large quantity

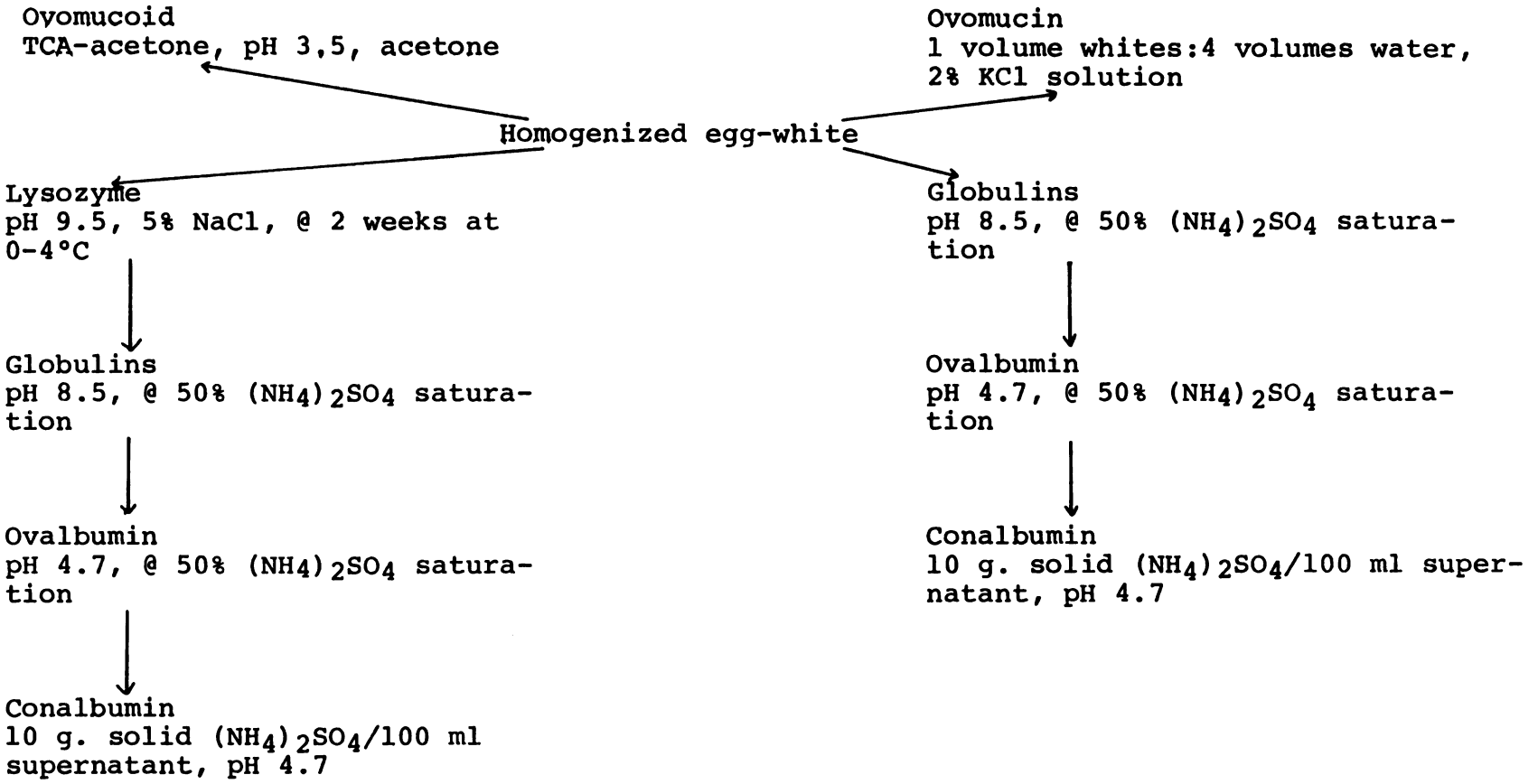


Fig. 2. Flow diagram of the isolation of egg albumen proteins.

of proteins required, several batches of whites were prepared to solve the bulk handling problems and minimize the deterioration of samples. Referring to Figure 2, a single batch was divided into two equal portions for the preparation of globulins and lysozyme. The supernatants obtained from the preparations were subsequently treated to prepare the other proteins. Separate egg white batches were used for isolation of ovomucoid and ovomucin.

Globulins

This protein fraction was isolated using a modification of the procedure described by MacDonnel et al. (1955). To approximately 1500.0 g of homogenized albumen an equal volume of a saturated ammonium sulfate solution was added and with constant stirring the pH of the resulting solution was adjusted to 8.5 with a 1N potassium hydroxide solution. After stirring proceeded for an additional 30 minutes, the solution was centrifuged in a Damon/IEC B-20A centrifuge, model 3444 at 10,000 rpm (15,000XG) for 5 minutes. The temperature of the centrifuge chamber was kept at 4°C throughout centrifugation. Following separation, the supernatant was saved for ovalbumin preparation, the precipitate was removed from the centrifuge bottles and redissolved in 600 ml of deionized water. To this solution an equal volume of saturated ammonium sulfate solution was added and the pH readjusted to 8.5. After being kept at 2-4°C overnight, the precipitate was removed by

centrifugation as previously described. This procedure was repeated four times to ensure the removal of the other proteins. The supernatants from the first and second washings were stored at 2-4°C for further treatment to obtain conalbumin. After the final wash the protein was redissolved in 400 ml of deionized water and poured into a dialysis membrane of 3.6 cm of diameter, and a molecular weight cut-off of 12,000 to 15,000. The ends were securely closed by tight knots and the tubing was placed in a large stainless steel bucket filled with deionized water. The dialysis set up was kept at 2-4°C and daily changes of the water was performed to accelerate the removal of the ammonium sulfate salt. The presence of the salt ions was monitored with barium chloride. To a small volume of the dialysis water a few drops of a barium chloride solution were added and the formation of an insoluble barium sulfate precipitate was taken as a qualitative measure of the presence of the salt ions. Dialysis continued until the water was free from sulfate ions. At the end of this period the protein solution was again centrifuged to remove a large precipitate usually formed during dialysis. The clear supernatant containing the soluble globulins fraction was transferred to several nine inch round aluminum pie pans and kept frozen at -23°C until further treatment.

Ovalbumin

The procedure used for the preparation of ovalbumin was a modification of the method used by Sörensen and Höyrup (1915-17). The supernatant obtained from the globulins preparation was adjusted to pH 4.7 with the gradual addition of 1N sulfuric acid solution and constant stirring. The resulting creamy white mixture was kept undisturbed at 2-4°C overnight. The precipitated protein was then removed by centrifugation using the same conditions as described for globulins preparation. The supernatant was put aside for further treatment to obtain conalbumin and the precipitate was dissolved in 800 ml of deionized water. An equal quantity of saturated ammonium sulfate solution was added, the mixture stirred, and kept for at least 15 hours at 2-4°C. Following this period, centrifugation was again performed, and the precipitated mass was redissolved in 800 ml of deionized water. Washings of the precipitated protein were repeated three additional times and then the protein fraction obtained was dissolved in 500 ml of deionized water and dialysed as previously described.

The supernatants from the first two washings were saved for conalbumin preparation. The salt free protein solution was equally divided into round aluminum pie containers, and stored at -23°C for further treatment.

Conalbumin

This protein was prepared as the iron-free complex following the procedure outlined by Warner and Weber (1951) with several modifications. The supernatants obtained from the ovalbumin fractionation were combined and 10 grams of solid ammonium sulfate salt per 100 ml of supernatant were added. Following complete dissolution with constant stirring, the mixture was allowed to stay at room temperature for two hours, and then centrifuged at 10,000 rpm (15,000XG) for 5 minutes and at 4°C. The supernatants from globulins washing were adjusted to pH 4.7 with 1N sulfuric acid solution and then conalbumin was salted out with the addition of solid ammonium sulfate (10 grams/100 ml supernatant). This mixture was left undisturbed for two hours at room temperature followed by centrifugation at the end of this period. The precipitates obtained from both batches were combined, dissolved in 600 ml of deionized water and dialysed at 2-4°C against frequent changes of deionized water. After the majority of the salt ions were removed, usually after three days, the solution was centrifuged to separate a small precipitate formed during dialysis. The clear solution was then adjusted to pH 6.0 with 1N potassium hydroxide solution and made 0.02 M sodium chloride with a 1M sodium chloride solution. With constant monitoring of the solution pH, a 50% ethanol-0.02 M sodium chloride solution was gradually added to a final concentration of

20% ethanol-0.02 M sodium chloride. This solution was then kept at 2-4°C for two to five days to effect the crystallization of the protein. After a fairly large precipitate was formed, the mixture was centrifuged at 12,000 rpm (20,000XG) for eight minutes and at a temperature of -10°C. The mixture was kept below 0°C at all times during centrifugation to prevent redissolution of the protein and maximize its recovery. The supernatant was discarded and the material obtained was dissolved in 500 ml of deionized water. This solution was again made 20% ethanol-0.02M sodium chloride, pH 6.0, and the entire procedure was performed 4 additional times to remove other proteins. After the final wash the protein solution was dialysed at 2-4°C against deionized water until the dialysate was free from chloride ions. The presence of these ions was detected with a silver nitrate solution.

Following dialysis, the yellowish clear protein solution was transferred to round aluminum pie pans and stored at -23°C.

Lysozyme

Lysozyme was obtained utilizing the crystallization method of Alderton and Fevold (1946). To approximately 1500.0 grams of homogenized egg white was added solid sodium chloride to a final concentration of 5%. After complete dissolution of the salt, the pH was adjusted to 9.5 with a 1N potassium hydroxide solution. A very small

amount of crystalline lysozyme was added without stirring, and the prepared albumen was left at 2-4°C for at least two weeks to effect the crystallization of the protein. During this period the pH was checked from time to time and readjusted to 9.5 when necessary. The crystalline material formed was then removed by centrifugation at 8,000 rpm (9,500XG) for five minutes and at a temperature of 4°C. The supernatant was further used for the preparation of globulins, ovalbumin, and conalbumin, as previously described, while the crystalline mass was dissolved in 500 ml of 0.1 M acetate buffer, pH 4.3. The insoluble material in the solution was removed by centrifugation at the conditions described above. The clear supernatant was again made 5% with respect to sodium chloride, the pH adjusted to 9.5, and placed in the refrigerator. Subsequent crystallizations usually occurred after 3-4 days at 2-4°C and were repeated three times. After the last crystallization, the material obtained was dissolved in 400 ml of the acetate buffer and dialysed against deionized water. The dialysis membrane used was a spectrapor membrane tubing with a diameter of 7.64 cm and a molecular weight cut off of 6,000-8,000. After removal of the salt ions, the protein solution was placed in round aluminum pie containers and kept at -23°C until further treatment.

Ovomucoid

The procedure utilized for the fractionation of

ovomucoid was a slight modified method outlined by Lineweaver and Murray (1947). To portions of homogenized whites ranging from 1000.0 to 1500.0 grams were added an equal volume of a trichloroacetic acid-acetone solution or until a pH of 3.5 was attained. The trichloroacetic acid-acetone solution consisted of a mixture of 1 volume of 0.5 M trichloroacetic acid and 2 volumes of acetone. The thick creamy white mass was centrifuged at 8,000 rpm (9,500 XG) for five minutes at 4°C, and the precipitate discarded. To the greenish-yellow clear supernatant were added approximately 2.5 volumes of acetone. The mixture was left undisturbed for approximately 15 minutes or until the precipitate settled. The clear upper solution was removed by suction with a glass tubing connected to a water pump. Three additional acetone washings were done, the residual acetone removed by centrifugation, and the precipitate dissolved in 500 ml of deionized water. The protein solution was adjusted to pH 4.5 with a 1N potassium hydroxide solution, and dialysed, using the same procedure as described for globulins, for approximately eight days. The protein solution was then placed in aluminum pie containers and kept frozen at -23°C.

Ovomucin

The procedure adopted for obtaining ovomucin has been described by Robinson and Monsey (1971). Since

drying or freezing adversely affect the solubility of this protein, fractionation was performed just prior to its use. A small-scale separation was done to test the adequacy of the purification procedure. The protein obtained in this preparation was stored at -23°C for further testing.

To approximately 1000.0 grams of homogenized whites were added four volumes of deionized water and the resulting mixture was stirred for 15-20 minutes. Following this period the precipitate was removed by centrifugation at 13,000 rpm (21,000 X G) for five minutes at 4°C. The supernatant was discarded, the precipitated material dispersed in 2000 ml of deionized water, and centrifuged again. The protein mass was redispersed in 2000.0 ml of a 2% potassium chloride solution, the mixture stirred for 30 minutes and then centrifuged. Washings with the potassium chloride solution were repeated several times until the wash solution contained no measurable amount of proteins. The presence of these proteins was detected by measuring the absorbance of the wash solution on a Beckman spectrophotometer, model DB-G, at 290 nm. After the contaminating proteins were removed from the amorphous ovomucin mass, approximately 2000.0 ml of deionized water were added, the mixture stirred for about 30 minutes and then centrifuged. After this stage it was very difficult to separate the translucent ovomucin mass by centrifugation; therefore the wash water was removed by filtering the mixture through 4 layers of cheese cloth. This procedure was repeated until the wash water, monitored with a silver nitrate

solution, was free from chloride ions. The pH of the preparation was checked, adjusted to 8.0 with 1N potassium hydroxide, and moisture was determined to estimate the protein concentration. For preparing the cake containing only ovomucin, the excess moisture was removed under vacuum with frequent changes of phosphorus pentoxide at 2-4°C.

Freeze-Drying

The frozen protein preparations were covered with perforated wrapping films and subsequently freeze-dried for 3-4 days in a Virtis Unitrap II freeze-drier with a capacity of eight liters, and equipped with a clear drum of dimensions 43.2 cm high x 30.5 cm diameter (17 x 12 in). The samples were dried at a system pressure of $4 - 6 \times 10^{-2}$ Torr and trays temperature of approximately 40-50°C. The dried materials were weighed and reduced to a fine powder by blending in a Osterizer blender, model 857-05 JX, set at high speed, except for the globulins fraction which was broken into small pieces. The proteins were placed in tightly covered glass jars and stored at -23°C.

Removal of Iron from Conalbumin

Small amounts of iron present in the conalbumin preparation were removed by treatment with a resin as

described by Warner and Weber (1951). Dowex-1, 1 x 8 - 50, chloride form, was recycled by suspending in a 10% sodium chloride, following by stirring for 15 minutes. The excess chloride ions were removed by several washes with deionized water. About 4 g of protein were dissolved in 200 ml of a 0.01 M potassium citrate buffer, pH 4.7, and approximately 10 g of the recycled resin were added to the resulting solution. After stirring for 30 minutes the mixture was filtered through filter paper no. 4, followed by three washes of the resin with approximately 10 ml of citrate buffer. The filtrate was again treated with fresh resin two additional times and then dialysed and freeze-dried as previously described. The dried protein mass was reduced to a fine powder and stored in securely closed glass jars at -23°C.

Assessment of Purity-Purification

Each individual batch of prepared protein was tested for purity by a disc-type polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). If impurities were estimated to exceed 10%, the proteins were purified by employing the same washing or crystallization procedure used for their isolation. Relatively pure batches (over 90%) of the same protein were combined to form a common uniform lot for the functionality tests. Purification by gel filtration of small quantities of proteins

containing between 5-10% of contaminating proteins were performed for testing of the individual proteins functional properties.

Electrophoresis. PAGE was performed using a modified procedure described by Ornstein (1964), and Davis (1964), and the gels for SDS-PAGE were prepared according to the method outlined by Laemmli (1970) except that spacer gel was not used in both preparations. The electrophoresis apparatus was a Buchler Polyanalist, model 3-1750, connected to a Beckman Duostat power supply, model RD. Glass tubes of dimensions 10.0 cm long x 0.5 cm I.D. x 0.8 cm O.D. were carefully cleaned by immersing in a chromerge solution for 24 hours, followed by rinsing with cold tap water and distilled water. A final rinse with a photoflo solution (1:200) was done, the tubes drained and allowed to dry.

For PAGE the stock gel consisted of a 7.5% polyacrylamide (cyanogum 41) solution in a 0.38 M tris-hydrochloric acid buffer, pH 8.91, the running buffer a 0.055 M tris-0.767 M glycine buffer, pH 8.3, and the sample buffer a 0.062 M tris-hydrochloric acid buffer, pH 6.7. The stock gel and sample buffer were kept refrigerated between uses, and the chamber buffer was freshly prepared.

To 28 ml of stock gel were added 35 ul of N, N, N′, N′-tetra methylethylenediamine (TEMED) and 130 ul of a 5% ammonium persulfate solution, freshly made. The solution

was swirled to mix the chemicals and immediately poured with a syringe filling the prepared tubes to about 1.5 cm from the top. Water was then carefully layered on top of the gel solution to exclude oxygen and allow for polymerization to take place (usually completed after 20 to 30 minutes). The samples were prepared by dissolving approximately 1 mg of protein in 1 ml of the sample buffer contining 0.2 ml of a saturated sucrose solution and 0.01 ml of a bromophenol blue solution. Approximately 20 to 50 ug of each protein were layered on top of the gels, and the upper and lower reservoirs were filled with the chamber buffer. Electrophoresis was run at 1 mA/tube for 1 hour and then at 2 mA/tube until the tracking dye was at the bottom of the tube. The gels were removed from the tubes by squeezing water between the gel and the tube wall with a microsyringe connected to a tap water supply. Gels were stained with a 1% amido black, 50% methanol, 10% acetic acid solution for five minutes and destained by diffusion in a 7% acetic acid solution.

For SDS-PAGE, stock solutions consisted of a stock gel prepared with a 37.5:1:150 mixture of acrylamide, N,N′-methylenebisacrylamide (Bis) and water, on a weight basis, a 1.5M tris-hydrochloric acid buffer, pH 8.8, a 0.2M ethylenedinitrilo tetraacetic acid disodium salt (EDTA) solution and a 10% sodium dodecyl sulfate (SDS) solution. The first three solutions were kept refrigerated while the SDS solution was stored at room

temperature. The sample buffer was a 1% sodium dodecyl sulfate, 0.05 M tris-hydrochloric acid, pH 6.72, 0.05% mercaptoethanol, 0.001% bromophenol blue, containing 10 ml of a saturated sucrose solution. This buffer was stored in a plastic bottle in the freezer. For the gel preparation, to 10 ml of the stock gel were added 3 ml of the tris-HCl buffer, 0.3 ml each of the SDS and EDTA solutions, 0.015 ml of TEMED, 11.3 ml of water and 0.6 ml of a 5% ammonium persulfate solution, freshly prepared. The solution was immediately poured with a syringe, filling the tubes to 1.5 cm from the top. Oxygen was excluded by layering n-butanol on top of the gel solution and polymerization was allowed to take place. After the gel had hardened the butanol was removed by several rinses with distilled water and then with the chamber buffer. This buffer consisted of a 0.05 M tris - 0.38 M glycine solution, pH 8.41, and containing 10 ml each of 10% SDS and 0.2 M EDTA solutions. Samples were prepared by dissolving from 0.9 to 1.3 mg of each protein in 5 to 10 ml of the sample buffer and incubating the resulting solution in a 40°C water bath for one hour. From 1 to 6 ug of the cooled samples were layered on top of the gels, the upper and lower reservoirs filled with the running buffer and electrophoresis was run at 1 mA/tube for 3-4 hours or until the tracking dye reached the bottom of the gel. The gels were removed using the previously described technique, and stained overnight with a 0.03% coomassie brilliant

blue (R-250), 50% methanol, 7% acetic acid solution. The gels were destained by diffusion with frequent changes of a 5% methanol - 7.5% acetic acid solution.

The purity of each final common lot (all batches of a single protein combined) was determined quantitatively with the SDS-PAGE by measuring the absorbance of the protein bands in a densitometer at 550 nm. The instrument consisted of a Beckman DU spectrophotometer, model 2400, coupled to a Gilford gel scanner, model 2520, and a Hewlett Packard integrator, model 33805. Gels were scanned at a rate of 2 cm/min while the chart was set to a speed of 1 cm/min.

Purification. Conalbumin, ovomucoid, and globulins were purified by gel filtration with Sephadex G-75-120. The resin was equilibrated with a 0.1M sodium acetate - 0.1M sodium chloride buffer, pH 8.0, for three days at 2-4°C and then poured into a glass column of dimensions 3.5 cm I.D. x 140 cm long, equipped with a glass stopcock. The column was allowed to stabilize for a week by constantly running the acetate buffer through it. At this point the resin had settled to a constant height of 112 cm.

About 1.5 g of protein were dissolved in 5 ml of the acetate buffer containing 1 ml of a concentrated blue dextran solution (5 mg/ml) and 2 ml of a saturated sucrose solution. The prepared sample was carefully layered on the surface of the resin through the buffer, the buffer

tank was connected to the top of the column, and then separation was performed at a flow rate of 0.5 - 0.7 ml/min. Soon after blue dextran was eluted from the column, collection started with a GM chromatography automatic collector. Fraction size collected was 8 ml/tube and the protein concentration was monitored at 280 nm on a Beckman DB-G spectrophotometer.

Chemical Analyses

Duplicate determinations were performed for all chemical tests.

Moisture Analysis

Moisture content determination was carried out in a Hotpack vacuum oven, model 633, at 100°C and a vacuum of 660-711 Torr (26-28 in. Hg) following the official AOAC method 1007 (1975) except that approximately 2.0 g of liquid sample were used. Samples were dried to a constant weight and the dried weight was expressed as the percentage of total solids.

Total Nitrogen

A micro-Kjeldahl method described by McKenzie (1970) was used to determine total nitrogen. Approximately 30 mg of dry sample were digested with 2.5 ml of concentrated

sulfuric acid, 1.5 g of potassium sulfate and 0.5 ml of a 0.46 M mercuric sulfate in 2M sulfuric acid solution. Following digestion the samples were cooled, diluted with about 20 ml of ammonia-free water, and transferred to a micro-Kjeldahl distillation apparatus. To the digest were added 10 ml of a 12.5 M sodium hydroxide - 0.13 M sodium thiosulfate solution and collection of the steam distillate started soon after immersing the condenser tip into 5 ml of a boric acid indicator solution placed in a 50 ml beaker. This solution was prepared by combining 20 g of boric acid and 6.67 mg of methylene blue, dissolved in 800 ml of water, with 13.3 mg of methyl red dissolved in 10 ml of ethyl alcohol, and completing the volume to one liter.

After the distillate-indicator solution reached the 40 ml mark, the beaker was lowered from the condenser tip and distillation proceeded until about 5 ml more of distillate were collected. The tip was rinsed with a few ml of distilled water, the solution quantitatively transferred to a 125 ml erlenmeyer flask and titrated with a 0.02N hydrochloric acid solution to a grey-lilac end point.

Recoveries of nitrogen were determined with dl - tryptophan dried over phosphorus pentoxide for three days. Blanks were run along with the samples and the percentage of nitrogen was calculated with the formula:

$$\%N = \frac{(\text{sample-blank})\text{ml HCl} \times \text{normality(HCl)} \times 14.007 \times 100}{\text{mg sample}}$$

After adjusting the values for the percentage recovery the protein content was calculated by multiplying the corrected nitrogen values by the factor 6.25.

Sulfhydryl Groups

A modification of the method described by Ellman (1959) was used to determine "free" sulfhydryl groups. About 3 to 5 mg of sample were dissolved in 1 ml of distilled water, then 4 ml of a 0.01 M sodium phosphate buffer, pH 8.0 - 1% sodium lauryl sulfate - 0.4% EDTA solution were added. The samples were boiled for 30 minutes, allowed to cool, and then 0.2 ml of a 0.01M 5, 5' dithiobis - 2-nitrobenzoic acid (DTNB) solution in 0.1M sodium phosphate buffer, pH 7.0, was added. The color was allowed to develop for one hour and then the absorbance of the solution was measured at 412 nm in a Beckman DB-G spectrophotometer. Blank determinations were run parallel to the tests and concentration of the sulfyhdryl groups was calculated using a extinction coefficient of 13,600 with the formula:

$$\text{moles SH/g protein} = \frac{(\text{sample-blank})\text{absorbance} \times \text{final volume}}{13{,}600 \times \text{mg sample}}$$

The procedure described above was used for all samples except that for the gel samples a few modifications were introduced. The samples were dissolved in 5 ml of the buffer solution containing 2% SDS. The concentration of

this chemical was raised to prevent reassociation of the polypeptide chains upon cooling. Solutions that were still cloudy were filtered through Whatman filter paper no. 4 before absorbance measurement.

Total Sulfhydryl - Disulfide Groups

The number of disulfide groups was determined by assessing the total sulfhydryl content according to the method of Cavallini et al. (1966). To approximately 1.0 mg of sample were added 1 ml of a 0.05 M sodium phosphate - 0.001 M EDTA buffer, pH 7.4, 0.5 ml of 1-octanol, and 1 ml of a freshly prepared 40:1:40 urea: sodium borohydride:water solution. The resulting solution was incubated in a water bath at 40°C for 30 minutes. Samples were cooled and 0.5 ml of a 1 M potassium phosphate - hydrochloric acid buffer, pH 2.7, was added drop wise to prevent excessive foaming. After five minutes,1 ml of acetone was added and the tubes shaken to complete borohydride destruction. To this solution, 0.2 ml of a 0.01 M DTNB solution in 0.1 M sodium phosphate buffer, pH 7.0, was added and color was allowed to develop for one hour. Before measuring the absorbance at 412 nm the solutions were diluted with 5-8 ml of deionized water, and filtered through filter paper no. 4 to remove 1-octanol. Blank tests were run with the samples and the total sulfhydryl content, expressed as moles of SH/g of protein, was calculated using the formula for the "free" sulfhydryl determination. For

proteins of known molecular wieght, the number of sulfhydryl groups was calculated with the equation:

$$N = \frac{M.W. \times A \times V}{12{,}000 \times m}$$

where MW = Molecular Weight
A = Absorbance
V = final Volume
m = sample weight in mg

The disulfide content was estimated from the difference of total sulfhydryl and "free" sulfhydryl contents.

Elemental Analyses

Trace elements were determined at the Ohio Agricultural Research and Development Center (Wooster, Ohio), using a inductively coupled plasma - optical emission spectroscopy technique as described by Fassel and Kniseley (1974). Samples were prepared according to Kenworthy (1960).

Chlorine and sulfur content were determined by a private food testing laboratory (Micro-Tech Laboratories, Inc., Skokie, IL) using a combustion and titration technique. For chlorine, titration was done with a silver perchlorate solution with dichlorofluorescein as indicator, and for sulfur the samples were titrated with a barium perchlorate solution using dimethylsulfonazo-III as indicator.

The concentration of the minerals present in dried egg-white and the various isolated proteins is shown in Table 7. These values were used to calculate the mineral content of the protein mixtures to be used in the

Table 7. Mineral Composition of Dried Egg-White and Several Isolated Proteins

Mineral	Egg-white	Ovomucin	Lysozyme	Globulins	Ovomucoid	Conalbumin	Ovalbumin
				mg/g			
OARDC[a]							
Potassium	11.41	13.82	1.73	1.11	4.44	2.88	1.00
Calcium	1.10	2.42	0.07	0.16	0.76	0.60	0.30
Sodium	6.09	0.07	0.17	0.12	0.16	0.16	0.08
Phosphorus	1.15	0.22	<0.01	0.23	0.47	0.51	0.77
Magnesium	0.93	0.11	<0.01	0.03	0.19	0.12	0.06
Manganese	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Iron	<0.01	0.05	<0.01	0.01	0.01	0.02	0.01
Boron	<0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01
Copper	<0.01	0.06	<0.01	<0.01	<0.01	<0.01	<0.01
Zinc	<0.01	0.03	0.01	0.01	0.01	0.01	0.01
Aluminum	<0.01	0.15	<0.01	0.01	<0.01	<0.01	<0.01
Strontium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Barium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M-TL[b]							
Chlorine	13.40	3.50	6.35	ND[c]	0.30	0.10	ND[c]
Sulfur	9.04	3.00	5.78	6.50	0.30	4.70	11.40

a - Ohio Agricultural Research and Development Center.

b - Micro-Tech Laboratories

c - not detectable.

functionality tests. Potassium, calcium, sodium, and chlorine were replaced to their original values (levels found in egg-white) with the addition of sodium chloride, calcium chloride, and potassium hydroxide, all in 1N solutions.

Experimental Design

The extreme vertices design for experiments with mixtures described by McLean and Anderson (1966) was used to determine the levels of the various proteins in the formulation of the protein solutions to be used in the angel food cake system. Selection of this design was based on its unique feature of maintaining the sum of the mixture components at a constant value (i.e., $\sum_{i=1}^{k} X_i = 1$ or 100%) while allowing for variation of the component levels within specific ranges, or constraints, chosen by the experimenter. The concentration in albumen of the six proteins of interest are listed in Table 8 (Powrie, 1977). These values were adjusted by a factor to 100% to account for the other minor albumen proteins not used in the mixtures. In the same table are listed the minimum and maximum levels within which each protein was allowed to vary. The maximum value was set at approximately 1.5 times the normal adjusted levels of each protein. The set of treatment combinations found (protein formulations) described a geometrical region with five dimensional faces

Table 8. Proximate Protein Composition of Egg Albumen and Level Range Values of Each Protein

Protein	Normal Values[a]	Adjusted Values[b]	Range[c]	
			Minimum	Maximum
	%	%	%	
Ovomucin	1.50	1.65	0.00	2.50
Lysozyme	3.50	3.80	0.00	6.00
Globulins	8.00	8.80	3.00	13.00
Ovomucoid	11.00	12.10	5.00	18.00
Conalbumin	13.00	14.30	6.00	21.00
Ovalbumin	54.00	59.30	30.00	89.00

a - Powrie, 1977.

b - normal values adjusted to 100%.

c - constraints placed on each protein.

(K-1) with the formulations located at the vertices and centroids of this region. To reduce the number of observations only the centroids of the third and fifth dimensional faces were used. The procedure to find the vertices were to first construct all treatment combinations ($K.2^{K-1}$) with the minimum and maximum levels and leaving one factor's level in blank, e.g. X_1, X_2, --, X_4, X_5, X_6. Next, the blanks were filled with admissible values for each protein (within the constraints) to make the sum of the levels equal to 100% (or unity). This procedure yielded a total

of 32 vertices. The centroids of the 3-dimensional face were found by averaging the four remaining factor levels of the vertices with any two constant factor levels. The 5-dimensional face centroid was obtained by averaging the levels of all the treatments found. Table 9 displays the vertices and centroids of the design used to prepare the cakes, and in the appendix the steps and calculations involved in developing the design are shown in more detail.

For testing each factor separately, additional treatments with 100% of each protein were performed, and a control cake was prepared with the adjusted levels of each protein listed in Table 8.

For the model system representing a custard type gel each factor was allowed to vary from 0 to 100% and tested individually and in combination of two or five proteins. The control gel was prepared with the adjusted levels of the proteins. Table 10 lists the treatments used for preparing the gels.

Angel Food Cake System

The formulation and procedure used to prepare the cakes were adapted from Dam et al. (1970). The quantities of the ingredients were reduced to 50% of their basic recipe and consisted of 30.0 g of protein solution, 30.5 of sugar, 11.0 g of cake flour, and 0.15 g of solid sodium chloride. Variable quantities of cream of tartar

Table 9. Treatment Levels Combinations of Six Proteins Used for the Preparation of Angel Food Cakes.

Treatment Number	Protein					
	Ovomucin	Lysozyme	Globulins	Ovomucoid	Conalbumin	Ovalbumin
			%			
Vertices						
1	0.00	0.00	3.00	5.00	6.00	86.00
2	0.00	0.00	3.00	5.00	21.00	71.00
3	0.00	0.00	3.00	18.00	6.00	73.00
4	0.00	0.00	3.00	18.00	21.00	58.00
5	0.00	0.00	13.00	5.00	6.00	76.00
6	0.00	0.00	13.00	5.00	21.00	61.00
7	0.00	0.00	13.00	18.00	6.00	63.00
8	0.00	0.00	13.00	18.00	21.00	48.00
9	0.00	6.00	3.00	5.00	6.00	80.00
10	0.00	6.00	3.00	5.00	21.00	65.00
11	0.00	6.00	3.00	18.00	6.00	67.00
12	0.00	6.00	3.00	18.00	21.00	52.00
13	0.00	6.00	13.00	5.00	6.00	70.00
14	0.00	6.00	13.00	5.00	21.00	55.00
15	0.00	6.00	13.00	18.00	6.00	57.00
16	0.00	6.00	13.00	18.00	21.00	42.00
17	2.50	0.00	3.00	5.00	6.00	83.50
18	2.50	0.00	3.00	5.00	21.00	68.50
19	2.50	0.00	3.00	18.00	6.00	70.50
20	2.50	0.00	3.00	18.00	21.00	55.50
21	2.50	0.00	13.00	5.00	6.00	73.50
22	2.50	0.00	13.00	5.00	21.00	58.50
23	2.50	0.00	13.00	18.00	6.00	60.50
24	2.50	0.00	13.00	18.00	21.00	45.50
25	2.50	6.00	3.00	5.00	6.00	77.50

Table 9 Continued

Treatment Number	Protein					
	Ovomucin	Lysozyme	Globulins	Ovomucoid	Conalbumin	Ovalbumin
			%			
Vertices						
26	2.50	6.00	3.00	5.00	21.00	62.50
27	2.50	6.00	3.00	18.00	6.00	64.50
28	2.50	6.00	3.00	18.00	21.00	49.50
29	2.50	6.00	13.00	5.00	6.00	67.50
30	2.50	6.00	13.00	5.00	21.00	52.50
31	2.50	6.00	13.00	18.00	6.00	54.50
32	2.50	6.00	13.00	18.00	21.00	39.50
Centroids						
33	0.00	0.00	8.00	11.50	13.50	67.00
34	0.00	6.00	8.00	11.50	13.50	61.00
35	2.50	0.00	8.00	11.50	13.50	64.50
36	2.50	6.00	8.00	11.50	13.50	58.50
37	0.00	3.00	3.00	11.50	13.50	69.00
38	0.00	3.00	13.00	11.50	13.50	59.00
39	2.50	3.00	3.00	11.50	13.50	66.50
40	2.50	3.00	13.00	11.50	13.50	56.50
41	0.00	3.00	8.00	5.00	13.50	70.50
42	0.00	3.00	8.00	18.00	13.50	57.50
43	2.50	3.00	8.00	5.00	13.50	68.00
44	2.50	3.00	8.00	18.00	13.50	55.00
45	0.00	3.00	8.00	11.50	6.00	71.50
46	0.00	3.00	8.00	11.50	21.00	56.50
47	2.50	3.00	8.00	11.50	6.00	69.00
48	1.25	0.00	3.00	11.50	13.50	70.75

Table 9 Continued

Treatment Number	Protein					
	Ovomucin	Lysozyme	Globulins	Ovomucoid	Conalbumin	Ovalbumin
			%			
Centroids						
49	1.25	0.00	13.00	11.50	13.50	60.75
50	1.25	6.00	3.00	11.50	13.50	64.75
51	1.25	6.00	13.00	11.50	13.50	54.75
52	1.25	0.00	8.00	5.00	13.50	72.25
53	1.25	0.00	8.00	18.00	13.50	59.25
54	1.25	6.00	8.00	5.00	13.50	66.25
55	1.25	6.00	8.00	18.00	13.50	53.25
56	1.25	0.00	8.00	11.50	6.00	73.25
57	1.25	0.00	8.00	11.50	21.00	58.25
58	1.25	6.00	8.00	11.50	6.00	67.25
59	1.25	3.00	8.00	11.50	13.50	62.75

Table 10. Treatment Levels Combinations of Six Proteins Used for the Preparation of Custard Type Gels.

Treatment	Protein					
	Ovomucin	Lysozyme	Globulins	Ovomucoid	Conalbumin	Ovalbumin
			%			
1	100.00	0.00	0.00	0.00	0.00	0.00
2	0.00	100.00	0.00	0.00	0.00	0.00
3	0.00	0.00	100.00	0.00	0.00	0.00
4	0.00	0.00	0.00	100.00	0.00	0.00
5	0.00	0.00	0.00	0.00	100.00	0.00
6	0.00	0.00	0.00	0.00	0.00	100.00
7	0.00	50.00	50.00	0.00	0.00	0.00
8	0.00	50.00	0.00	50.00	0.00	0.00
9	0.00	50.00	0.00	0.00	50.00	0.00
10	0.00	50.00	0.00	0.00	0.00	50.00
11	0.00	0.00	50.00	50.00	0.00	0.00
12	0.00	0.00	50.00	0.00	50.00	0.00
13	0.00	0.00	50.00	0.00	0.00	50.00
14	0.00	0.00	0.00	50.00	50.00	0.00
15	0.00	0.00	0.00	50.00	0.00	50.00
16	0.00	0.00	0.00	0.00	50.00	50.00
17	20.00	20.00	20.00	20.00	20.00	0.00
18	20.00	20.00	20.00	20.00	0.00	20.00
19	20.00	20.00	20.00	0.00	20.00	20.00
20	20.00	20.00	0.00	20.00	20.00	20.00
21	20.00	0.00	20.00	20.00	20.00	20.00
22	0.00	20.00	20.00	20.00	20.00	20.00
23	16.66	16.66	16.66	16.66	16.66	16.66
Control	1.65	3.85	8.80	12.10	14.30	59.30

were added to the protein solution to adjust the pH to 5.7. The ionic strength of these solutions ranged from 0.204 to 0.253. One cake was prepared per treatment.

Protein Solution Preparation

A total of 3.65 g of protein was used to prepare the equivalent of 35.0 g of egg white (10.4% protein, liquid basis). Sodium chloride, calcium chloride, and potassium hydroxide, all in 1N solutions were added to replace the ions normally present in egg albumen.

For preparing the solutions, certain precautions had to be taken to prevent precipitation or complexing of lysozyme with conalbumin and/or ovomucin. For mixtures containing no ovomucin, the insolubilization problem was solved by dissolving the weighed globulins, ovomucoid, conalbumin, ovalbumin, and lysozyme, one at a time, in 10 ml of deionized water containing the sodium chloride and calcium chloride solutions, as well as the solid sodium chloride. Lastly, additional water with the potassium hydroxide solution was added to complete the weight to 35.0 g.

For solutions with the maximum level of ovomucin, 50-60% of the alkali and the salt solutions were added to the weighed ovomucin mass contained in a 50 ml erlenmeyer flask. After thorough mixing, globulins, ovomucoid, conalbumin, ovalbumin, and solid sodium chloride were

added one at a time, and carefully dissolved. Finally, to this resulting solution was added the previously dissolved lysozyme in the remaining water and potassium hydroxide solution.

For mixtures with medium ovomucin content, globulins, ovomucoid, conalbumin, ovalbumin, and lysozyme were dissolved in 10 ml of deionized water with the salt solutions and solid sodium chloride. After complete mixing, the previously prepared ovomucin-alkali mixture was slowly poured into the protein solution and the weight completed to 35.0 g with additional water.

Following preparation, the viscosity and surface tension of the protein solutions were determined. The viscosity was measured on a Nametre Direct Readout Viscometer, model 7.006, at a temperature of 25 ± 0.01°C, maintained by a Neslab Exacal circulating water bath, model Ex 100. The surface tension determination was performed on a Fisher Surface Tensiometer, model 20. The container for the protein solution was a crystallizing dish of 4.5 cm I.D. and surface tension forces were measured with a platinum-iridium ring of 6.0 cm of circumference.

After these determinations, the pH of the protein solutions was adjusted to 5.7 by the addition of solid potassium acid tartrate (cream of tartar), the flasks covered with food wrapping film, and stored overnight in the refrigerator.

Cake Preparation

A Kitchen Aid mixer, model K5-A, connected to a Gralab Universal Timer, model 171, was used to prepare the cakes. After the protein solution reached room temperature, 30.0 g were weighed into the mixer bowl, and whipped at speed 10 (210 rpm) until an optimum foam stage for the particular solution was attained. At this point, a portion of the foam was placed in a crystallizing dish of dimensions 4.5 cm I.D. x 3.5 cm height, leveled with a spatula, and weighed to the nearest 0.01 g for foam specific volume determination. The foam was placed back into the mixer bowl and rewhipped for two to five seconds. One third of the sugar was sprinkled over the foam and mixed by whipping at speed 6 (145 rpm) for four seconds. The remaining two portions of sugar were incorporated into the foam in the same manner, after which the sides of the bowl were scraped. The flour-sugar mixture (one fourth of the sugar sifted with the flour twice) was then folded into the foam in four additions. Each portion was sifted over the meringue and gently folded into it using 10 strokes of a stainless-steel spatula. A 55 g portion of the batter was weighed into a tared, ungreased mini loaf pan of dimensions 9 x 5 cm at the base, 5.5 cm height, and 12 x 7.5 cm at the top. The cake was baked at 175°C (350°F) for 25 minutes in a National Reel type test baking oven. After baking the cake, it was allowed to cool, in an inverted position, on cooling racks.

Before addition of sugar, a one level teaspoon of

foam was removed, placed in a vial, and immediately frozen in dry ice. The remaining unused portion of the protein solution was also placed in vials and stored at -23°C along with the foams. These samples were further freeze-dried for sulfhydryl determinations.

Volume Determination

The top of the cooled cake was dusted with flour and the volume determined by rape-seed displacement before removing the cake from the pan. The seeds were placed over the cake, filling the pan to the top. The surface was leveled with a spatula and the remaining seeds were poured into a collecting pan. The volume of these seeds was determined by pouring into a 100 ml graduated cylinder from a constant height. Cake volume was expressed in cm^3 and calculated from the difference between the total pan volume (determined with rape-seeds) and the volume of the seeds.

After volume determination, the cakes were removed from the pans, wrapped in plastic film, and stored at -23°C until further determinations.

Tenderness Determination

The slices for tenderness testing were prepared while the cakes were still frozen to prevent collapse of

the fragile structure. Cakes were cut vertically through the center; one-half was wrapped again and stored at -23°C, and from the other half a 1.3 cm (0.5 in.) thick section was cut horizontally from the bottom. Each slice was wrapped in plastic film and allowed to defrost at room temperature. The slices were then unwrapped, weighed to the nearest 0.01 g and sheared with a multiple blade compression cell in an Allo-Kramer shear press, model SP12. Measurements were performed with either the 100 lb or 3,000 lb maximum load transducer connected to the Food Technology Corporation Texturecorder, model TR 3. The areas of the curves outlined were calculated by triangulation and tenderness was expressed as the work, in lb x cm, required to shear through 1 g of sample.

Compressibility Determination

The unused upper portion of the cake prepared for tenderness determination was shaped by cutting with a 5.2 cm I.D. circular mold, wrapped, and allowed to defrost. After the slices reached room temperature, the minimum and maximum heights of each slice were measured with a caliper, and then compressed to 0.8 cm in an Allo-Kramer shear press, model SP12, equipped with a Food Technology Inc. Texturecorder, model TR3. For most determinations, the 100 lb maximum load transducer was used at a recorder range of 100, and a few samples required the use of the

3000 lb maximum load transducer at recorder ranges of either 10 or 33.

The areas of the curves obtained were measured with a Keuffel and Esser Compensating Polar Planimeter. The average height of the slices was used for the calculations and compressibility was expressed as the work, in lb x cm, required to compress 1 cm of the sample.

Foaming Index Determination

Foaming index values were calculated by dividing the foam specific volume, in cm^3/g, by the whip time, in minutes, of each protein solution. This ratio compensated for the variations in specific gravity and whip time and allowed for an easier comparison among samples. These values also correlated directly with foamability, e.g., larger numbers represented better air incorporation.

Texture

Photographs were used to show the textural characteristics of selected samples.

Custard Model System

To prevent any possible interactions among milk, yolk, and albumen proteins, as well as visual interferences at the ultrastructural level, yolk and milk were eliminated

from the system. Additional egg white proteins were used to replace the yolk proteins and a salt solution with a similar ionic strength (0.165), substituted for milk. This solution was prepared according to the milk mineral composition listed in the Michigan State University Nutrient Data Bank (1978). The salts used were calcium, sodium, magnesium, potassium, and ferric, all in the chloride form. Duplicate gels were prepared per treatment.

The formula used to prepare the gels consisted of a 1:4 mixture of protein solution:salt solution, resulting in a 1.27% protein concentration in the final mixture. A total of 0.78 g of proteins was dissolved in deionized water with added sodium and calcium chloride solutions to reconstitute the equivalent of 6.0 g of whole egg (13% protein, liquid basis). To this mixture was added the salt solution replacing milk, and with constant stirring, the pH was adjusted to 8.0 with 1N potassium hydroxide solution. The resulting solution was transferred to a 50 ml beaker which had been previously sprayed with silicone to prevent sticking of the gel to the container walls. The beaker was covered with a double layer of Saran$^{(R)}$ wrapping film, and placed in a pan of dimensions 10 x 10 x 4.5 cm, filled with an 8% sodium chloride solution. This salt solution was used as the bath medium to prevent excessive evaporation of the water during heating. The beaker was held in place by positioning it in between two thick wires running across the top and around the

sides of the pan, and a flat piece of porcelain was placed under the container to prevent direct contact with the hot pan. The heating system consisted of two Corning Hot Plate Stirrer units, model PC-351, each connected to a Staco Variable Autotransformer, type 2PF 1010, set to 80. The water bath set-up containing the sample, was placed on the previously heated hot plates and a uniform temperature throughout the liquid was ensured by constant agitation with two magnetic stirrers. To monitor the temperature of the protein solution and the water bath, a thermocouple was securely positioned into the protein solution to a depth of 0.5 cm from the surface, and another into the water bath. Temperature recording immediately started using a Honeywell Brown Electronik Potentiometer, model 153 x 65 - P12H - II - III - 81 - A8, equipped with 12 thermocouple terminals. The terminals were connected in parallel into two sets with the same type of wire used for the thermocouples to shorten the elapsed time between each temperature recording to 15 second intervals.

For attainment of similar heating rates of the different protein solutions, particularly during coagulation, the hot plate's controls were set as displayed in Table 11. The settings were changed at water bath temperatures of 65°C (intermediate setting) and 79-80°C (final setting). At these conditions, the heating rate during coagulation of the various protein solutions, was approximately 0.74°C/min. For the mixtures the control

settings varied accordingly with the proteins present.

After the end-point temperature of the gel was attained, it was removed from the water bath, covered with a plastic film and stored at 2-4°C for further determinations.

Gel Strength Determination

Gel strength was determined the day following gel preparation using an Instron Universal testing instrument, floor model TT-BM. An Instron tension load cell, model D30-36 was adapted for the measurements since a compression load cell of equivalent sensitivity was not available.

Table 11. Hot Plate Control Settings Used to Prepare the Custard Type Gels.

Protein Solution	Setting		
	Initial	Intermediate	Final
Ovomucin	6.5	no change	high
Lysozyme	6.2	no change	6.5
Globulins	6.0	no change	6.5
Ovomucoid	6.5	no change	high
Conalbumin	4.2	no change	no change
Ovalbumin	6.0	no change	6.5
Control	4.2	6.0	6.5

A probe was made with a 100 g reference weight and a micro syringe glass plunger of 0.35 cm of diameter and 6.8 cm

long firmly attached to it. The instrument was set to zero, the probe hooked to the tension cell, and the recorder pen brought back to zero with the balance fine knob. For calibration, a 10 g standard weight was added to the cell and calibrated to full scale (at instrument range 1) with the calibration knob. Upon removal of the standard weight, the pen returned to zero, after which it was moved up to full scale with the balance fine knob. This reversed polarity position was used as the reference point for measurement of gel firmness. At such position, application of a force upwards against the plunger would decrease the tension force on the cell and result in a curve with the peak maximum directed towards the normal zero.

Force resistance curves of the gels plunged with the devised probe, were obtained at a cross-head speed of 5 cm/min. with the gear shift set to high. After reaching room temperature, the sample was placed on the platform attached above the cross-head and slowly raised towards the probe until 1.0 cm of the plunger penetrated the gel. At this point the cross-head was stopped and immediately lowered. Force curves were obtained from two opposite locations on the gel at a chart speed of 20 cm/min. The area of the curves was determined with a Keuffel & Esser compensating polar planimeter and gel strength was expressed as the work, in g x cm, required to plunge through 1 cm of the sample.

Percentage Drainage

Following gel strength determination, the coagulums were carefully removed from the beakers onto a pre-weighed dish set-up devised for collecting the drained liquid. This set-up consisted of a medium size weighing boat to which a copper wire screen with 10 cm of diameter and seven openings/cm was molded. A Whatman filter paper no. 4 of 11.0 cm of diameter was then placed over the screen. The dishes containing the gels were covered with stainless steel bowls and the liquid allowed to drain for one hour. After this period the total gel weight and the liquid weight were determined. The percentage drainage was calculated by dividing the liquid weight by the sample total weight and multiplying by 100.

Small gel portions of approximately 1 cm on a side were frozen in dry ice before the liquid was drained and further freeze-dried for sulfhydryl determinations.

pH Determination

The protein coagulum and drained liquid obtained from the previous determination were combined, and the curd was broken into small pieces with a metal spatula until a slurry was formed. The pH of this mixture was measured on a Beckman Expandomatic pH meter, model 76A.

Electron Microscopy

A transmission electron microscope (TEM) was used to examine foams and a scanning electron microscope (SEM) was used for both foams and gels examination.

Transmission Electron Microscopy

Selected freeze-dried foams were fixed with osmium tetroxide vapors. A pyrex dish filled with a 2% osmium tetroxide solution was placed into a medium size petri-dish, and the samples, contained in small plastic vial caps, were positioned around the fixative. The petri dish was then covered, carefully sealed with an adhesive tape, and left under the hood for 72 hours. After fixation, the samples were transferred to small capped vials (4.5 ml capacity) and infiltrated for 24 hours with anhydrous, TEM grade acetone. An Epon-Araldite: ERL epoxy resin mixture (1:1), prepared according to Hooper et al. (1979), was used as the embedding medium. The resin mix was dissolved in acetone (1:2 mixture), and added to the sample vials to a final 1:5 resin:acetone concentration. The vials were capped, gently swirled and left standing for 24 hours. After this period, the vials were uncapped and placed under the hood for four hours. At the end of this period, additional resin-acetone mix was added to 2/3 of the vial capacity, the vials gently agitated to ensure complete mixing, and left uncapped under the hood

grids were rinsed with a 0.02 N sodium hydroxide solution and two additional distilled water rinses of one minute each. The prepared grids were examined in a Philips 201 TEM at an accelerating voltage of 60kV.

Scanning Electron Microscopy

Small pieces of freeze-dried samples were mounted on aluminum stubs and gold coated with a sputter coater for nine to twelve minutes. The prepared samples were examined in a Japan Electron Optics Limited SEM, model JSM 35C, at an accelerating voltage of 15kV.

Statistical Analyses of the Data

The data obtained from the cakes were treated by multiple regression analysis using a least squares program available at Michigan State University Computer Center. For most dependent variables examined, the possible subset models contained only linear terms and interaction terms with two independent variables whereas, for foamability, a few interaction terms with three independent variables were introduced. A technique described by Becker (1968) was also used to detect components with a linear blending behavior resulting in models with non-polynomial forms. The procedure involved transformation of all interaction terms to the form $X_iX_j/X_i + X_j$, and

components exhibiting linear blending were then detected through their improved correlation coefficients significance. The "best" final subset of variables was found by eliminating variables of poor significance to improve the standard error of the estimate (S), the coefficient of multiple determination (R^2), and the sum of squares of the residuals, simultaneously.

The prediction equations for volume and foaming index parameters obtained with the least squares method were used to estimate regions of optimum response through response surface analysis. The contour plots and perspective response surfaces were obtained as a function of two independent variables by a Surface II Graphics System program developed by Sampson (1975), also available at Michigan State University Computer Center. Ovomucin and lysozyme were used as the independent variables and ovomucoid, conalbumin, and ovalbumin levels were fixed to their normal values. Globulins levels of the mixture treatments plotted were obtained by the difference of the sum of the other five protein levels from 1.

Gel data were analyzed by the Student's t-statistics for comparison of two means with unknown, but equal variances, with the following formula (Gill, 1978):

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S\sqrt{(1/N_1) + (1/N_2)}}$$

where

$$S = + \sqrt{\frac{\{[\Sigma X^2_1 - \frac{(\Sigma X_1)^2}{N_1}] + [\Sigma X^2_2 - \frac{(\Sigma X_2)^2}{N_2}]}{(N_1 + N_2 - 2)}}$$

RESULTS AND DISCUSSION

Protein Fractionation

Determination of moisture and nitrogen contents were performed on several batches of prepared albumen. Table 12 displays data from these evaluations and corresponding calculated protein concentration. The average percent protein value was used to obtain yields and recoveries of the isolated fractions as well as for preparation of protein solutions used in the angel food cake system.

Table 13 lists yields and recoveries of the various proteins isolated by the described fractionation procedure. Except for conalbumin and globulins, recoveries were satisfactory, with values ranging from 66 to 76%. Conalbumin was prepared as the iron-free protein complex. Therefore, owing to its higher solubility, only partial crystallization was achieved resulting in low recoveries. The low recoveries of globulins appeared to be associated with formation of a large precipitate during dialysis. This highly insoluble material contained very high molecular weight protein fractions, as estimated by electrophoresis, and some globulins. In addition, considerable quantities of conalbumin present in globulins preparations necessitated purification of the material at less than half

Table 12. Average Moisture, Nitrogen, and Protein Contents of Egg Albumen.[a]

Albumen Batch	Moisture	Nitrogen[b] (Dry Weight Basis)	Protein (Wet Weight Basis)
	%	%	%
1	88.4	13.05	10.39
2	88.4	13.13	10.34
3	88.5	13.23	10.39
4	88.2	13.23	10.65
Average	88.3	13.16	10.44

[a]Values are average of two determinations.

[b]Determined with the Kjeldahl method.

Table 13. Yield and Recovery of Protein Fractions Isolated from Egg Albumen.

Protein Fraction	Unfractionated Albumen[a]		Yield[b]	Recovery
	%[c]	g	g	%
Ovomucin	1.5	1.6	1.1	73.4
Lysozyme	3.5	5.5	4.1	76.0
Globulins	8.0[d]	12.5	2.6	20.5
Ovomucoid	11.0	17.2	11.3	66.0
Conalbumin	13.0	20.3	4.4	21.9
Ovalbumin	54.0	84.2	58.0	68.9

[a]Albumen Weight 1250 g, protein content 130 g.

[b]Average of all fractionations.

[c]Source: Powrie (1977).

[d]Source: Parkinson (1966). Include globulins G_2 and G_3.

saturated ammonium sulfate solution (approximately 40-45%). Under these conditions some globulins also remained in solution.

The various protein preparations were tested for purity with PAGE and SDS-PAGE. Figure 3 A and B show SDS-PAGE and PAGE, respectively, of the preparations used for functionality tests. The degree of purity of all proteins was estimated to be over 90% visually and the relative intensity of the protein bands of SDS-PAGE gels was determined from densitometer tracings.

Under the conditions used in SDS-PAGE globulins appeared to have the same relative mobility as that of ovalbumin (Figure 3A, gels 2 and 5). This suggested that both proteins have comparable molecular weights.

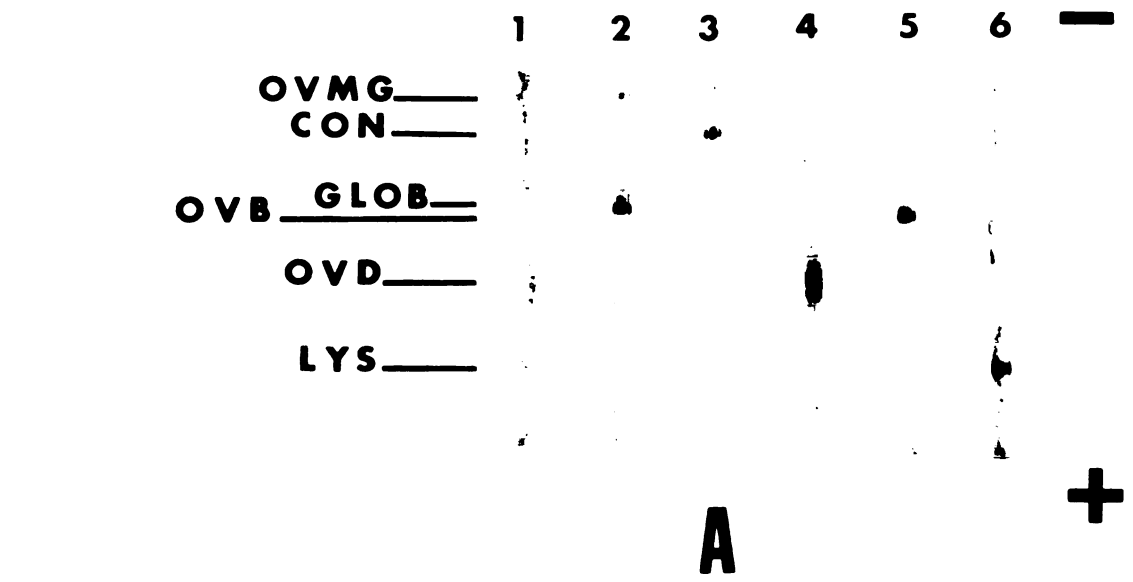
The composition and relative purity of the protein fractions are summarized in Table 14. Although dye binding ability varies among proteins, in general densitometric quantifications were comparable to gels visual inspection. An exception was observed with globulins preparation which appeared to contain a relatively high amount of ovomacroglobulin, calculated to be about 24% (Table 14). This apparent high concentration may have resulted from the combined effects of higher dye binding ability of ovomacroglobulin and the compactness of the protein band (Figure 3A, gel 2).

For evaluation of foaming properties of individual proteins, fractions with relative purity below 95%

Fig. 3. Electrophoretogram of egg white proteins.

a) SDS PAGE. 1. Ovomucin; 2. Globulins; 3. Conalbumin; 4. Ovomucoid; 5. Ovalbumin; 6. Lysozyme.

b) PAGE. 1. Conalbumin; 2. Ovomucoid; 3. Globulins; 4. Ovalbumin; 5. Lysozyme; 6. Ovomucin.


1
2
3
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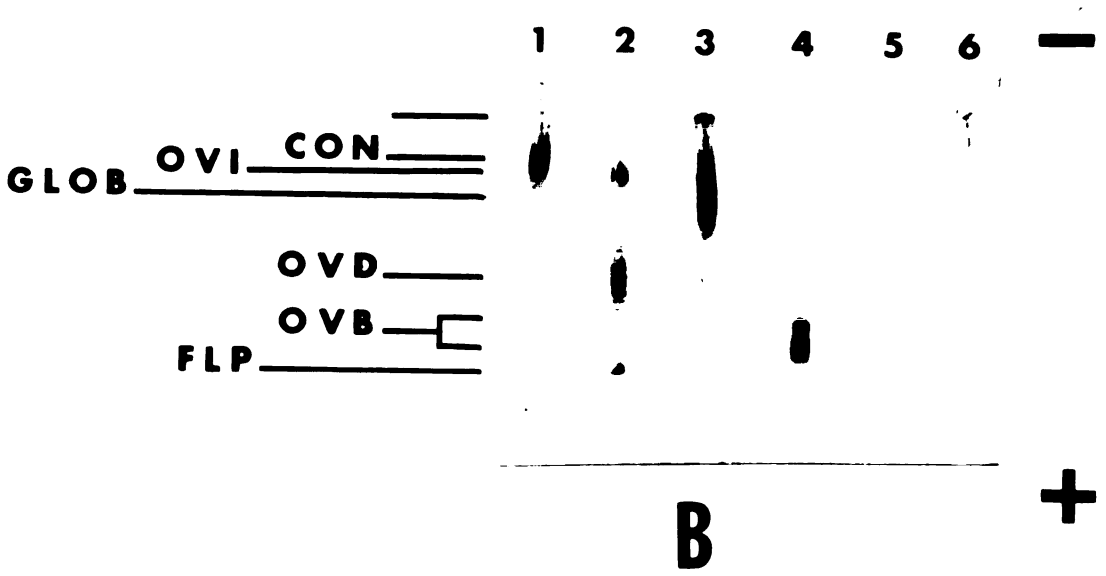
5
6
−
OVMG
CON
OVB
GLOB
OVD
LYS
+
A


1
2
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6
−
GLOB
OVI
CON
OVD
OVB
FLP
+
B

Table 14. Composition[a] and Relative Purity[b] of the Isolated Albumen Protein Fractions.

Protein Fraction	Egg Albumen Proteins									
	Ovo-mucin	Lyso-zyme	Glob-ulins	Ovo-mucoid	Conal-bumin	Oval-bumin	Ovomacro-globulin	Ovo-inhibitor	Flavo-protein	Other Proteins
						%				
Ovomucin	96.7		0.7							2.5
Lysozyme		99.0+								
Globulins		1.6	71.4		1.0	0.4	24.1		1.5	
Ovo-mucoid		2.6		94.4				3.0		
Conal-bumin		3.6			92.6				3.7	
Oval-bumin					3.0	97.0				

[a]Determined from relative mobilities in SDS-PAGE.

[b]Calculated from densitometer tracings.

(Table 14) were purified by gel filtration. For interaction studies the protein preparations were used without purification.

Sulfhydryl (SH) and disulfide (SS) contents were determined on the isolated fractions and these values are listed in Table 15. All protein preparations contained some SH groups and had relatively high SS contents. Ovalbumin contained most of the SH groups, 3.73 SH residues/mole, whereas less than one SH group was present in the other proteins. The absence of thiol groups in conalbumin, lysozyme, and ovomucoid (Powrie, 1977), and in globulins (Feeney et al., 1963) has been reported. Therefore, the measurable SH content in these proteins probably resulted from slight denaturation during fractionation.

The disulfide content obtained for most proteins was well within literature values. Lysozyme contained 4 SS groups, ovomucoid 8, conalbumin 15, and ovalbumin 1.35, while reported values are 4 (Jollès et al., 1963), 8.15 (Davis et al., 1971), 14.55 (Wenn and Williams, 1968), and 1 (Smith and Back, 1970), respectively.

Angel Food Cake System

The physico-chemical characteristics of the protein solutions used for testing foaming properties and the control solution are illustrated in Table 16. The

Table 15. Sulfhydryl and Disulfide Contents[a] of Albumen Proteins.

Protein	Sulfhydryl		Disulfide	
	moles/ 10^4g protein	moles/ mole protein	mole/ 10^4g protein	moles/ mole protein
Ovomucin	0.12		3.79	
Lysozyme	0.12	0.20	2.74	4.00
Globulins	0.36		1.51	
Ovomucoid	0.10	0.31	3.00	8.00
Conalbumin	0.10	0.09	1.85	15.00
Ovalbumin	0.73	3.73	0.30	1.35

[a]Values are average of two determinations.

parameters of angel food cakes prepared with these solutions are also summarized in Table 16. Viscosities ranged from 2.77 cps x g/cm^3 for the globulins solutions, to 1.53 cps x g/cm^3 for lysozyme solution. Viscosity and surface tension of ovomucin solution were not determined owing to the very poor flowing properties of the solution. However, it was evident that ovomucin had the highest viscosity of all proteins in solution. The control solution exhibited a viscosity of 2.02 cps x g/cm^3, which was primarily imparted by ovomucin.

The influence of ovomucin on viscosity appears to conform with its very high molecular size and carbohydrate content. With these characteristics, the protein is

Table 16. Physico-chemical characteristics of albumen proteins solutions[a] and angel food cake parameters.

Protein	Viscosity	Surface Tension	Foaming Index	Sulfhydryl Content[b]		Volume	Tenderness	Compressibility
				Solution	Foam			
	cps x g/cm^3	Dynes/cm	cm^3/g/min	moles/10^{5}g	sample	cm^3	work/g[e]	work/cm[e]
Ovomucin	ND[c]	ND[c]	0.00[d]	0.79±0.06	0.79±0.04	52	6.703	2.158
Lysozyme	1.53	42.0	0.12	0.81±0.00	0.65±0.02	107	11.915	6.465
Globulins	2.77	45.4	4.71	3.76±0.09	3.30±0.03	330	4.275	0.796
Ovomucoid	1.98	39.0	0.00[d]	0.98±0.03	0.98±0.03	54	16.982	2.320
Conalbumin	1.55	42.4	0.34	0.86±0.02	0.82±0.01	157	8.347	4.738
Ovalbumin	1.62	51.8	0.59	6.14±0.12	5.66±0.02	308	4.535	0.567
Control	2.02	46.7	3.08	3.92±0.17	3.78±0.10	272	4.047	0.750

[a]Protein concentration = 10.4%; I ≃ 0.20 to 0.25, pH = 5.7.

[b]Values are average of two determinations.

[c]Not Determined.

[d]Value connotes no foaming capacity.

[e]work = lb x cm.

capable of absorbing considerably large quantities of water, swelling tremendously in the process.

Likewise, the ability of ovomucoid to promote increases in viscosity may be related to the high quantity of carbohydrate prosthetic groups attached to the molecule. These groups represent about 20-25% of the protein weight (Davis et al., 1971).

The underlying causes for the high viscosity of globulins solution were not apparent. As evidenced from SDS-PAGE, globulins have relatively small molecular size ($\sim$ 45,000 daltons). The possibility that some carbohydrate moieties may be attached to the polypeptide chains and/or that the proteins may associate in solution forming larger complexes, could justify their effects on viscosity.

The proteins exhibited varying degrees of surface tension lowering effects. Ovalbumin was the least effective with a surface tension value of 51.8 dynes/cm whereas ovomucoid reduced the surface tension of water from about 72.0 dynes/cm to 39.0 dynes/cm. Lysozyme and conalbumin solutions surface tension values were quite similar, 42.0 dynes/cm and 42.4 dynes/cm, respectively. Globulins solution surface tension was 45.4 dynes/cm whereas the control solution showed a surface tension of 46.7 dynes/cm.

For four of the proteins, the foaming index of the solutions correlated positively with viscosity, whereas surface tension did not seem to affect foamability. Globulins showed the highest foaming capacity (4.71 $cm^3/g/min.$).

The other proteins had rather poor foaming ability with values of 0.59 $cm^3/g/min$ for ovalbumin, 0.24 $cm^3/g/min$ for conalbumin, and 0.12 $cm^3/g/min$ for lysozyme. The absence of air incorporation ability of ovomucin again resulted from the extremely high viscosity and lack of flowing properties of the solution.

Ovomucoid also exhibited no foaming capacity although its viscosity and surface tension characteristics favored foam formation. A certain degree of denaturation is necessary for formation of a cohesive film, and Adam (1941) indicated that the spreading ability of a protein at the liquid surface depends on the protein conformation. Ovomucoid has extremely high resistance to heat denaturation (Fredericq and Deutsch, 1949) and apparently, the mechanical whipping action also did not alter the protein conformation. This high resistance to denaturation may be related to the large number of disulfide linkages (8) stabilizing the protein structure. It was observed that the same type of correlation between disulfide groups and foaminess was present in the other proteins. Therefore, lysozyme, with four disulfide bonds present in the molecule, also showed very poor foaming power, whereas ovalbumin, with one disulfide group, exhibited slightly better air incorporation.

Whipping reduced the number of sulfhydryl groups of most proteins. A significant reduction of about 20% was observed for lysozyme ($P < 0.01$), of 12% for globulins

($P < 0.05$), and of 8% for ovalbumin ($P < 0.05$). Although a decrease in SH content of whipped conalbumin and control solutions was also observed, the differences were not significant.

No apparent effects of whipping on the sulfhydryl content of ovomucin and ovomucoid were observed. The reduction of SH groups for the other proteins, however, suggested that inter and/or intramolecular sulfhydryl-disulfide interchange reactions were involved in foam formation.

Great variability in volume of angel food cakes was obtained when the different porteins were used for foam preparation. Globulins produced the cake with the largest volume (330 cm^3) followed by ovalbumin (308 cm^3) and control (272 cm^3). Volumes of 157 cm^3 for conalbumin and 107 cm^3 for lysozyme cakes resulted from fewer air inclusions and instability of the foams, whereas volumes of 54 cm^3 for ovomucoid, and 52 cm^3 for ovomucin cakes reflected complete lack of aeration.

Tenderness and compressibility measurements reflected the degree of aeration of the cakes. Tenderness of globulins, ovalbumin, and control cakes varied little, but ovalbumin produced a slightly tougher cake, with tenderness value of 4.535 work/g. Cakes prepared with the control mixture and with globulins showed tenderness values of 4.047 and 4.275 work/g, respectively. Conalbumin, lysozyme, ovomucin, and ovomucoid cakes were considerably

less tender with tenderness values of 8.347, 11.915, 6.703, and 16.982 work/g, respectively. It was interesting to note that ovomucin cake was very moist and "mushy" and these characteristics apparently lowered the work required to shear the cake. Compressibility values of globulins and control cakes were quite similar, 0.769 and 0.750 work/g, respectively. The work required to compress the cakes prepared with ovalbumin, conalbumin, lysozyme, ovomucin, and ovomucoid were 0.567, 4.738, 6.465, 2.158 and 2.320 work/g, respectively. The somewhat lower compressibility values of ovomucin and ovomucoid cakes probably resulted from improper compression since these cakes had very low heights.

Figure 4 illustrates the cakes prepared with the various albumen proteins. Cake prepared with ovalbumin showed very coarse texture with several large air cells and thick cell walls, whereas cake prepared with globulins had numerous smaller air cells with thin walls. The control cake appeared to combine the effects of adding the various proteins, particularly globulins and ovalbumin. Textural characteristics of the other cakes varied accordingly with the degree of aeration in these products.

Protein Interactions

The effect of variations in levels of six albumen proteins on viscosity, surface tension, and foaming index of

Fig. 4. Angel Food Cakes Prepared with Various Albumen Protein Solutions. Protein Concentration 10.4%, pH 5.7, Ionic Strength 0.20.

CONTROL
OVALBUMIN
GLOBULINS
CONALBUMIN
LYSOZYME
OVOMUCOID
OVOMUCIN

protein solutions, and on volume, tenderness, and compressibility of angel food cakes were evaluated. Table 17 summarizes the significant simple correlations among protein mixtures components, physical characteristics, and cake parameters.

The observed and predicted values of protein mixtures physical parameters and angel food cake parameters are summarized in Tables 32 and 33 in the appendix B section.

Ovomucin and combinations of ovomucin with the other five proteins correlated positively with viscosity at $P < 0.0005$. Globulins and interaction terms of globulins with lysozyme, ovomucoid, and ovalbumin effectively reduced the surface tension of the solutions ($P < 0.05$).

Several linear and interacting variables affected foaming index. Positive correlations were observed with ovomucin ($P < 0.0005$), globulins ($P < 0.01$), the interacting variables of ovomucin with globulins, ovomucoid, conalbumin, and ovalbumin ($P < 0.05$). Viscosity also correlated positively with foaminess at $P < 0.0005$. Lysozyme, and the interacting variables of lysozyme with globulins, ovomucoid, conalbumin, and ovalbumin correlated negatively ($P < 0.05$) with foaming index.

Ovomucin, and interaction terms of ovomucin with globulins, ovomucoid, conalbumin, and ovalbumin correlated negatively with volume. Viscosity and foaming index also showed a negative correlation with volume. In contrast, lysozyme, and interaction of lysozyme with globulins,

Table 17. Significant Simple Correlation Coefficients Among Protein Mixtures Components, Physical Characteristics, and Cake Parameters.

	Viscosity	Surface Tension	Foaming Index	Volume	Tenderness	Compressibility
Ovomucin	0.91***		0.48***	-0.59***	0.29*	0.32**
Lysozyme			-0.39**	0.47***		-0.24*
Globulins		-0.28*	0.30**		-0.27*	
Ovomucoid					-0.28*	
Conalbumin						
Ovalbumin					0.30**	
OVN X LYS	0.49***				0.27*	
OVN X GLOB	0.88***		0.54***	-0.60***	0.24*	0.32**
OVN X OVD	0.89***		0.48***	-0.63***	0.24*	0.33**
OVN X CON	0.79***		0.43***	-0.50***		0.29*
OVN X OVB	0.87***		0.45***	-0.56***	0.35**	0.31**
LYS X GLOB		-0.34**	-0.25*	0.29*		
LYS X OVD			-0.31**	0.30**		
LYS X CON			-0.32**	0.37**		
LYS X OVB			-0.39**	0.50***		-0.25*
GLOB X OVD		-0.26*	0.24*		-0.41**	
GLOB X CON			0.23*		-0.22*	
GLOB X OVB		-0.26*	0.28*			
OVD X CON					-0.32**	
OVD X OVB					-0.25*	
CON X OVB						
Viscosity			0.55***	-0.61***		0.37**
Surface Tension						0.28*
Foaming Index				-0.60***		0.24*
Volume						-0.51***

*Significant at $P < 0.05$; **Significant at $P < 0.01$; ***Significant at $P < 0.0005$.

ovomucoid, conalbumin, and ovalbumin positively affected volume.

The work required to shear the cakes (tenderness determination) was positively affected by ovomucin, ovalbumin, ovomucin x lysozyme, ovomucin x globulins, ovomucin x ovomucoid, and ovomucin x ovalbumin. Globulins, ovomucoid, globulins x ovalbumin, ovomucoid x ovalbumin, globulins x ovomucoid, and ovomucoid x conalbumin negatively correlated with work/g values (produced more tender cakes).

Compressibility showed a positive correlation with ovomucin, ovomucin x globulins, ovomucin x ovomucoid, ovomucin x ovalbumin, and viscosity at $P < 0.01$, and with ovomucin x conalbumin, surface tension, and foaming index at $P < 0.05$. Lysozyme and lysozyme x ovalbumin ($P < 0.05$), and volume ($P < 0.0005$) correlated negatively with compressibility.

Foaming Index. The effects of the various proteins on foaming index appeared to be related to viscosity and surface tension, and to other underlying effects apparently associated with lysozyme levels. The highly significant positive correlations of ovomucin with viscosity and with foaming index suggested this protein facilitated foam formation primarily by increasing the solution viscosity. Globulins appeared to positively influence foaminess, in part, by lowering surface tension.

Lysozyme and its interaction terms correlated

negatively with foaming index. The influence of this protein on foamability may be associated with the formation of a ovomucin-lysozyme complex. Garibaldi et al. (1968) observed that an ovomucin-lysozyme complex formation during heating was related to loss of foaming properties of egg white. Heat apparently accelerates complex formation reaction rates at normal levels of ovomucin and lysozyme. Kato et al. (1975) showed the dependence of lysozyme concentration on ovomucin-lysozyme interaction. These authors pointed out aggregation increased in proportion to lysozyme concentration and reached saturation when the concentration of the protein was twice that of ovomucin.

Variations in levels of ovomucin and lysozyme from low (0%) to high (1.5 times normal levels) clearly showed the effects of these proteins on foaminess. Figures 5 and 6 illustrate the correlations among ovomucin and lysozyme levels and foaming index. Ovomucin exponentially increased the foaming index of solutions which contained 0% lysozyme and various levels of globulins (Figure 5). In the presence of 6% lysozyme, the magnitude of foaming index values was much lower. Moreover, all solutions showed similar trends, in that the normal level of ovomucin caused a slight reduction in foaminess while higher levels of ovomucin improved foaminess.

The drastic depression of foaming capacity of solutions containing a high level of ovomucin (2.5%) with the addition of lysozyme is seen in Figure 6. In absence of ovomucin,

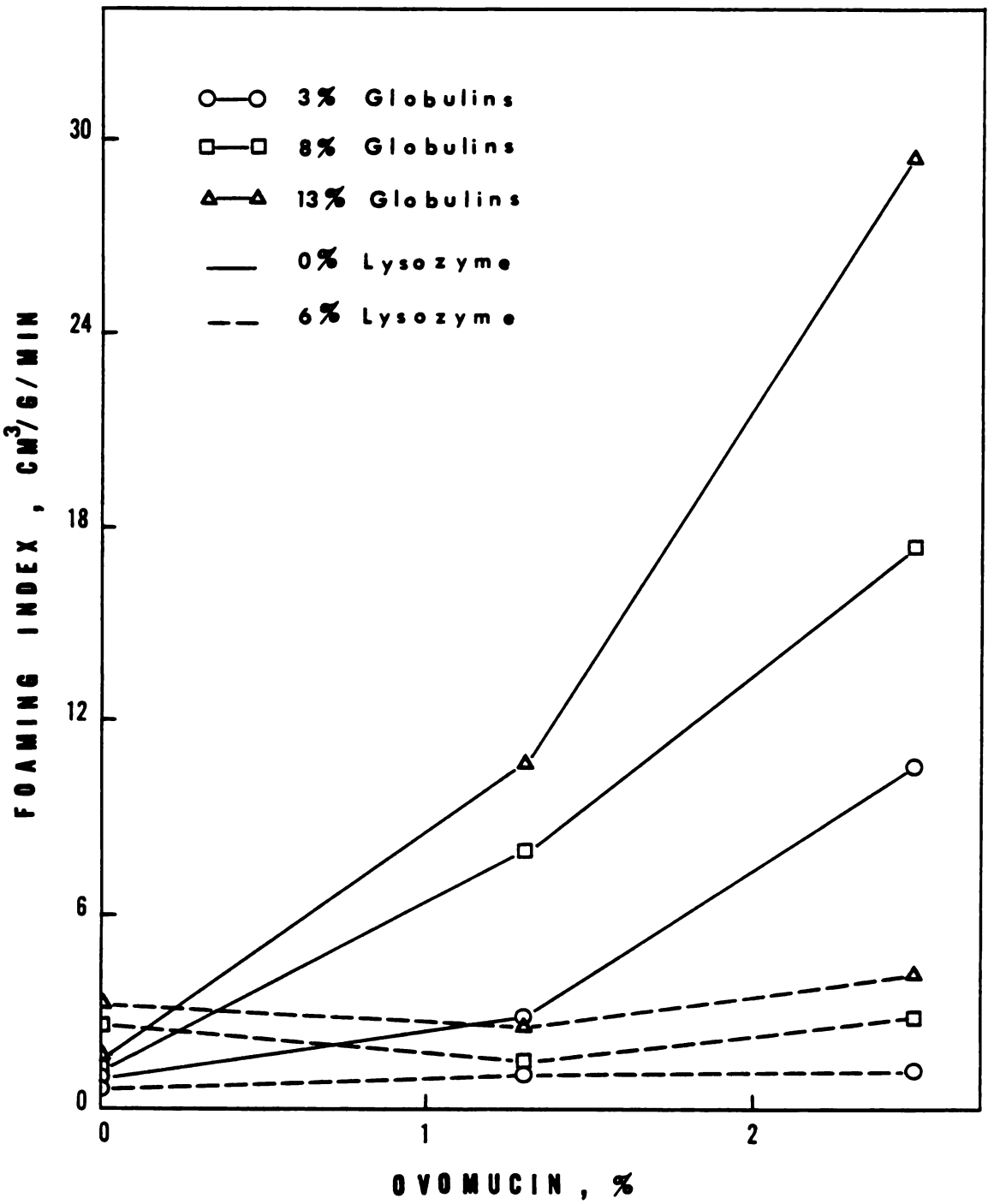


Fig. 5. Effect of ovomucin on the foaming ability of protein solutions containing various levels of globulins and lysozyme. Protein concentration 10.4%, pH 5.7, ionic strength 0.20. Ovomucoid = 11.5%; conalbumin = 13.5%; ovalbumin = 53.5 to 72.0%. N = 4.

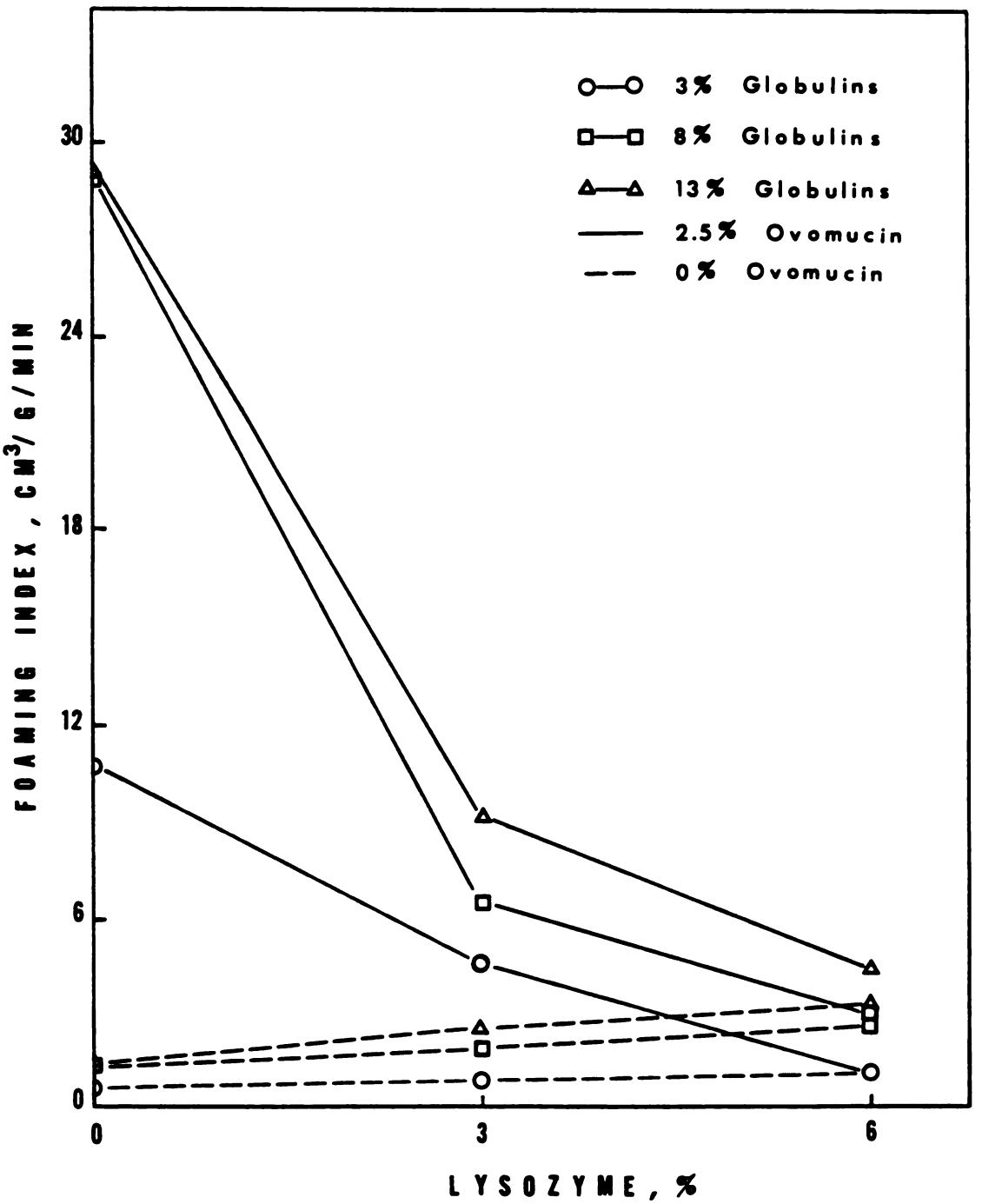


Fig. 6. Effect of lysozyme on the foaming capacity of protein solutions containing various levels of globulins and ovomucin. Protein concentration = 10.4%, pH = 5.7, ionic strength ≅ 0.20. Ovomucoid = 11.5%, conalbumin = 13.5%, ovalbumin = 53.5 to 72.0%. N = 4.

lysozyme improved foaminess of the solutions. This basic positively charged protein at pH 5.7 may interact electrostatically with the acidic proteins (ovomucoid and ovalbumin) and possibly with globulins facilitating foam formation.

The effect of globulins on foaming index is shown in Figure 7. Globulins increased foaming capacity of solutions with any level of lysozyme and ovomucin. Linearity was observed for most solutions. However, for solutions with 2.5% ovomucin and 0% lysozyme, a saturation point was reached at 8% globulins level.

No significant correlation between ovomucoid, conalbumin, and ovalbumin and foaming index was detected. This result suggested these proteins had little or no influence on foamability.

Volume. The influence of the various proteins on volume was associated with the effects they have on foaming capacity. The observable trend was an inverse correlation between volume and foaming index. Thus, ovomucin and its interactions, which positively correlated with foaming index, had high depressive effects on volume. Lysozyme and its double combinations, negatively correlated with foaming index, showed positive relationship with volume. Globulins and its interacting variables had no significant effects on volume.

The relationship between globulins, ovomucin, and

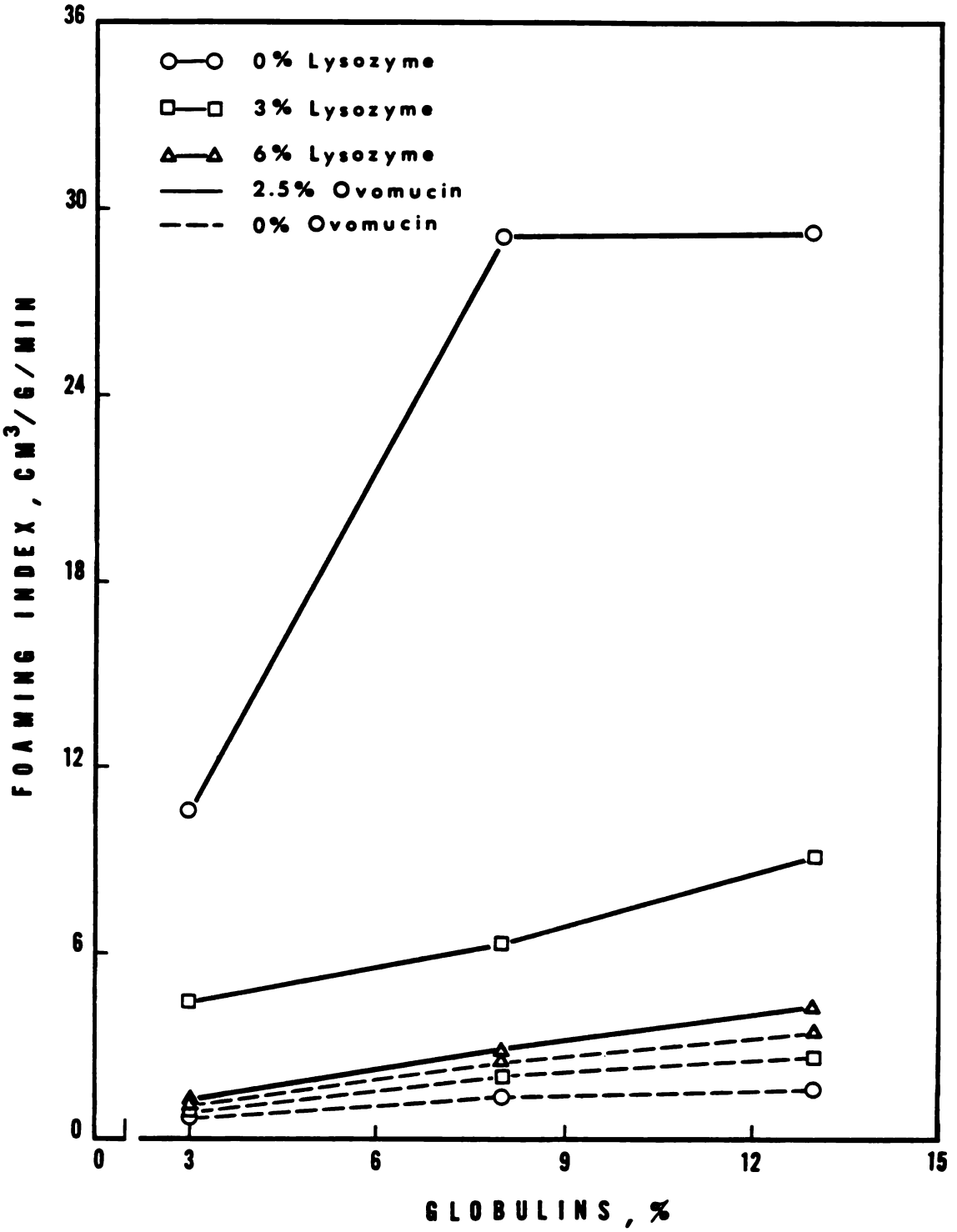


Fig. 7. Effect of globulins on the foaming capacity of protein solutions containing various levels of lysozyme and ovomucin. Protein concentration = 10.4%, pH = 5.7, ionic strength = 0.20. Ovomucoid = 11.5%, conalbumin = 13.5%, ovalbumin = 53.5 to 72.0%. N = 4.

lysozyme and volume is more readily seen in Figures 8, 9, and 10, respectively. Although globulins (Figure 8) did not significantly affect volume, it seemed that at normal levels (8%), better air incorporation resulted in slightly larger cakes for most treatments, whereas at higher levels (13%), a slight reduction in cake volume was observed. For the combination of 6% lysozyme with 2.5% ovomucin, a progressive increase in the level of globulins consistently reduced volume.

In general, with cakes that contained ovomucin (Figure 9) the trend was a decrease in volume with increasing levels of the protein, except that for treatments with 3% globulins and 6% lysozyme, higher volumes were obtained with the higher level of ovomucin. In contrast, for cakes with varying levels of lysozyme (Figure 10), considerable improvement of volume occurred for treatments with 2.5% ovomucin and 3, 8, and 13% globulins. For cakes with 0% ovomucin and 3, and 13% globulins increasing levels of lysozyme caused a slight improvement followed by a reduction of volume, whereas for treatment with 8% globulins, the inverse occurred.

As it was observed previously, the effects of globulins, ovomucin, and lysozyme on cake volume were clearly related to foaming capacities. Previously, MacDonnel et al. (1955) reported that smaller cakes were produced when prepared with egg white containing extra quantities of ovomucin. They implicated that excessive insolubilization of

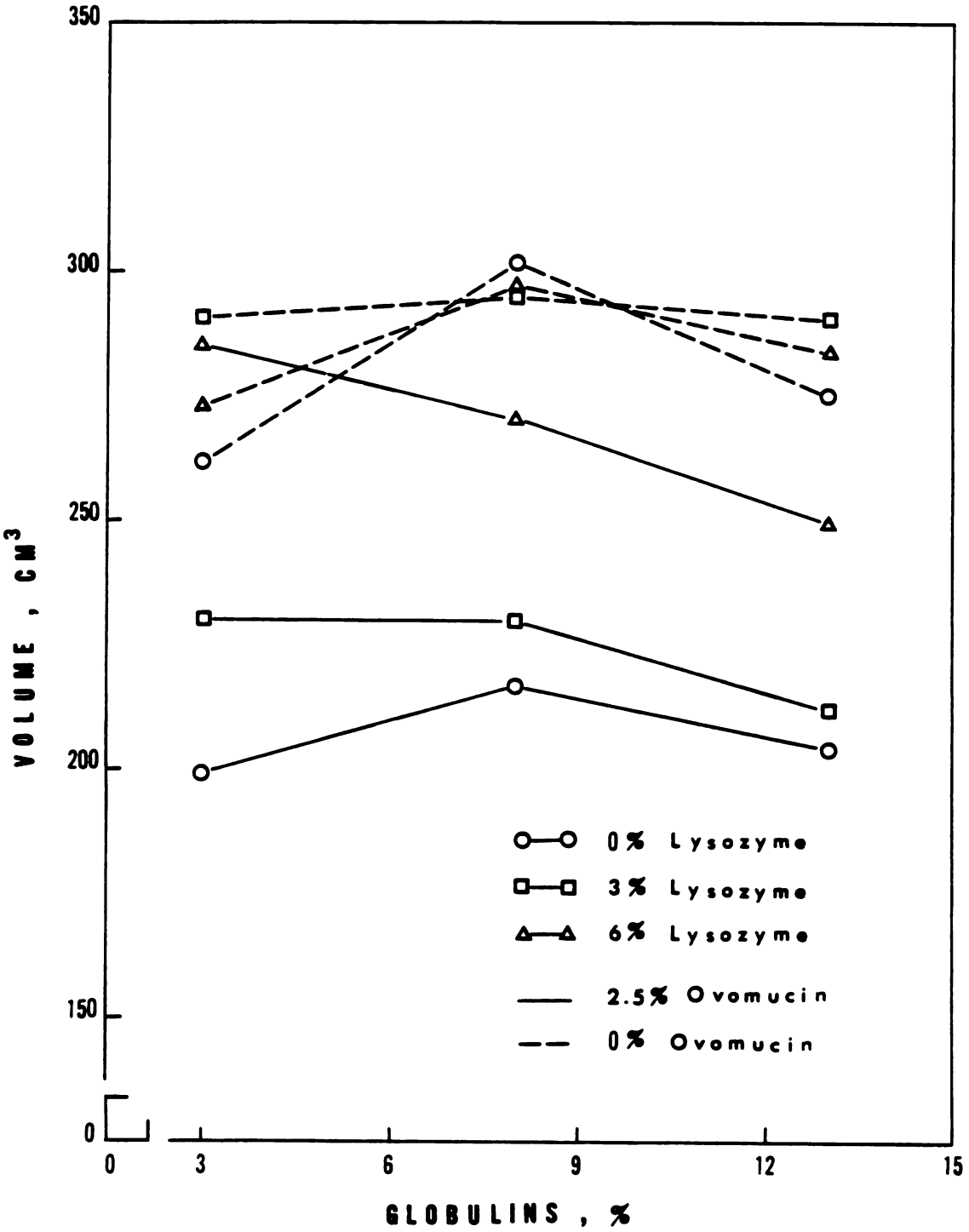


Fig. 8. Effect of globulins on volume of angel food cakes prepared with protein solutions containing varying levels of lysozyme and ovomucin. Protein concentration 10.4%, pH 5.7, ionic strength 0.20. Ovomucoid = 11.5%, conalbumin = 13.5%, ovalbumin = 53.5 to 72.0%. N =4.

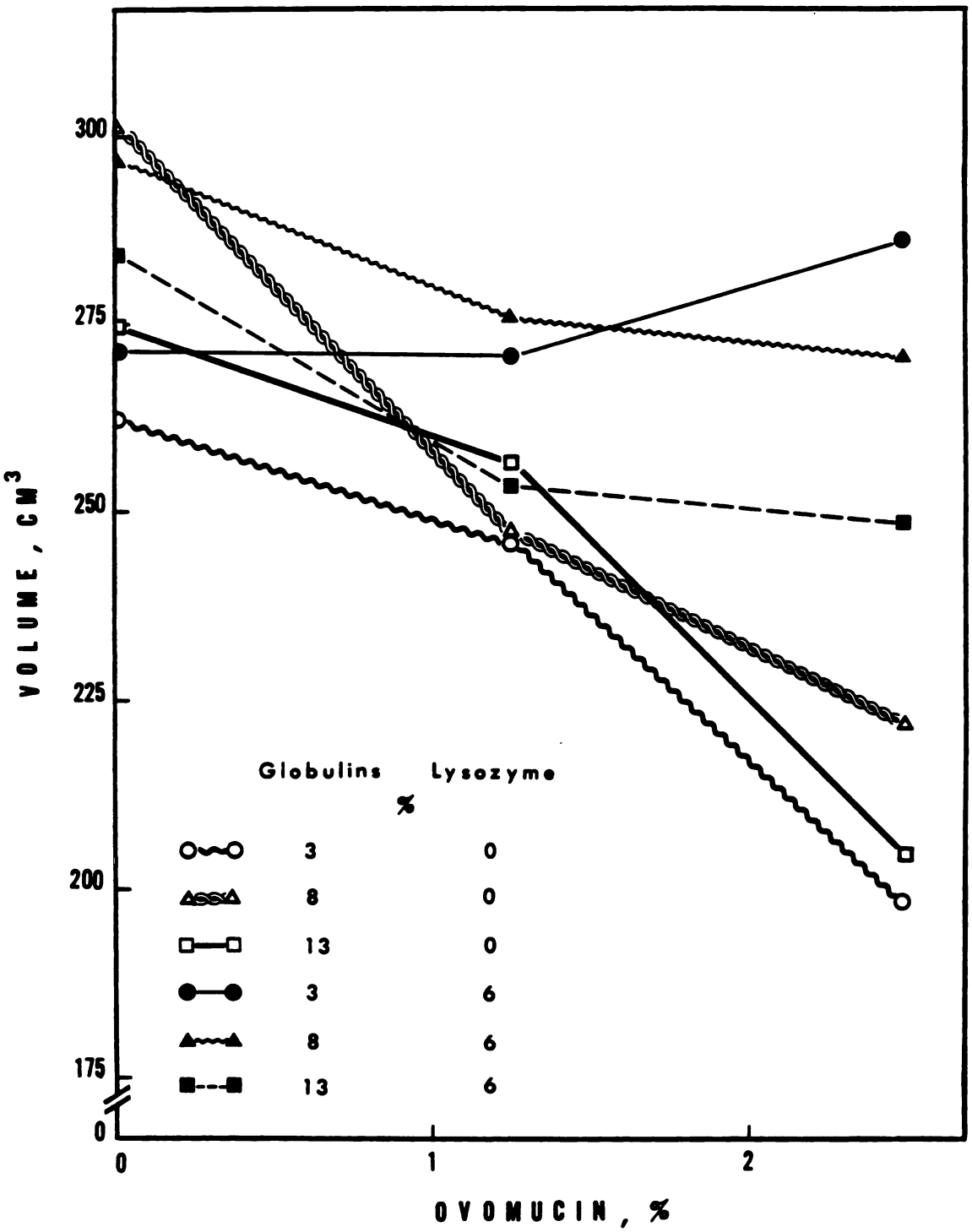


Fig. 9. Effect of ovomucin on volume of angel food cakes prepared with protein solutions containing varying levels of globulins and lysozyme. Protein concentration = 10.4%, pH = 5.7, ionic strength ≅ 0.20. Ovomucoid = 11.5%, conalbumin = 13.5%, ovalbumin = 53.5 to 72.0%. N = 4.

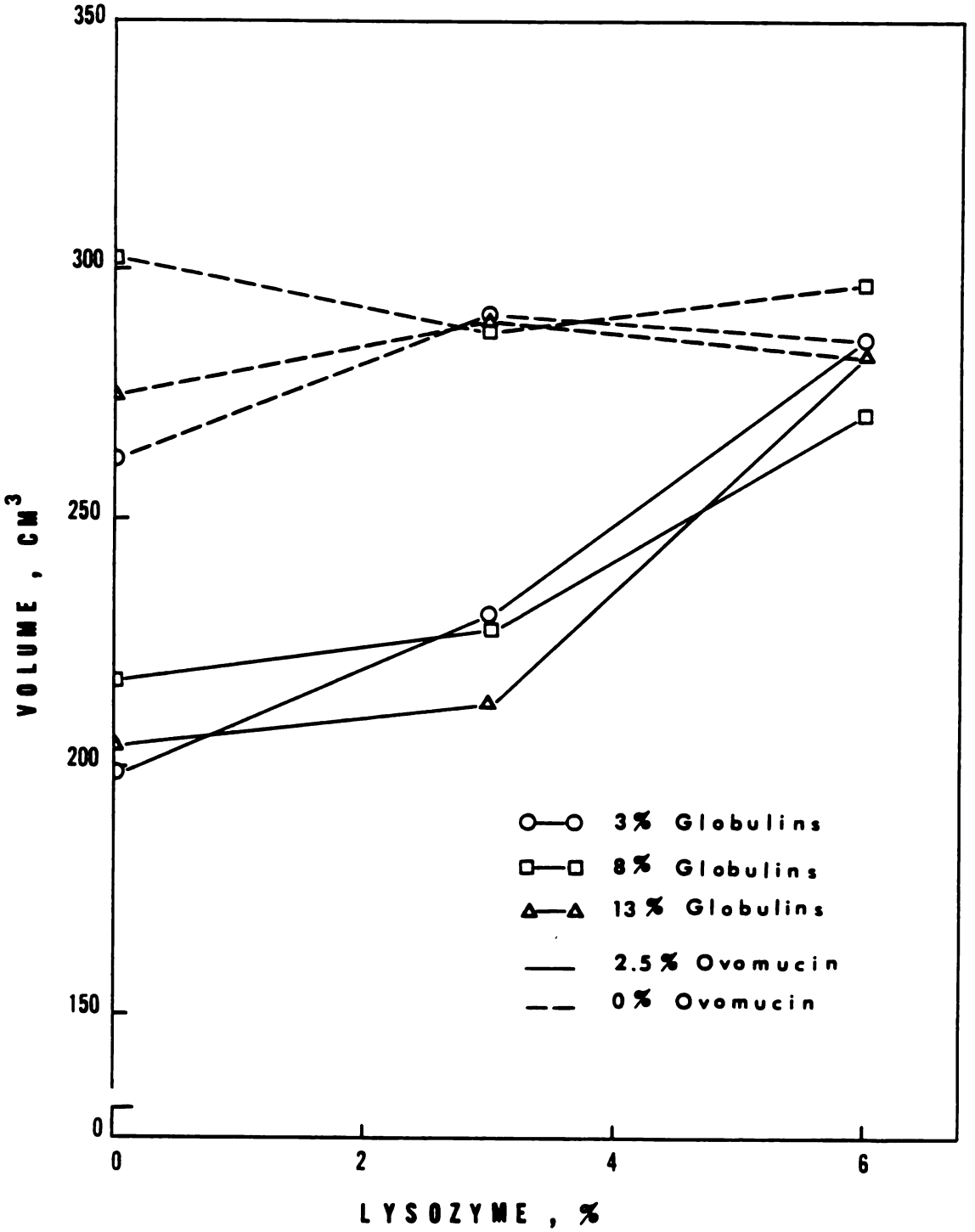


Fig. 10. Effect of lysozyme on volume of angel food cakes prepared with protein solutions containing varying levels of lysozyme and ovomucin. Protein concentration 10.4°, pH 5.7, ionic strength 0.20. Ovomucoid = 11.5%, conalbumin = 13.5%, ovalbumin = 53.5 to 72.0%. N = 4.

ovomucin at the bubble surface decreased film elasticity and prevented cake expansion during baking.

In the current study it was observed that cakes prepared with 2.5% ovomucin and 0% lysozyme expanded normally during baking, but then collapsed at the last stage of baking. This suggested that reduced heat coagulative properties of the film surrounding the air cells was primarily responsible for low cake volumes. In addition, ovomucin has been reported to lack heat coagulative properties (Cunningham and Lineweaver, 1965; MacDonnell et al., 1953).

The "protective" effect of lysozyme on volume seemed to correlate with formation of ovomucin-lysozyme complex. Apparently, this complex either does not insolubilize excessively at the bubble surface or has normal heat coagulative properties. The evidence for the reductive effects of high levels of globulins on cake volume points out this protein fraction may also insolubilize (denature) excessively at the air-albumen interface.

Tenderness and Compressibility. Determination of tenderness and compressibility may indicate the degree of aeration of baked products. In general, highly aerated products exhibit low tenderness (numerical value) and compressibility scores. Compressibility correlated positively with viscosity and foaming index and negatively with volume, although there were no apparent correlations of these parameters with tenderness.

As expected, ovomucin and its interaction terms correlated positively with both tenderness and compressibility. This indicated that cakes prepared with these variables were more compact. Lysozyme's aerating effect correlated negatively with compressibility values.

Globulins and ovomucoid appeared to produce more tender cakes. This effect was also observed for the following interaction terms: GLOB x OVD, GLOB x CON, OVD x CON, and OVD x OVB.

Sulfhydryl Groups

The sulfhydryl content of the various protein solutions before and after whipping are summarized in Table 18. The sulfhydryl content of the solutions varied accordingly with the concentration of ovalbumin since the majority or all sulfhydryl groups in egg white are present in this protein (Powrie, 1977).

In general, whipping reduced the number of sulfhydryl groups. This effect suggested that formation of the protein layer at the surface of the film involved sulfhydryl-disulfide interchange type reactions, with formation of disulfide bonds.

Although some mixtures exhibited more pronounced reductions in the number of sulfhydryl groups than others, the specific reasons for these effects were not apparent. Additionally, the fact that extensive sulfhydryl-disulfide

Table 18. Effect of Whipping on the Sulfhydryl Content[a] of Albumen Proteins Solutions.

Treatment[b]						Solution Mean± Standard Deviation	Foam Mean± Standard Deviation	t-statistic[c]
OVN	LYS	GLOB	OVD	CON	OVB			
						moles/10^5g sample		
L	L	L	L	L	H	5.83±0.02	5.52±0.18	NS
L	L	L	L	H	H	4.41±0.04	4.73±0.09	-4.595*
L	L	L	H	L	H	5.41±0.22	5.30±0.08	NS
L	L	L	H	H	N	3.97±0.08	3.78±0.16	NS
L	L	H	L	H	N	4.01±0.03	3.81±0.12	NS
L	L	H	H	H	L	3.76±0.09	3.69±0.08	NS
L	H	L	L	L	H	4.79±0.05	4.68±0.06	NS
L	H	L	L	H	N-H	4.14±0.29	4.01±0.11	NS
L	H	L	H	L	N-H	4.12±0.04	4.02±0.00	4.003*
L	H	L	H	H	N-L	3.58±0.19	3.42±0.07	NS
L	H	H	L	L	H	5.28±0.24	4.48±0.24	3.333*
L	H	H	L	H	N	3.80±0.09	3.63±0.18	NS
L	H	H	H	L	N	3.94±0.00	4.17±0.12	NS
L	H	H	H	H	L	3.12±0.09	2.85±0.03	4.025*
H	L	L	L	L	H	5.53±0.32	5.11±0.06	NS
H	L	L	L	H	N-H	4.35±0.13	4.28±0.06	NS
H	L	L	H	L	H	4.38±0.07	4.00±0.34	NS
H	L	L	H	H	N	3.41±0.04	3.89±0.14	-4.662*
H	L	H	L	L	H	4.79±0.25	4.83±0.07	NS
H	L	H	L	H	N	3.80±0.00	3.99±0.17	NS
H	L	H	H	L	N	4.58±0.10	4.36±0.04	NS
H	L	H	H	H	L	4.00±0.01	3.31±0.02	43.639***
H	H	L	L	L	H	4.58±0.20	4.77±0.29	NS
H	H	L	L	H	N	3.91±0.05	3.53±0.24	NS
H	H	L	H	L	N-H	4.31±0.08	3.73±0.16	4.585*
H	H	L	H	H	N-L	3.10±0.02	3.10±0.03	NS
H	H	H	L	L	N-H	5.18±0.05	4.95±0.13	NS
H	H	H	L	H	N-L	3.61±0.06	3.16±0.09	NS
H	H	H	H	L	N	3.72±0.25	3.65±0.04	NS
H	H	H	H	H	L	2.95±0.08	2.70±0.38	NS
L	L	N	N	N	N-H	4.54±0.04	4.36±0.07	3.157*
L	H	N	N	N	N	4.01±0.06	4.08±0.02	NS
H	L	N	N	N	N-H	4.10±0.05	3.86±0.05	4.800*
H	H	N	N	N	N	4.27±0.12	4.09±0.01	NS
L	N	L	N	N	N-H	4.25±0.16	3.75±0.06	4.138*
L	N	H	N	N	N	4.37±0.17	3.94±0.23	NS
H	N	L	N	N	N-H	4.03±0.00	3.94±0.04	NS
H	N	H	N	N	N	3.81±0.12	3.79±0.00	NS
L	N	N	L	N	H	4.44±0.02	4.43±0.18	NS
L	N	N	H	N	N	4.25±0.23	3.99±0.04	NS
H	N	N	L	N	N-H	4.56±0.14	4.38±0.16	NS
H	N	N	H	N	N	3.76±0.19	3.49±0.35	NS
L	N	N	N	L	H	4.96±0.14	4.70±0.10	NS
L	N	N	N	H	N	4.11±0.04	3.36±0.31	3.393*
H	N	N	N	L	H	5.07±0.30	4.72±0.29	NS
N	L	L	N	N	H	5.01±0.22	4.36±0.09	3.867*
N	L	H	N	N	N	4.15±0.13	3.91±0.08	NS
N	H	L	N	N	N-H	4.26±0.13	4.23±0.03	NS
N	H	H	N	N	N-L	4.33±0.08	4.22±0.07	NS
N	L	N	L	N	H	4.94±0.17	4.74±0.00	NS
N	L	N	H	N	N	4.93±0.09	4.02±0.15	7.357**
N	H	N	L	N	N-H	4.53±0.22	4.50±0.03	NS
N	H	N	H	N	N-L	3.48±0.02	3.51±0.12	NS
N	L	N	N	L	H	4.67±0.17	4.47±0.36	NS
N	L	N	N	H	N	4.34±0.03	4.55±0.08	-3.476*
N	H	N	N	L	N-H	4.86±0.21	4.27±0.03	3.933*
N	N	N	N	N	N	4.72±0.07	4.16±0.13	5.460*

[a] N=2

[b] OVN = ovomucin, LYS=lysozyme, GLOB=globulins, OVD=ovomucoid, CON=conalbumin; OVB= ovalbumin. L=low, N=normal, H=high. Numerical values for low, normal, and high levels are listed in Table 9.

*Significant at P < 0.05.

**Significant at P < 0.01.

***Significant at P < 0.0005.

[c] NS-not significant at P = 0.05.

interchange reactions may take place without a significant decrease in sulfhydryl groups (Jensen, 1959) make it more difficult to pinpoint which solutions favored the interchange reaction. The few mixtures which showed an increase in sulfhydryl content may reflect more extensive denaturation of the proteins, but most likely resulted from variations in experimental conditions.

Response Surface Analyses

The effect of variations in levels of six albumen proteins on specific functional properties was analyzed. Significant effects on viscosity and foaming index of protein solutions (Table 19) and on volume and tenderness of angel food cakes (Table 20) were observed. Changes in surface tension (Table 19) and in compressibility (Table 20) were not significantly associated with variations in protein levels.

The partial regression coefficients and standard errors, and partial correlation coefficients of the variables influencing viscosity and foaming index of protein solutions, and cake volume and tenderness are summarized in Tables 21 through 24. Variables positively affecting viscosity (Table 21) were ovomucin ($P < 0.005$) and ovalbumin ($P < 0.0005$). Conalbumin and the interacting variables of ovomucin with the other five proteins negatively affected viscosity at $P < 0.05$.

Table 19. Mean Square Values and F-ratio Significance of Viscosity, Surface Tension, and Foaming Index of Albumen Proteins Mixtures.

Source	Degrees of Freedom	Mean Squares		
		Viscosity	Surface Tension	Foaming Index
Regression (about mean)	20	.20***	2.00	136.85**
Residual[a]	39	.01	2.01	8.92
Total	59			

***Significant at P < 0.0005.

[a]Error term.

Table 20. Mean Square Values and F-Ratios Significance of Volume, Tenderness, and Compressibility of Angel Food Cakes.

Dependent Variable	Degrees of Freedom			Mean Square Value	
	Regression	Residual[a]	Total	Regression	Residual[a]
Volume	19	40	59	2494.01***	180.07
Tenderness	18	41	59	0.39**	0.12
Compressibility	20	39	59	0.07	0.05

**Significant at P < 0.001.

***Significant at P < 0.0005.

[a]Error Term.

Table 21. Partial Regression Coefficients (bi), Standard Errors (S_b), Partial Correlation Coefficients, and t-test Significance for Viscosity of Protein Mixtures.

Variable Type Coded[a]	Regression Coefficient (bi)	Standard Error (S_b)	Partial Correlation Coefficient
Linear OVN	707.52	264.58	0.39*
LYS	31.08	44.22	0.11
GLOB	9.08	12.88	0.11
OVD	4.91	6.85	0.11
CON	- 11.04	5.05	-0.33*
OVB	1.55	0.18	0.80***
Interaction			
OVN x LYS	-745.99	296.65	-0.37*
OVN x GLOB	-709.75	267.83	-0.39*
OVN x OVD	-701.77	270.36	-0.38*
OVN x CON	-667.34	270.13	-0.36*
OVN x OVB	-704.80	271.41	-0.38*
LYS x GLOB	- 46.77	45.55	-0.16
LYS x OVD	- 40.08	46.71	-0.13
LYS x CON	- 18.24	46.35	-0.06
LYS x OVB	- 30.70	47.08	-0.10
GLOB x OVD	- 18.02	18.32	-0.15
GLOB x CON	16.23	16.47	0.15
GLOB x OVB	- 8.47	15.22	-0.09
OVD x CON	11.56	10.54	0.17
OVD x OVB	- 3.11	8.76	-0.06
CON x OVB	16.62	6.77	0.36*

*Significant at $P < 0.05$; ***Significant at $P < 0.0005$; a-OVN=ovomucin, LYS=lysozyme, GLOB=globulins, OVD=ovomucoid, CON=conalbumin, and OVB=ovalbumin.

Table 22. Partial Regression Coefficients (bi), Standard Errors (S_b), Partial Correlation Coefficients, and t-test Significance for Foaming Index of Protein Mixtures.

Variable Type	Variable Coded[a]	Regression Coefficient (bi)	Standard Error (S_b)	Partial Correlation Coefficient
Linear	OVN	5057.97	997.83	0.63***
	LYS	1238.19	1071.79	0.18
	GLOB	99.71	77.07	0.20
	OVD	- 93.09	91.65	-0.16
	CON	- 125.74	97.50	-0.20
	OVB	- 2.11	4.75	-0.07
Interaction	OVNxOVB	-3537.14	682.86	-0.64***
	LYSxGLOB	-1031.31	1157.93	-0.14
	LYSxOVD	-1117.07	1114.12	-0.16
	LYSxCON	-1314.68	1164.93	-0.18
	LYSxOVB	-1317.51	1165.03	-0.18
	GLOBxOVB	- 139.14	117.37	-0.19
	OVDxOVB	100.73	127.08	0.13
	OVNxLYSxGLOB	-126893.04	26699.49	-0.61***
	OVNxLYSxOVD	- 24077.62	20310.13	-0.19
Becker's Model	$\frac{OVNxLYS}{(OVN+LYS)}$	- 296.27	211.32	-0.22
	$\frac{OVNxGLOB}{(OVN+GLOB)}$	1102.82	325.59	0.48**
	$\frac{OVNxOVD}{(OVN+OVD)}$	- 1179.36	464.65	-0.38*
	$\frac{OVNxCON}{(OVN+CON)}$	- 2407.80	563.48	-0.56***
	$\frac{OVDxCON}{(OCD+CON)}$	81.83	69.77	0.18
	$\frac{CONxOVB}{(CON+OVB)}$	158.84	133.79	0.18

*Significant at P 0.05.
**Significant at P 0.01.
***Significant at P 0.0005.

[a]OVN=ovomucin, LYS=lysozyme, GLOB=globulins, OVD=ovomucoid, CON=conalbumin, OVB=ovalbumin.

Table 23. Partial Regression Coefficients (bi), Standard Errors (S_b), Partial Correlation Coefficients, and t-test Significance for Cake Volume.

Variable Type	Variable Coded[a]	Regression Coefficient(bi)	Standard Error (S_b)	Partial Correlation Coefficient
Linear	OVN	13806.63	5077.49	0.39**
	LYS	16474.39	4166.61	0.53****
	GLOB	- 2223.09	1523.21	-0.22
	OVD	- 1296.85	660.70	-0.30
	CON	542.80	346.51	0.24
	OVB	256.85	21.11	0.88****
Interaction	OVNxCON	- 6099.54	4614.42	-0.20
	OVNxOVB	- 8281.42	3893.64	-0.32*
	LYSxGLOB	-17926.07	4551.40	-0.53****
	LYSxOVD	-15941.07	4542.21	-0.49***
	LYSxCON	-17036.42	4543.87	-0.50***
	LYSxOVB	-16935.44	4459.28	-0.51****
	GLOBxOVD	3654.34	2003.10	0.27
	GLOBxCON	2764.02	1928.81	0.22
	GLOBxOVB	3164.59	1801.81	0.26
	CONxOVB	- 515.07	525.27	-0.15
Becker's Model	$\frac{OVNxLYS}{(OVN+LYS)}$	983.13	606.06	0.25
	$\frac{OVNxGLOB}{(OVN+GLOB)}$	- 3840.27	1271.79	-0.43**
	$\frac{OVNxOVD}{(OVN+OVD)}$	- 9142.23	2275.22	-0.54****
	$\frac{OVDxOVB}{(OVD+OVB)}$	2002.96	829.16	0.36*

*Significant at P < 0.05; **Significant at P < 0.01; ***Significant at P < 0.001; **** Significant at P < 0.0005.

[a]OVN=ovomucin, LYS=lysozyme, GLOB=globulins, OVD=ovomucoid, CON=conalbumin, OVB=ovalbumin.

Table 24. Partial Regression Coefficients (bi), Standard Errors (S_b), Partial Correlation Coefficients, and t-test Significance For Cake Tenderness.

Variable Type	Variable Coded[a]	Regression Coefficient(bi)	Standard Error (S_b)	Partial Correlation Coefficient
Linear	OVN	- 1531.04	828.27	-0.28
	LYS	- 11.18	29.78	-0.06
	GLOB	- 31.68	24.61	-0.20
	OVD	- 4.60	14.91	-0.05
	CON	46.53	14.93	0.44**
	OVB	3.56	0.51	0.74***
Interaction	OVNxLYS	1938.02	872.71	0.33*
	OVNxGLOB	1592.56	852.22	0.28
	OVNxOVD	1486.13	850.57	0.26
	OVCxCON	1511.82	843.72	0.27
	OVNxOVB	1594.45	849.94	0.28
	LYSxOVB	29.74	27.96	0.16
	GLOBxCON	- 28.14	26.72	-0.16
	OVDxCON	- 72.50	24.09	-0.43**
	CONxOVB	- 55.45	20.33	-0.39**
Becker's Model	$\frac{LYSxOVD}{(LYS+OVD)}$	25.87	14.49	0.27
	$\frac{LYSxCON}{(LYS+CON)}$	- 44.72	14.71	-0.43**
	$\frac{GLOBxOVB}{(GLOB+OVB)}$	38.85	29.45	0.20
	$\frac{OVDxOVB}{(OVD+OVB)}$	13.56	19.30	0.11

*Significant at P < 0.05; **Significant at P < 0.01; ***Significant at P < 0.0005.

[a]OVN=ovomucin, LYS=lysozyme, GLOB=globulins, OVD=ovomucoid, CON=conalbumin, OVB=ovalbumin.

The linear term ovomucin and the additive (Becker's model) term of ovomucin and globulins positively affected foaming index ($P < 0.01$). The interaction terms OVN x OVB, and OVN x LYS x GLOB, and the additive terms of ovomucin with ovomucoid and conalbumin showed negative significant effects on foaming index.

Cake volume (Table 23) showed significant partial positive correlation with ovomucin, globulins and ovalbumin. The interaction terms of OVN x OVB and of lysozyme with globulins, ovomucoid, conalbumin, and ovalbumin negatively correlated with volume. The additive (Becker's model) terms of ovomucin with globulins and ovomucoid significantly reduced cake volume.

Significant variables affecting cake tenderness (Table 24) positively were conalbumin, ovalbumin, and the interacting OVN x LYS variable. The interaction terms OVD x CON, and CON x OVB negatively correlated with tenderness. Lysozyme and conalbumin additive variables also significantly reduced tenderness.

The effects of the various protein mixtures on viscosity, foaming index, cake volume, and tenderness when evaluated by multiple regression analysis, revealed complex interactions among the individual mixture components. The underlying effects of the individual components, however, cannot be analysed as such, and the overall mixture response should be considered for evaluating functional performance. The prediction equations of non-polynomial form for the

significant protein solutions physical parameters and cake parameters are illustrated in Tables 25 and 26, respectively. The prediction equations for foaming index and volume were used for drawing contour response surface and perspective response surface with variations in ovomucin, lysozyme, and globulins. Figures 11 and 12 illustrate the effects of ovomucin, lysozyme, and globulins, on the response surfaces of foaming index and cake volume, respectively.

Nearly linear correlations between ovomucin and lysozyme levels with foaming index values of three and above were observed (Figure 11A). Increasing lysozyme concentration progressively decreased foaming index. Minimum foaming index values were characterized by high levels of lysozyme and normal levels of ovomucin. The region of optimum response was localized in the corner defined by low levels of lysozyme and high levels of ovomucin.

Figure 11B illustrates the three-dimensional correlation among ovomucin, lysozyme, and foaming index. It is clearly seen that the optimum response was in the region of high levels of ovomucin and low levels of lysozyme. The minimum extreme was observed in the region of normal levels of ovomucin and high lysozyme content.

The effects of lysozyme and ovomucin on volume are seen in Figure 12. Increasing ovomucin levels consistently decreased volume, whereas, with lysozyme, the inverse occurred. Maximum volume responses were observed in the neighborhood of the regions with low ovomucin levels and

Table 25. Prediction Equations for Significant*** Physical Parameters of Protein Mixtures, Standard Error of the Estimate (S), and Coefficients of Multiple Determination (R^2).

Parameter	Equation[a]	S	R^2
Foaming Index	$Y = 5057.97X_1+1238.19X_2+99.71X_3-93.09X_4-125.74X_5-2.11X_6-3537.14X_1X_6-1031.31X_2X_3-1117.07X_2X_4-1314.68X_2X_5-1317.51X_2X_6-139.14X_3X_6+100.73X_4X_6-126893.04X_1X_2X_3-24077.62X_1X_2X_4-296.27\frac{X_1X_2}{(X_1+X_2)}+1102.82\frac{X_1X_3}{(X_1+X_3)}-1179.36\frac{X_1X_4}{(X_1+X_4)}-2407.80\frac{X_1X_5}{(X_1+X_5)}+81.33\frac{X_4X_5}{(X_4+X_5)}+158.84\frac{X_5X_6}{(X_5+X_6)}$	2.99	0.89
Viscosity	$Y = 707.52X_1 + 31.08X_2+9.08X_3+4.91X_4-11.04X_5+1.55X_6-745.99X_1X_2-709.75X_1X_3-701.77X_1X_4-667.34X_1X_5-704.80X_1X_6-46.77X_2X_3-40.08X_2X_4-18.24X_2X_5-30.70X_2X_6-18.02X_3X_4+16.23X_3X_5-8.47X_3X_6+11.56X_4X_5-3.11X_4X_6+16.62X_5X_6$	0.11	0.89

***Significant at $P < 0.0005$.

[a] X_1 = ovomucin, X_2 = lysozyme, X_3 = globulins, X_4 = ovomucoid, X_5 = conalbumin, X_6 = ovalbumin.

Table 26. Prediction Equations for Significant Cake Parameters, Standard Error of the Estimate (S), and Coefficients of Multiple Determination (R^2).

Parameter	Equation[a]	S	R^2
Volume***	$Y = 13806.63X_1+16474.39X_2-2223.09X_3-1296.85X_4+542.80X_5+256.85X_6-6099.54X_1X_5-8281.42X_1X_6-17926.07X_2X_3-15941.07X_2X_4-17036.42X_2X_5-16935.44X_2X_6+3654.34X_3X_4+2764.02X_3X_5+3164.59X_3X_6-515.07X_5X_6+983.13\frac{X_1X_2}{(X_1+X_2)}-3840.27\frac{X_1X_3}{(X_1+X_3)}-9142.23\frac{X_1X_4}{(X_1+X_4)}+2002.96\frac{X_4X_6}{(X_4+X_6)}$	13.42	0.86
Tenderness**	$Y = -1531.04X_1-11.18X_2-31.68X_3-4.60X_4+46.53X_5+3.56X_6+1938.02X_1X_2+1592.56X_1X_3+1486.13X_1X_4+1511.82X_1X_5+1594.45X_1X_6+29.74X_2X_6-28.14X_3X_5-72.50X_4X_5-55.45X_5X_6+25.87\frac{X_2X_4}{(X_2+X_4)}-44.72\frac{X_2X_5}{(X_2+X_5)}+38.85\frac{X_3X_6}{(X_3+X_6)}+13.56\frac{X_4X_6}{(X_4+X_6)}$	0.34	0.59

***Significant at $P < 0.0005$.

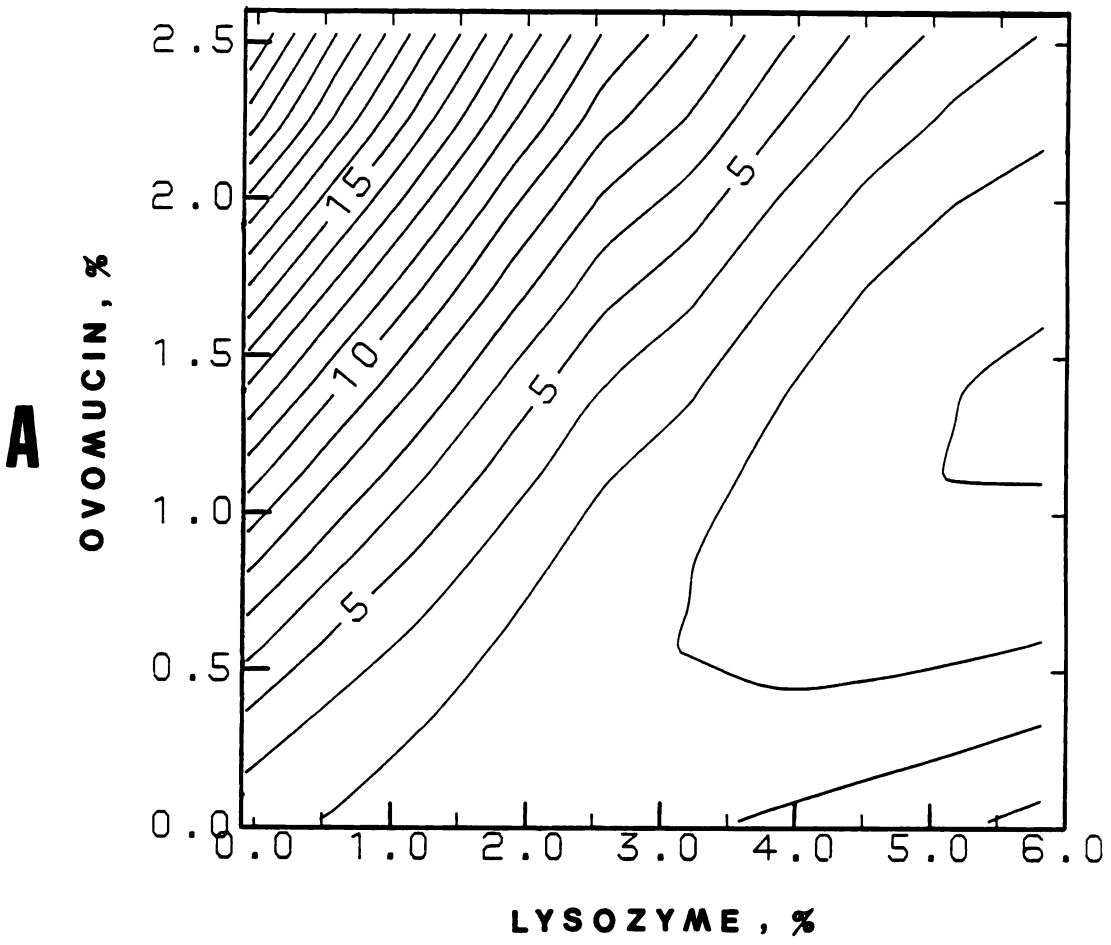
**Significant at $P = 0.001$.

[a] X_1 = ovomucin, X_2 = lysozyme, X_3 = globulins, X_4 = ovomucoid, X_5 = conalbumin, X_6 = ovalbumin.

Fig. 11. Protein Mixture Foaming Index Response Surfaces as a Function of Lysozyme and Ovomucin. Other Protein Levels: Globulins 6.50-14.95%, Ovomucoid 12%, Conalbumin 14%, and Ovalbumin 59%.

a) Contour Surface.

b) Perspective Response Surface.


A
OVOMUCIN, %
2.5
2.0
1.5
1.0
0.5
0.0
15
10
5
5
5
0.0
1.0
2.0
3.0
4.0
5.0
6.0
LYSOZYME, %

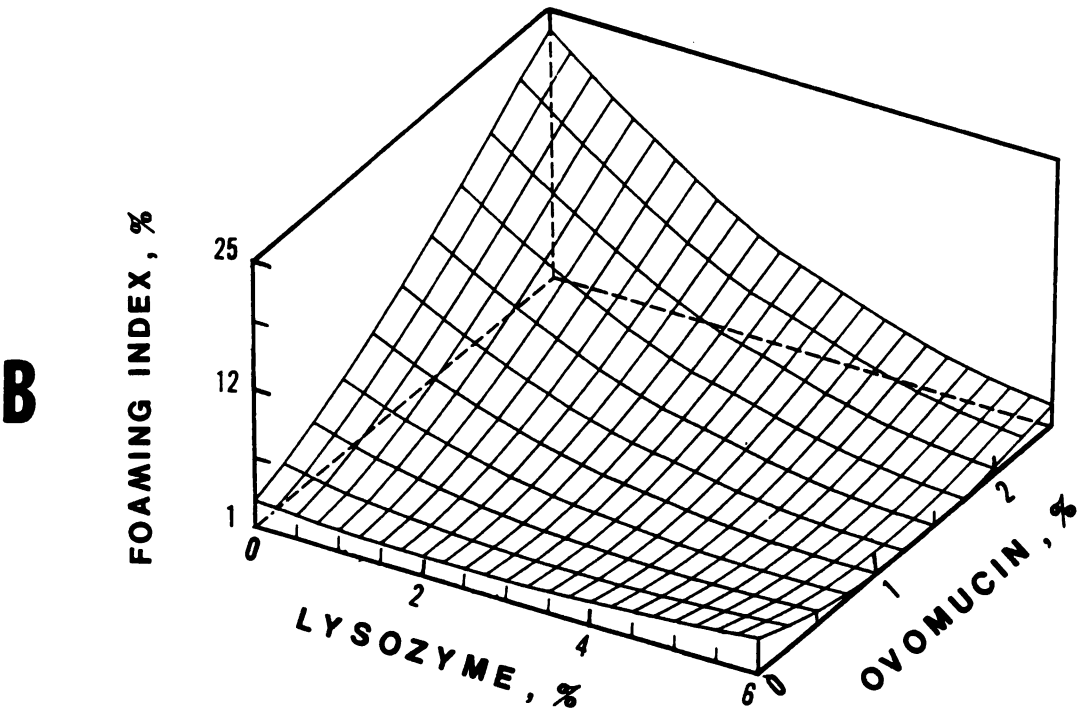
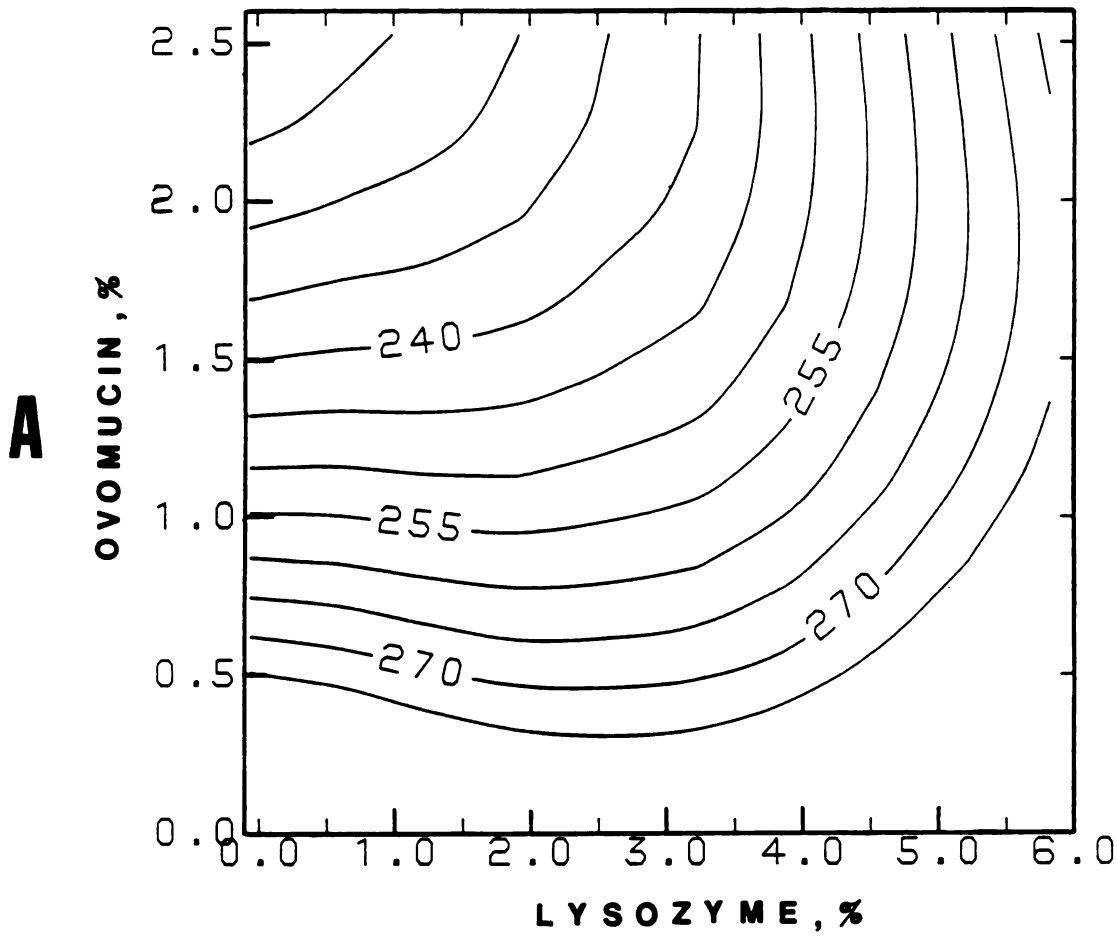
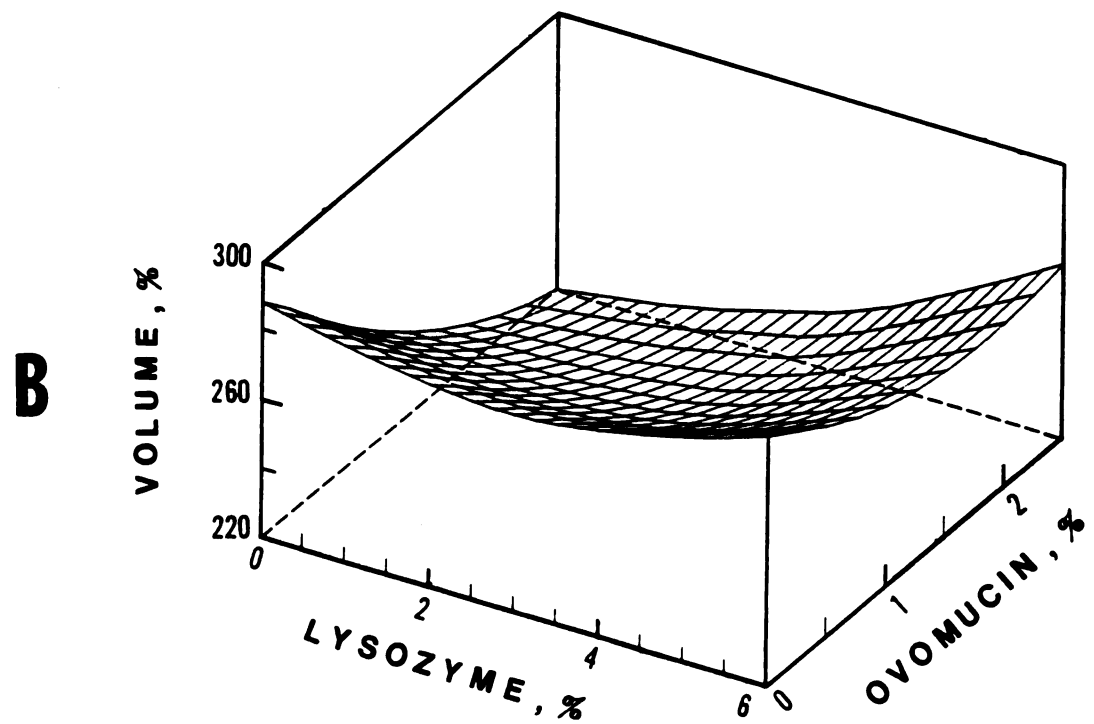

B
FOAMING INDEX, %
25
12
1
0
2
4
6
LYSOZYME, %
0
1
2
OVOMUCIN, %

Fig. 12. Angel Food Cake Volume Response Surfaces as a Function of Lysozyme and Ovomucin. Other Protein Levels: Globulins 6.50-14.95%, Ovomucoid 12%, Conalbumin 14%, and Ovalbumin 59%.

a) Contour Surface

b) Perspective Response Surface.


A
OVOMUCIN, %
2.5
2.0
1.5
1.0
0.5
0.0
240
255
255
270
270
0.0 1.0 2.0 3.0 4.0 5.0 6.0
LYSOZYME, %


B
VOLUME, %
300
260
220
0
2
4
6
LYSOZYME, %
0
1
2
OVOMUCIN, %

low and high lysozyme levels.

For optimization of foaming index and volume simultaneously Figures 11A and 12A were overlaid to designate the point or regions of maximum response (Figure 13). Since foaming index and volume correlated inversely, the regions of satisfactory response, that is, the region delineated by volume values ranging from 255 cm^3 and above and foaming index values above 4 $cm^3/g/min.$, were considered. This region was localized near the corner with low levels of ovomucin (0.2-1.0%) and lysozyme (0.0-1.8%). Accordingly, globulins levels ranged from 12.2 to 14.8%. The normal levels of these proteins in egg-white are 1.5, 3.5, and 8% for ovomucin, lysozyme and globulins, respectively.

Custard Model System

The time-temperature relationships for gels prepared with lysozyme, globulins, conalbumin, and ovalbumin are illustrated in Figure 14. A rapid increase in temperature followed by a decrease and levelling off during heating was observed for all gels. The decrease in temperature marked the onset of coagulation and also showed that aggregation of the polypeptides proceeded with absorption of energy from the system (endothermic process).

The coagulation temperature ranges, firmness, and percentage drainage of gels prepared with lysozyme, globulins, conalbumin, and ovalbumin, and t-statistics

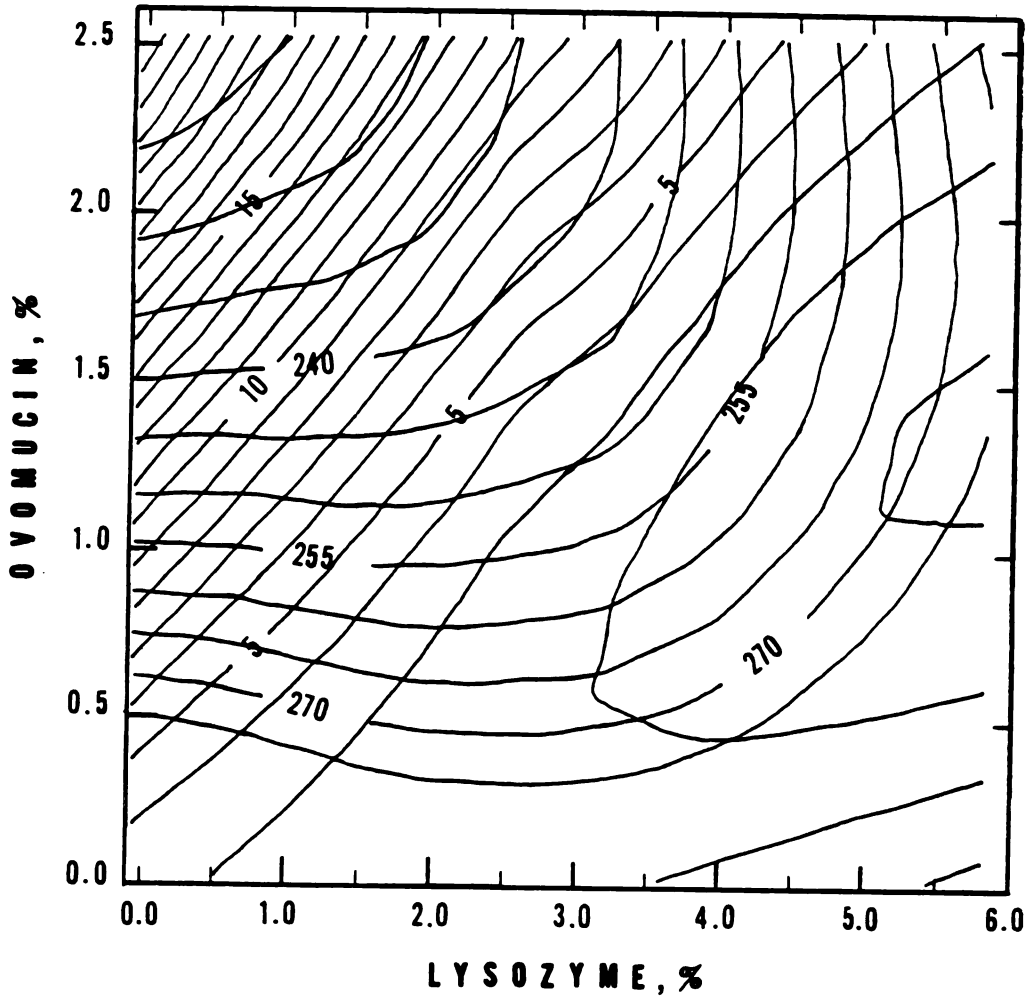


Fig. 13. Foaming index and volume contour plots overlay for designation of levels of ovomucin and lysozyme in angel food cake system.

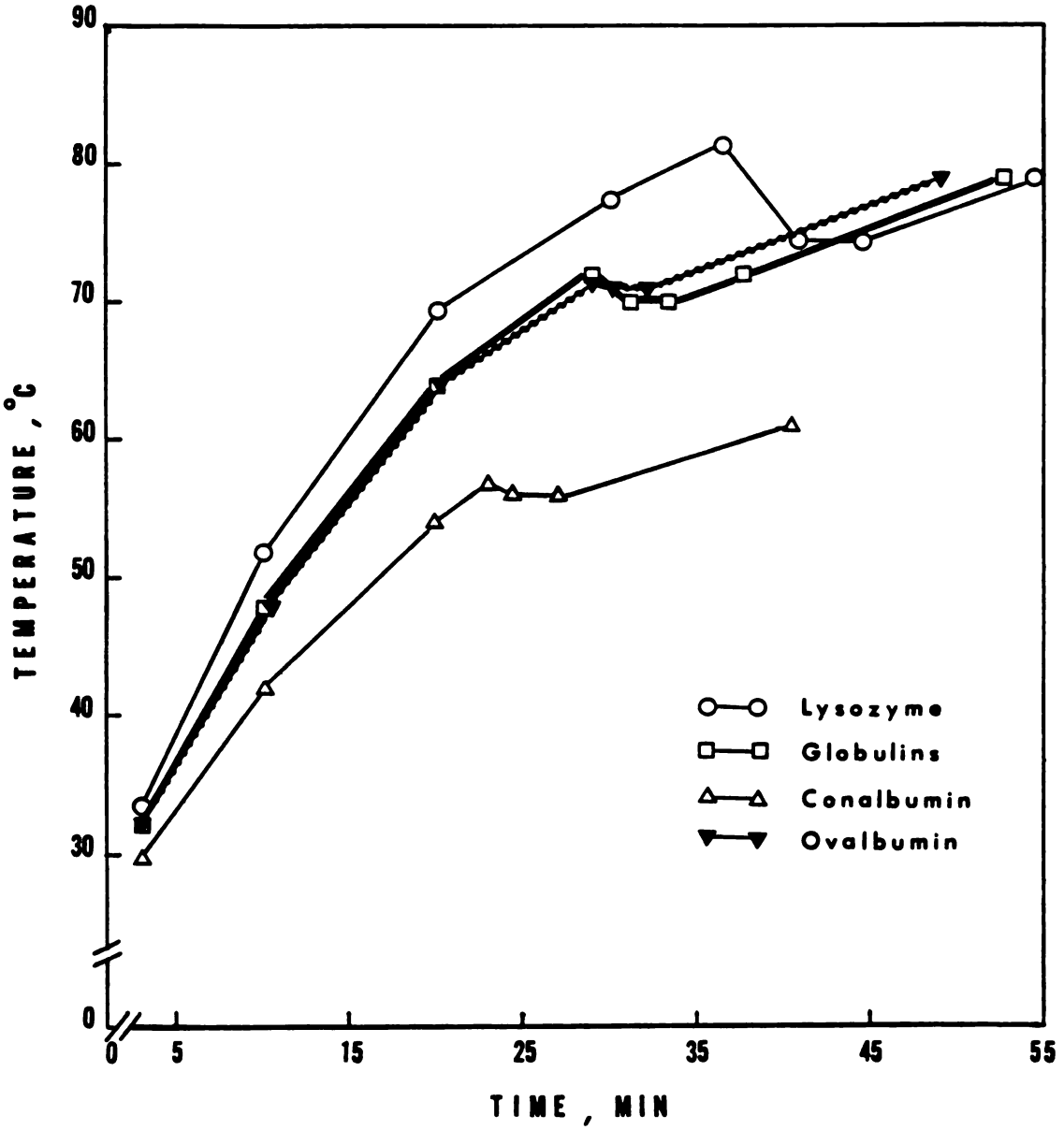


Fig. 14. Time-temperature curves of several albumen protein solutions heated at a rate of 0.74°C/min. Protein concentration = 1.27%, ionic strength = 0.275, pH = 8.0.

are shown in Tables 27 through 29. Conalbumin coagulation initiated at 57.3°C and aggregation ensued promoting a drop of 1.3°C in temperature.[b] Ovalbumin coagulation temperature ranged from 71.5 to 71.0°C. Aggregation of the polypeptides proceeded with a modest drop of 0.5°C in temperature. A temperature range of 72.0-69.9°C for globulins coagulation was observed with a drop of about 2°C in temperature. Lysozyme showed the highest thermal requirement for unfolding of the polypeptides, 81.5°C. Formation of the coagulum ensued with a drop of 7°C in temperature. The initial coagulation temperatures were significantly different among the various gels except for ovalbumin and globulins gels.

Ovomucin and ovomucoid showed no gelation properties under the conditions used for evaluations. The observed heat stability of these proteins is in close agreement with the earlier findings of MacDonnell et al. (1953) and Lineweaver and Murray (1947) for ovomucin and ovomucoid, respectively.

Significant differences among the various protein gel firmness were observed (Table 28). Lysozyme produced the firmest gel (43.98 g x cm) of all proteins, followed by globulins (14.26 g x cm), ovalbumin (5.68 g x cm) and conalbumin (3.61 g x cm).

Percentage drainage measurements reflect the ability of gels to hold liquid in the interstices and this in turn is dependent on gel strength. As expected, lysozyme

Table 27. Coagulation Temperature Ranges of Albumen Proteins Solutions[a] and T-Statistic.

Protein Solution[b]	Temperature Range[c]		t-statistic[d]			
	Initial	Drop	Lysozyme	Globulins	Conalbumin	Ovalbumin
	°C					
Ovomucin	NC[e]					
Ovomucoid	NC[e]					
Lysozyme	81.5±0.7	74.5±0.0		13.435**	43.379***	20.000**
Globulins	72.0±0.7	69.9±0.1			26.385**	NS[f]
Conalbumin	57.3±0.4	56.0±0.0				-56.993***
Ovalbumin	71.5±0.0	71.0±0.0				
LYS-OVD	NC[e]					
LYS-GLOB	67.5±0.0	66.3±0.4	-28.000**	-9.000**		
LYS-CON	58.3±0.4	56.8±0.4	-41.590***		NS[f]	
LYS-OVB	70.6±0.5	69.8±0.7	-17.400**			NS[f]
GLOB-OVD	76.1±0.2	75.1±0.2		8.004**		
GLOB-CON	57.8±0.0	57.0±0.0		-28.500**	NS[f]	
GLOB-OVB	70.4±0.2	69.0±0.0		-3.153*		-8.999**
OVD-CON	58.8±0.4	57.8±0.4			4.242*	
OVD-OVB	73.5±0.7	72.6±0.5				4.000*
CON-OVB	58.1±0.2	57.1±0.2			3.130*	-106.986***

[a]Protein concentration = 1.27%, pH = 8.0, ionic strength = 0.275.

[b]Mixtures are 1:1 on a weight basis; LYS = lysozyme, OVD = ovomucoid, GLOB = globulins, CON = conalbumin, OVB = ovalbumin.

[c]N = 2.

[d]Comparisons between initial temperature means.

[e]NC = no coagulation.

[f]NS = not significant at P = 0.05.

*significant at P < 0.05.

**significant at P < 0.01.

***significant at P < 0.0005.

Table 28. Firmness of Coagulums Prepared with Albumen Proteins Solutions[a] and T-Statistic.

Protein[b]	Gel Strength[c]	t-statistic			
		Lysozyme	Globulins	Conalbumin	Ovalbumin
	g x cm				
Lysozyme	43.98±4.51		9.299**	12.597**	11.984**
Globulins	14.26±0.23			31.878***	36.587***
Conalbumin	3.61±0.41				-6.202*
Ovalbumin	5.68±0.23				
LYS-GLOB	26.55±0.09	-5.460*	69.397***		
LYS-CON	11.59±0.50	-10.088**		17.484**	
LYS-OVB	10.59±0.97	-10.229**			6.980**
GLOB-OVD	3.58±0.10		-59.028***		
GLOB-CON	10.57±0.29		-13.898	19.531**	
GLOB-OVB	10.80±2.23		NS[d]		3.232*
OVD-CON	2.51±0.03			-3.776*	
OVD-OVB	1.67±0.04				-23.768**
CON-OVB	4.17±0.56			NS[d]	-3.541*

[a]Protein concentration = 1.27%, pH = 8.0, ionic strength = 0.275.

[b]Mixtures are 1:1 on a weight basis.

[c]N = 2.

[d]NS = not significant at P = 0.05.

*significant at P < 0.05.

**significant at P < 0.01.

***significant at P < 0.0005.

Table 29. Percentage Drainage of Gels Made with Albumen Proteins Solutions and T-Statistic.

Protein[a]	Drainage[b]	t-statistic			
		Lysozyme	Globulins	Conalbumin	Ovalbumin
	%				
Lysozyme	57.2±0.8		-3.070*	-3.000*	NS[c]
Globulins	59.3±0.5			NS[c]	NS[c]
Conalbumin	64.3±3.2				NS[c]
Ovalbumin	59.3±0.9				
LYS-GLOB	62.9±0.0	9.545**	9.847**		
LYS-CON	63.9±4.3	NS[c]		NS[c]	
LYS-OVB	61.4±1.5	3.509*			NS[c]
GLOB-OVD	70.2±0.5		22.465**		
GLOB-CON	65.1±3.9		NS[c]	NS[c]	
GLOB-OVB	59.0±0.8		NS[c]		NS[c]
OVD-CON	75.5±0.6			4.835*	
OVD-OVB	72.7±1.7				10.060**
CON-OVB	63.9±0.2			NS[c]	7.337**

[a]Mixtures are 1:1 on a weight basis; LYS = lysozyme, GLOB = globulins, CON = conalbumin, OVB = ovalbumin, OVD = ovomucoid.

[b]N = 2.

[c]NS = not significant at P = 0.05.

*significant at P < 0.05.

**significant at P < 0.01.

coagulum showed the least loss of liquid (57.2%) (Table 29). Although globulins gel strength was relatively higher than that of ovalbumin, both coagulums showed similar percentage drainage of 59.3%. The highest loss of liquid, 64.3%, was exhibited by conalbumin gel. Significant differences between percentage drainages of lysozyme and globulins gels, lysozyme and conalbumin gels, were observed.

The great variability in temperature for initiation of denaturation observed for the various proteins is apparently associated with protein molecular conformation. Disulfide, as well as hydrogen, hydrophobic, and electrostatic bonds, and Van der Waals attractions stabilize the protein structure. The covalent SS linkage has fairly high heat of formation ranging from 30-100 Kcal/mole (Whitaker , 1977). Consequently, disruption also requires fairly high energy. Ionic attractions rank second with approximately 10-20 kcal/mole, followed by hydrogen bonds, 1-5 kcal/mole, and Van der Waals attractive forces, with 1-3 kcal/mole.

Proteins that are compact in shape, as well as bearing several disulfide bonds appear to show higher resistance to heat denaturation (Whitaker, 1977). Therefore, the relatively high denaturation temperature of lysozyme (81.5°C) may have resulted from its nearly spherical shape and the high concentration of SS groups, 2.74 moles/ 10^4 g protein, maintaining the protein structure.

The heat resistance exhibited by ovomucoid and

ovomucin was remarkable. Ovomucoid has 3 moles SS/10^4 g protein, and ovomucin 3.79 moles SS/10^4 g protein. The disulfide groups are evidently involved in stabilizing the protein structure at key points. Additionally, both proteins have a very high content of carbohydrate moieties. These groups may physically hinder formation of aggregates through swelling or expansion of the proteins.

Conalbumin has 1.85 moles SS/10^4 g protein. Apparently, the disulfide groups are either not involved in maintaining the higher structural level of the protein (quaternary) since denaturation takes place at a relatively low temperature ($\sim$ 57°C), or these groups are more susceptible to disruption. In addition, disruption of only hydrogen and/or hydrophobic bonds may be sufficient for formation of aggregates.

Globulins, with 1.51 moles SS/10^4 g protein, exhibited higher heat stability than conalbumin. Ovalbumin, with 0.3 moles SS/10^4 g protein (1 disulfide group/mole protein) had heat stability comparable to that of globulins. It appears that the single disulfide linkage in ovalbumin is situated at a key point in the polypeptide chain lending higher structural stability to the protein. Possibly, other types of molecular interactions, particularly electrostatic attractions contribute to maintenance of ovalbumin structure. Evidences for formation of ionic interactions in ovalbumin are not available, but these may very possibly occur since ovalbumin has various phosphate groups attached

to the molecule.

Formation of stronger gels may be related to more extensive cross-linking of the polypeptides. The drastic drop in temperature of 7°C during lysozyme coagulation paralleled by formation of the firmest gel suggests more extensive SS linkages were involved in the aggregation phenomenon of this protein.

Protein Interactions

The effects of the double combinations of lysozyme, globulins, ovomucoid, conalbumin, and ovalbumin on gels coagulation temperature, strength, and percentage drainage are shown in Tables 27 through 29. Data for the student t-test of these characteristics are also summarized in these tables. Time-temperature curves of the double combinations are illustrated in Figures 29 through 32 in the appendix C section.

As can be seen in Table 27, the presence of conalbumin, globulins, and ovalbumin significantly decreased the coagulation temperature range of lysozyme coagulums. Conalbumin showed the most intense effect, lowering the initial coagulation temperature of lysozyme from 81.5 to 58.3°C. Globulins ranked second (67.5°C), followed by ovalbumin (70.6°C). In addition, the reduction in temperature during formation of the aggregate in these double combinations was much less pronounced, 0.8-1.5°C compared to 7.0°C for

lysozyme. It was interesting to note that lysozyme-ovomucoid combination did not coagulate suggesting ovomucoid had a protective effect on lysozyme, thus preventing denaturation.

For interaction gels with globulins, lysozyme significantly reduced coagulation temprature of globulins from 72.0 to 67.5°C, conalbumin to 57.8°C, and ovalbumin to 70.4°C. Ovomucoid increased the initial coagulation temperature to 76.1°C.

The effects of the double combination treatments on the coagulation temperature of conalbumin (57.3°C) were not significant with lysozyme and globulins addition, whereas significant increases were observed with ovomucoid addition (58.8°C) and with ovalbumin addition (58.1°C). The changes in initial coagulation temperature of ovalbumin gel (71.5°C) were significant with the addition of globulins (70.4°C), ovomucoid (73.5°C), and conalbumin (58.1°C).

Table 28 presents the gel strength data of the double protein combinations. Globulins significantly reduced gel firmness of lysozyme from 43.98 to 26.55 g x cm, followed by conalbumin (11.59 g x cm), and ovalbumin (10.59 g x cm).

Firmness of coagulums containing globulins (14.26 g x cm) markedly increased when lysozyme (26.55 g x cm) was added, but a reduction was observed with the addition of ovomucoid (3.58 g x cm), conalbumin (10.57 g x cm), and ovalbumin (10.80 g x cm). These changes were significant except for the coagulum with globulins and ovalbumin.

Conalbumin coagulum firmness significantly improved with the addition of lysozyme from 3.61 to 11.59 g x cm, and with globulins (10.57 g x cm), but was significantly weakened when combined with ovomucoid (2.5 g x cm). Ovalbumin slightly increased firmness of conalbumin coagulum (4.17 g x cm).

Significantly firmer coagulums were obtained when ovalbumin (5.68 g x cm) was combined with lysozyme (10.59 g x cm) and globulins (10.8 g x cm). In combination with ovomucoid (1.67 g x cm) and conalbumin (4.17 g x cm), weakening of the gels was observed.

Data for percentage drainage of the coagulums prepared with the double combinations are shown in Table 29. A significant increase in liquid loss of lysozyme coagulum (57.2%) with the addition of globulins (62.9%), and ovalbumin (61.4%) was detected. Conalbumin addition to lysozyme gel induced the highest liquid loss (63.9%), but the difference was not significant, owing to the relatively high standard deviation.

Globulins gel percentage drainage increased from 59.3 to 62.9% with the addition of lysozyme, to 70.2% with ovomucoid, and to 65.1% with conalbumin. Ovalbumin did not show an apparent effect on liquid loss from globulins gel.

The double combination gels of conalbumin with lysozyme, globulins, and ovalbumin showed similar liquid loss to that of conalbumin coagulum. A significant increase in

percentage drainage of conalbumin gel from 64.3% to 75.5% was observed with ovomucoid addition.

Percentage drainage of ovalbumin gel (59.3%) was not significantly different from LYS-OVB combination gel (61.4%) and from GLOB-OVB combination gel (59.0%). In contrast, compared to OVD-OVB combination gel (72.2%) and to CON-OVB combination gel (63.9%) significant differences were detected.

Figures 15 through 18 illustrate the changes observed in the double protein combinations gels coagulation temperature, firmness, and percentage drainage.

As can be seen in Figure 15, the extent of the effects on lysozyme gel coagulation temperature and firmness was pronounced, whereas percentage drainage was moderately affected. Globulins, conalbumin, and ovalbumin reduced coagulation temperature and gel strength, and increased liquid loss.

The evidence for increased instability of lysozyme to heat denaturation with the inclusion of globulins, conalbumin, or ovalbumin may be related to a denaturating action exerted by these proteins. It seemed that once a few protein molecules were denatured, the process of aggregation was accelerated resulting in formation of coagulums. Earlier investigations have shown that addition of thiols to proteins containing SS and SH groups enhanced the rate of intermolecular SH-SS exchange leading to polymerization of the polypeptides (Huggins et al., 1951).

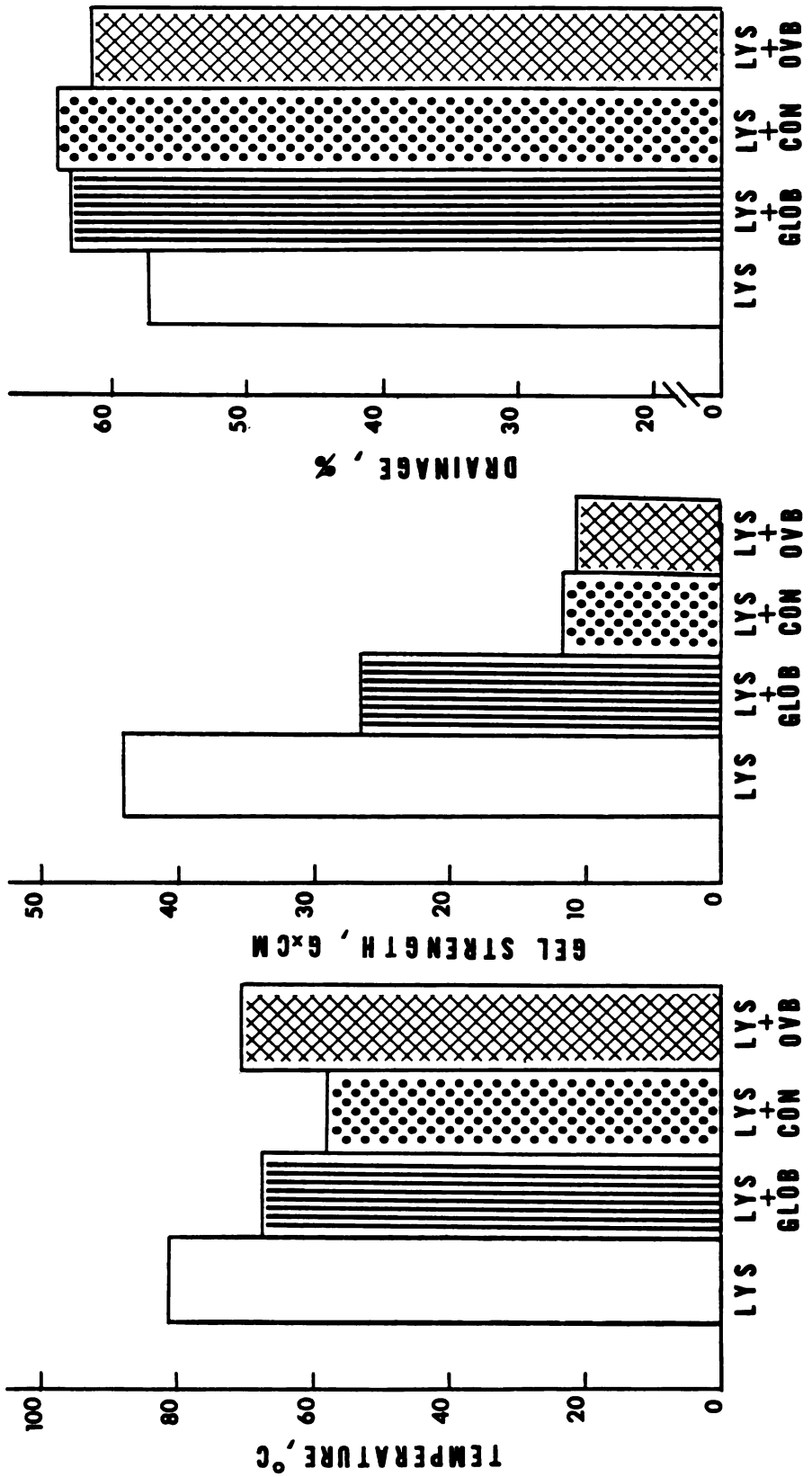


Fig.15. A comparison of changes in coagulation temperature, gel firmness, and percentage drainage of lysozyme (Lys) coagulum with the addition of globulins (Glob), conalbumin (Con), and ovalbumin (Ovb).

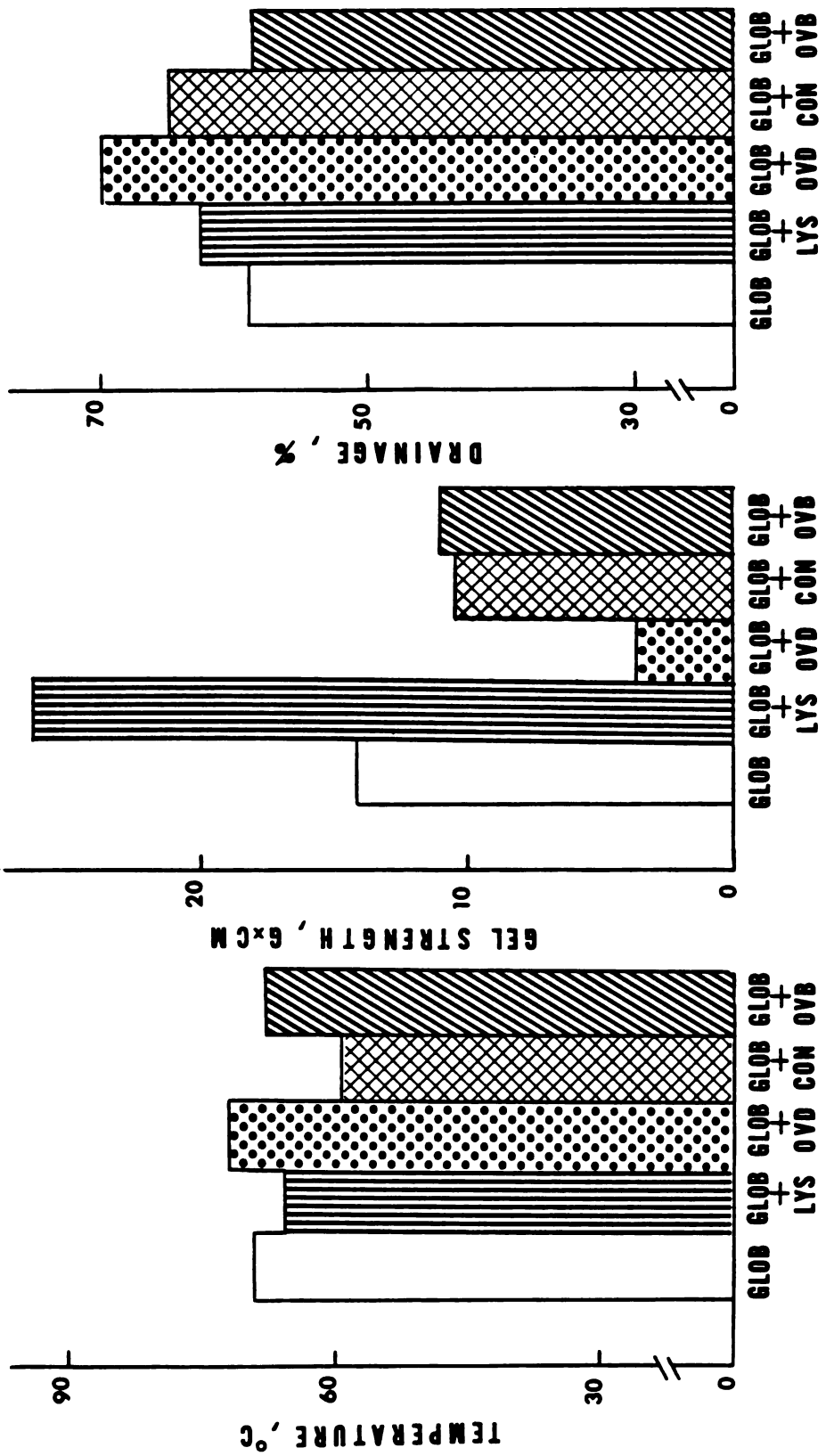


Fig.16. A comparison of changes in coagulation temperature, gel firmness, and percentage drainage of globulins (Glob) coagulum with the addition of lysozyme (Lys), ovomucoid (Ovd), conalbumin (Con), and ovalbumin (Ovb).

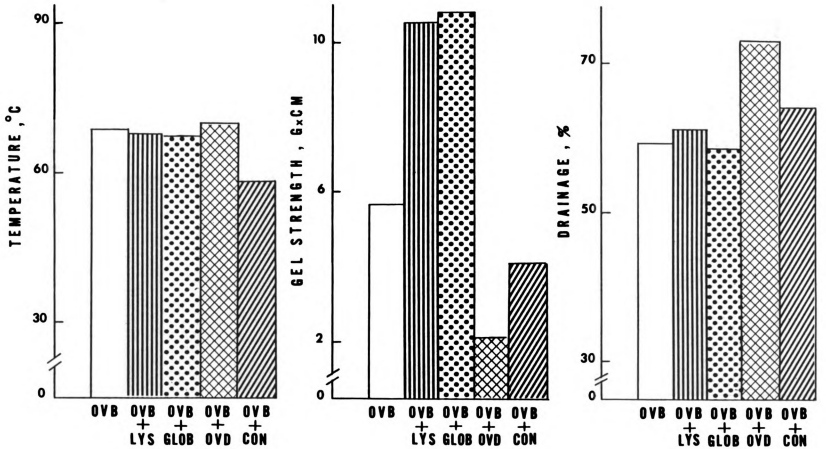


Fig.17. A comparison of changes in coagulation temperature, gel firmness, and percentage drainage of ovalbumin (Ovb) coagulum with the addition of lysozyme (Lys), globulins (Glob), ovomucoid (Ovd), and conalbumin (Con).

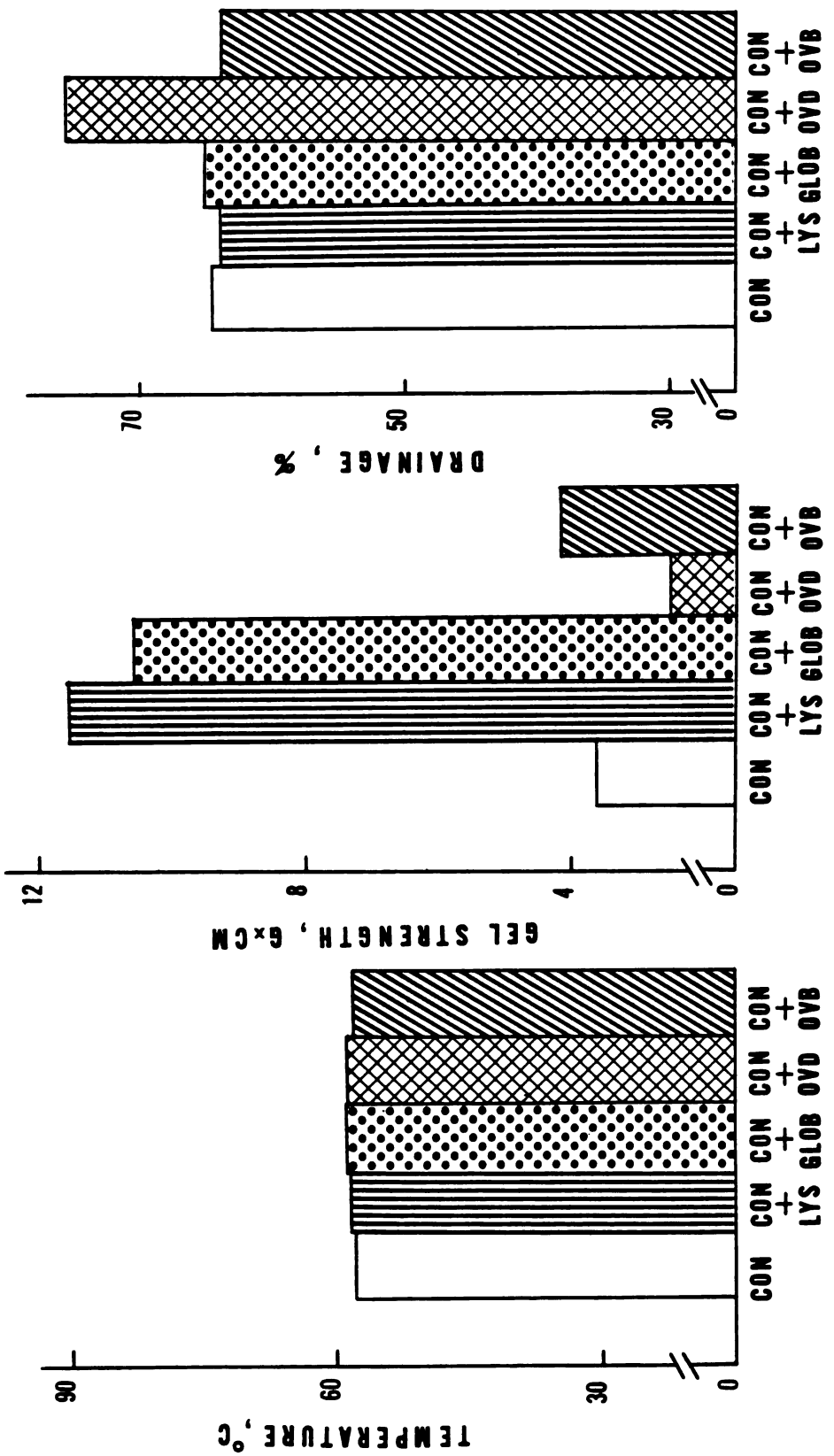


Fig. 18. A comparison of changes in coagulation temperature, gel firmness, and percentage drainage of conalbumin (Con) coagulum with the addition of lysozyme (Lys), globulins (Glob), ovomucoid (Ovd), and ovalbumin (Ovb).

The decrease in lysozyme gel strength reflected the combined effects of variable extent of aggregation of the different proteins and dilution effects. Increases in percentage drainage paralleled formation of weaker gels.

Similar trends were observed for the effects of double combinations in the other protein gels (Figures 16, 17, and 18). Ovomucoid raised the denaturation temperature of globulins, but gel strength decreased considerably. It appeared that ovomucoid did not participate in the aggregation phenomena leading to formation of a weaker gel, which also reflected in the poor liquid holding ability of the gel.

Conalbumin and its combinations consistently showed the lowest coagulation temperature. Lysozyme and globulins double combinations showed the highest gel strengths which indicated these proteins were actively involved in development of the coagulums.

The effect of heating on the sulfhydryl (SH) content and pH of the various protein solutions are summarized in Table 30. In general, heating decreased the number of SH groups of the coagulums. Significant reductions in SH groups of globulins, ovalbumin, and GLOB x OVB gels were observed. In contrast, heating significantly increased SH content of lysozyme, conalbumin, and OVD x CON gels. The occurrence of extensive unfolding of polypeptides without formation of inter and/or intramolecular SS bonds does not seem a viable possibility for these gels. In

Table 30. Effect of Heating on the Sulfhydryl Content and pH of Albumen Proteins Solutions.[a]

Protein[b]	Sulfhydryl Content[c] Solution	Coagulum	t-statistic	Coagulum pH
	moles/10^5 g sample			
Lysozyme	0.859 ± 0.028	1.123 ± 0.085	-4.172*	7.32 ± 0.00
Globulins	3.164 ± 0.003	2.097 ± 0.008	176.611***	7.89 ± 0.00
Conalbumin	1.040 ± 0.050	1.823 ± 0.057	-14.604*	7.74 ± 0.06
Ovalbumin	5.363 ± 0.078	4.805 ± 0.226	3.301*	7.52 ± 0.02
LYS-GLOB	2.011[d]	1.535 ± 0.016		7.55 ± 0.00
LYS-CON	0.949[d]	1.322 ± 0.015		7.50 ± 0.03
LYS-OVB	3.111[d]	2.027 ± 0.084		7.28 ± 0.00
GLOB-OVD	2.302[d]	1.414 ± 0.036		7.50 ± 0.00
GLOB-CON	2.102[d]	1.621 ± 0.196		7.59 ± 0.01
GLOB-OVB	4.008 ± 0.109	2.814 ± 0.177	8.123*	7.71 ± 0.01
OVD-CON	0.950[d] ± 0.008	1.196 ± 0.034	-9.960**	7.70 ± 0.17
OVD-OVB	3.401[d]	2.616 ± 0.079		7.72 ± 0.05
CON-OVB	3.202[d]	3.329 ± 0.094		7.52 ± 0.00

[a]Protein concentration = 1.27%, initial pH = 8.0, ionic strength = 0.275.

[b]Mixtures are 1:1 on a weight basis; LYS-lysozyme, GLOB-globulins, CON-conalbumin, OVB-ovalbumin, OVD-ovomucoid

[c]N=2

[d]Estimated values

[e]NS = not significant at P = 0.05

*Significant at P < 0.05.
**Significant at P < 0.01.
***Significant at P < 0.0005.

turn, the observed incomplete solubilization of the gels during the determinations may have caused higher absorbance readings, affecting the results.

A significant decrease in pH from 8.00 to 7.58 (treatment total) occurred with heating. This indicated more acidic groups were exposed with the treatment.

The time-temperature relationship of protein mixtures gels are illustrated in Figures 19 and 20. As can be seen in Figure 19, the mixture containing all proteins showed a slight decrease in temperature (0.5°C) after about 15 min. of heating followed by a fairly rapid increase in temperature. The mixture lacking ovomucin coagulated at two different temperatures at about 23 and 38 min. of heating. The other protein mixtures did not show an apparent drop in temperature during coagulation, but the slight decrease in rate of heat penetration, seen in the curves, marked the onset of coagulation.

The mixture with no globulins showed a slight drop in temperature during coagulation (Figure 20). It can be seen that the mixture lacking conalbumin coagulated after about 25 min. of heating, whereas without ovalbumin, coagulation took place earlier.

The characteristics of the gels prepared with the protein mixtures are summarized in Table 31. Mixtures containing ovomucin, lysozyme, and conalbumin simultaneously showed an initial coagulation temperature ranging from 55 to 59°C. Removal of conalbumin raised coagulation

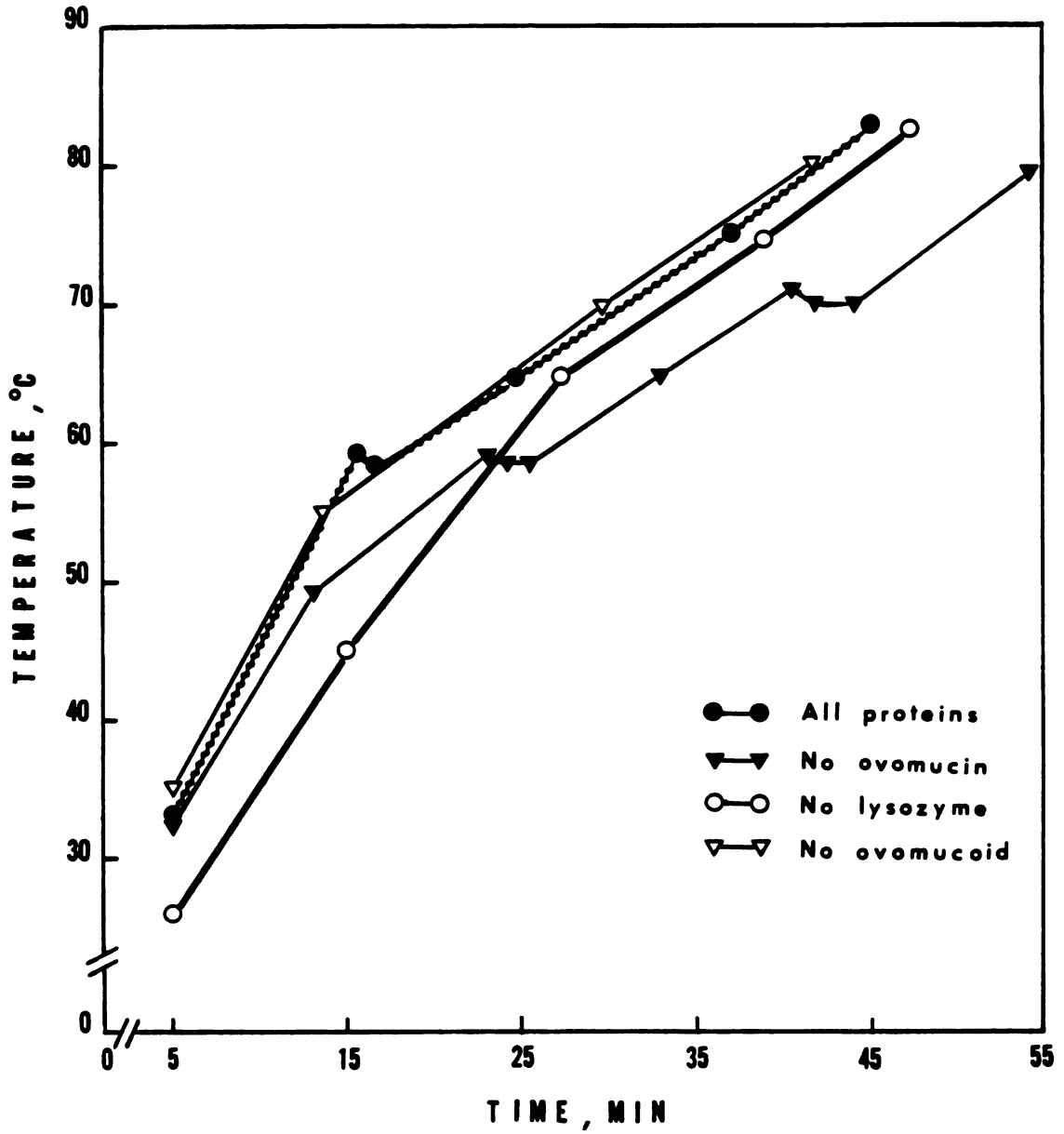


Fig. 19. Comparison of time-temperature curves of various solutions with different protein composition. Heating rate = 0.74°C/min, protein concentration = 1.27%, pH = 8.0, ionic strength = 0.275.

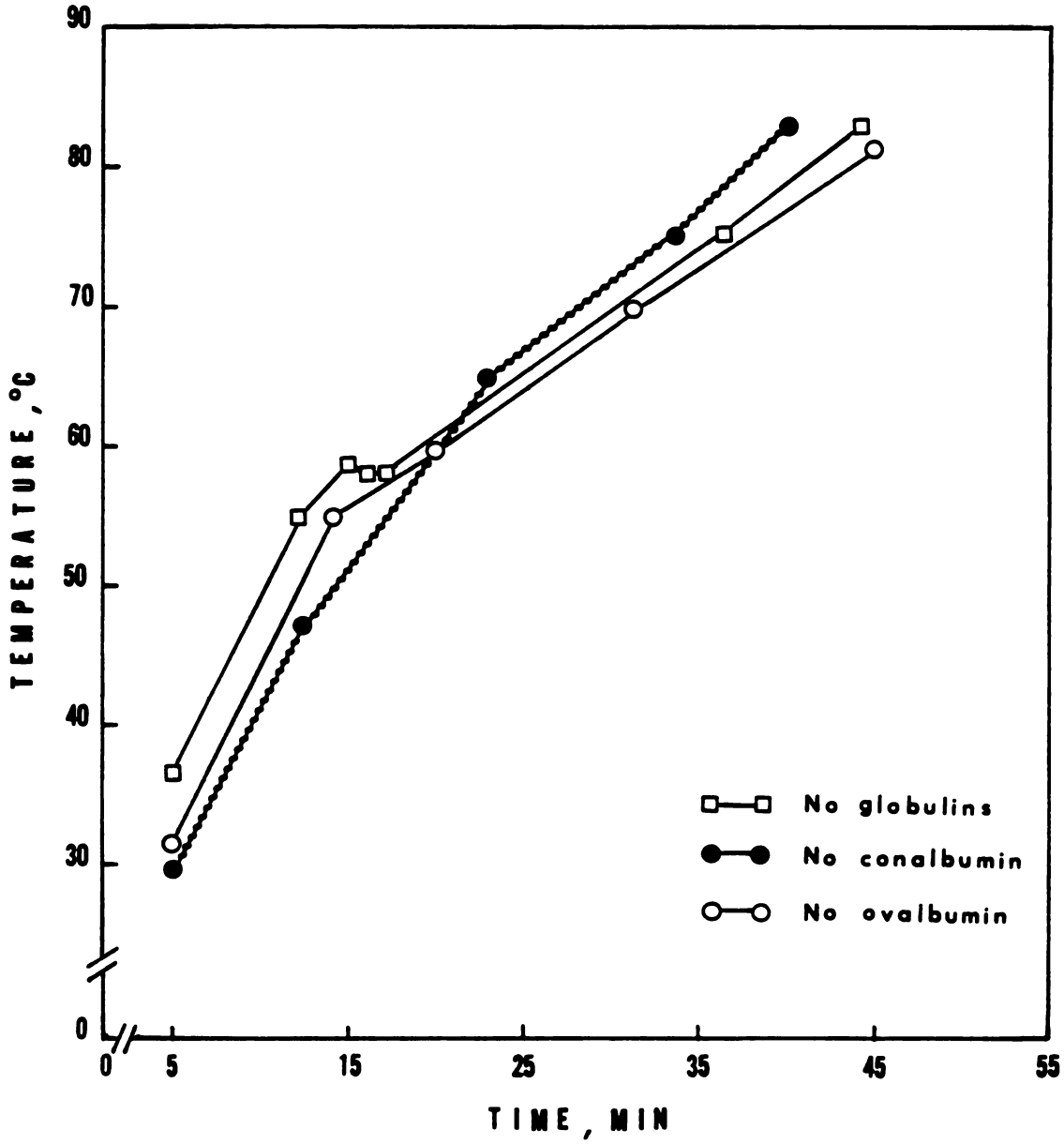


Fig. 20. Comparison of time-temperature curves of solutions with different protein composition. Heating rate = 0.74°C/min., protein concentration = 1.27%, pH = 8.0, ionic strength = 0.275.

Table 31. Physico-chemical Characteristics[a] of Gels Made With Egg Albumen Proteins Mixtures.[b]

Protein Mixture[c]	Coagulation Temperature Range	Gel Strength	Drainage	pH	Sulfhydryl Content Solution[f]	Sulfhydryl Content Coagulum
	°C	gxcm	%		moles/10^5 g sample	
ON-LY-GL-OD-CO	55.0-58.5	12.17±1.35*	53.3±1.0*	8.11±0.01	1.300	1.382±0.003
ON-LY-GL-OD-OB	65.0-75.0	3.79±0.91	56.8±3.0*	8.12±0.00	2.165	1.678±0.002
ON-LY-GL-CO-OB	55.0-60.0	10.48±0.28*	54.7±0.1*	7.90±0.07	2.085	1.889±0.083
ON-LY-OD-CO-OB	58.9-58.4	5.97±0.21	62.3±2.9	7.88±0.04	1.740	1.603±0.052
ON-GL-OD-CO-OB	65.0-75.0	4.36±0.43	0.0±0.0*	7.89±0.01	2.201	1.985±0.149
LY-GL-OD-CO-OB	{59.4-58.9 71.3-70.8	6.73±0.69*	66.2±1.0*	7.61±0.01	2.373	1.414±0.168
ON-LY-GL-OD-CO-OB	59.0-58.5	5.54±0.19	65.2±0.9*	7.90±0.03	1.978	1.340±0.085
Control	{61.5-62.5 73.0-71.0	6.34±0.06	61.4±0.8*	7.78±0.00	3.815	3.470±0.206

[a]Values are average of two determinations.

[b]Protein concentration = 1.27%, initial pH = 8.0, ionic strength = 0.275.

[c]Levels of the proteins in mixtures of 5 = 20%, in mixtures of 6 = 16.6%; ON-ovomucin, LY-lysozyme, GL-globulins, OD-ovomucoid, OB-ovalbumin.

*Values within a column are significant at $P < 0.05$.

[f]Estimated values.

temperature range to 65-75°C. Similar effect was observed with the mixture lacking lysozyme. Omitting ovomucin induced coagulation at two temperatures, 59.4-58.9°C, and 71.3-70.8°C. The control also displayed two coagulation temperature ranges, 61.5-62.5°C and 73.0-71.0°C. The first coagulation temperature range indicated conalbumin and possibly some other protein(s) denatured with partial aggregation, and coagulation completed at the second temperature range.

The destabilizing effect of the least heat stable protein (conalbumin) on the other proteins in a fashion similar to that of the double combination treatments was affirmed with the mixtures of five or six proteins. In the mixture lacking lysozyme, the observed higher coagulation temperature may have resulted from the extremely viscous nature of the solution. This characteristic appeared to have, in part, prevented intermolecular associations of conalbumin with the other proteins.

Firmest gels were produced with the mixtures with no ovalbumin (12.17 g x cm), and no ovomucoid (10.48 g x cm). Control and gel with no ovomucin ranked second, with values of 6.34 and 6.73 g x cm, respectively. Gel with no globulins (5.97 g x cm) and gel with all proteins (5.54 g x cm) ranked third, followed by the gel with no lysozyme (4.36 g x cm) and the gel with no conalbumin (3.79 g x cm).

In general, gels containing ovomucin in the five proteins combinations exhibited better liquid retention

with percentage drainage values ranging from 0-62%. No liquid drained from gel lacking lysozyme. Evidently ovomucin retained most of the liquid in the swollen carbohydrate moieties. This suggests that, in presence of lysozyme, formation of ovomucin-lysozyme complex results in decreased ability of ovomucin's water absorption capacity.

The gel with no ovomucin had the highest liquid loss (66%) followed by the gel containing all proteins (65%). The control gel lost about 61% liquid.

The observable trend in the effect of heating on pH was a decrease except that for gels with no ovalbumin or conalbumin, an increase was noted. The significance or causes for these higher pH values were not apparent.

The sulfhydryl content of the coagulums were consistently lower than that of the solutions. This supports the idea of SH-SS interchange reactions involvement in cross-linking of polypeptides.

Electron Microscopy

Ultrastructural examinations of protein foams and gels may provide some further insight as to the visual nature of protein-protein interactions in these systems. Selected foams were examined in a transmission electron microscope (TEM) and in a scanning electron microscope (SEM), whereas gels were examined in a scanning electron microscope.

Examination of Foams

The mechanism of foam formation has been postulated by many investigators (Peter and Bell, 1930; Adam, 1941; Thuman et al., 1949). These authors concurred that formation of a cohesive layer of insoluble protein at the air-liquid interface was involved in the process of air incorporation.

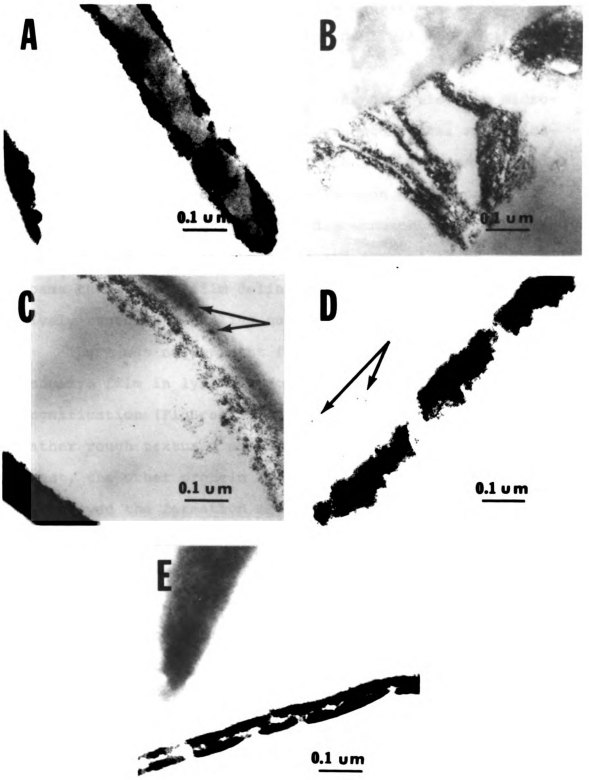
As can be seen in Figure 21, transmission electron micrographs of whipped protein solutions revealed the presence of a layer of high electron density at the film surface, sustaining foam theory. The character of the layers seemed roughly similar in lysozyme, globulins, conalbumin, and ovalbumin foams, with formation of conglomerates of unfolded polypeptides being evident. The protein clusters noticeably formed a network of parallel filaments that were easily dislodged from the liquid phase (micrograph B).

The surface film in the control foam varied in nature and displayed a double layering phenomenon of the unfolded polypeptides. The overall thickness of the film was diminished and the surface appeared smooth, but formation of conglomerates was still noticeable. There was no visual discernment of which proteins actively participated in formation of the film. In all likelihood, ovomucin, lysozyme, and globulins were involved.

In all films the liquid phase displayed a sandy

Fig. 21. Transmission electron micrographs of albumen protein foams.

a) Lysozyme
b) Globulins
c) Conalbumin
d) Ovalbumin
e) Control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background with no apparent preferencial distribution of the proteins. Occasionally, small clusters of medium electron density were visible in the light matrix (indicated by arrows).

Figures 22, 23, and 24 show scanning electron micrographs of the various protein foams at several magnifications. The severe breakdown of the fragile protein foams during handling concealed visualization of air cells (Figure 22). However, partial discernment of air inclusions was possible in globulins and control foams. In all foams the surface film delineating the air cells was extensively ruptured revealing numerous membrances underneath it.

There was no apparent formation of a thin membranous cohesive film in lysozyme foam when examined at a higher magnification (Figure 23, micrograph A). The film was rather rough textured and appeared quite rigid. In contrast, the other protein films, although severely interrupted, showed the formation of a continuous smooth sheet which appeared flexible in nature.

Some variability in membrane thickness was also observable. Globulins (micrograph B), ovalbumin (micrograph D), and control (micrograph E) films looked very thin, conalbumin (micrograph C) film seemed slightly thicker and less flexible, whereas lysozyme film was considerably thicker.

At the highest magnification (Figure 24) all protein films looked "textured," exhibiting numerous small granules.

Fig. 22. Scanning electron micrographs of albumen protein foams.

a) Lysozyme
b) Globulins
c) Conalbumin
d) Ovalbumin
e) Control

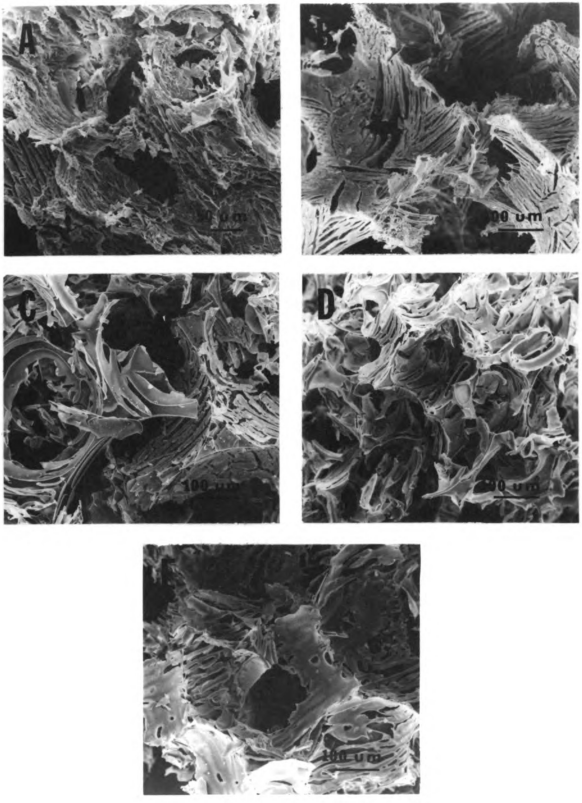
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Fig. 23. Scanning electron micrographs of albumen protein foams.

a) Lysozyme
b) Globulins
c) Conalbumin
d) Ovalbumin
e) Control

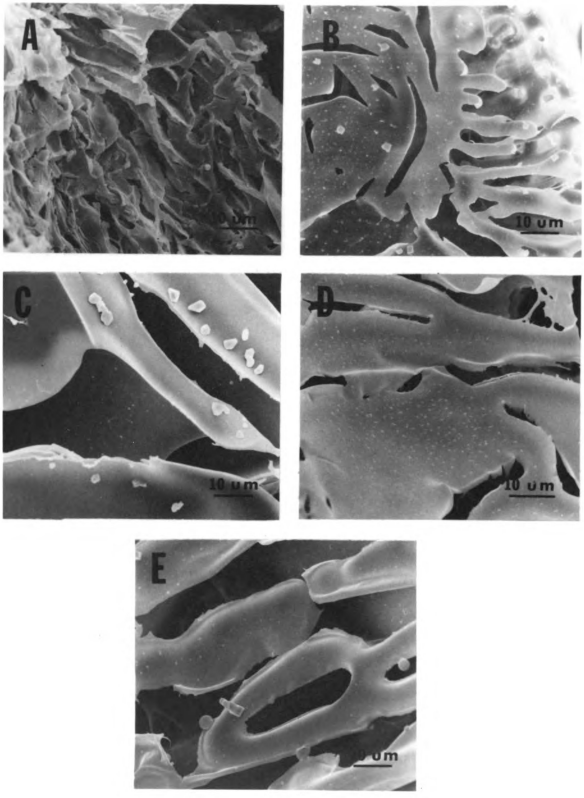
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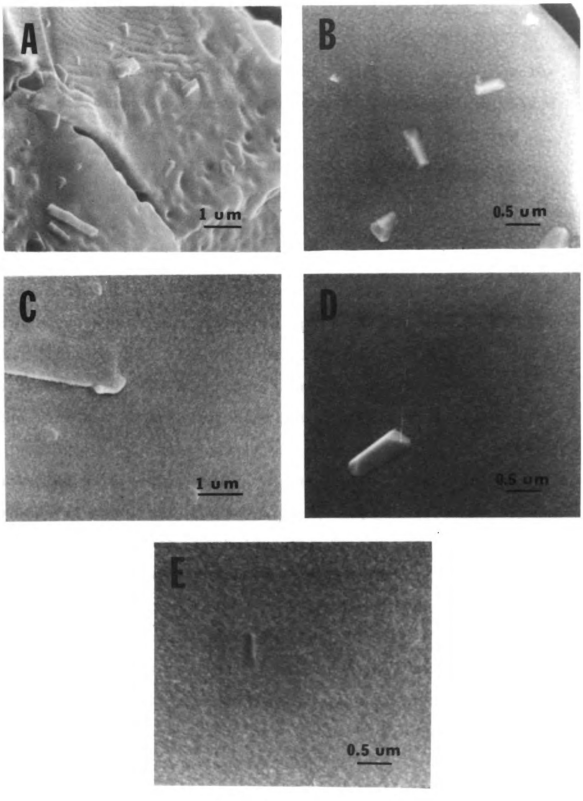
Fig. 24. Scanning electron micrographs of albumen protein foams.

a) Lysozyme
b) Globulins
c) Conalbumin
d) Ovalbumin
e) Control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These were probably unfolded polypeptide clusters forming the surface layer. A few salt crystals were also seen in most micrographs.

Scanning electron micrographs of whipped protein solutions differing in lysozyme content are displayed in Figure 25. Likewise, it was noted that breakdown of the foams occurred with manipulation. However, it was quite evident that the foam with high levels of ovomucin and no lysozyme was more severely disrupted (micrograph A). At higher magnification (micrograph B) it seemed that the membrane was less adhesive in nature, as well as thick and fluid, draping over the torn edges. Lysozyme appeared to strengthen the foam (micrograph C). There was less rupturing of the membraneous layer and at higher magnification (micrograph D) the film looked thin and somewhat flexible, but not amorphous.

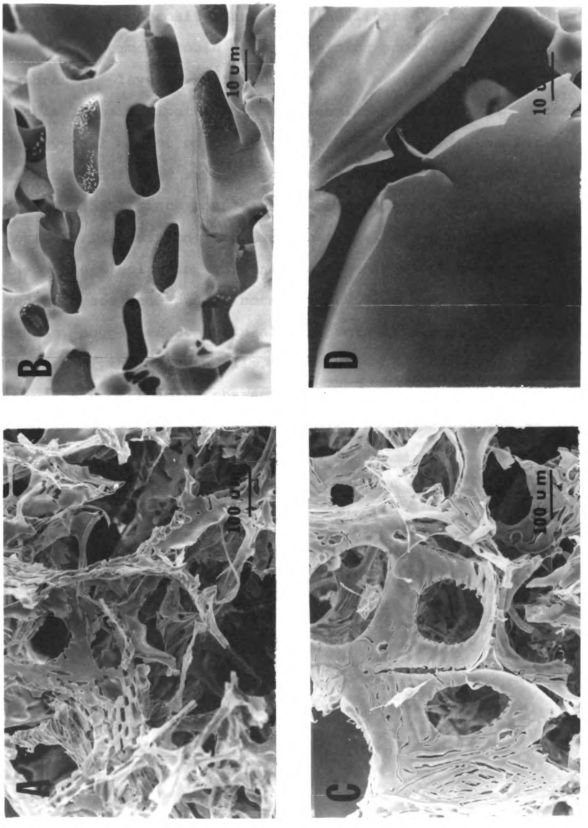
In previous testings it was determined that the protein solution with high ovomucin content and without lysozyme exhibited excellent foaming ability but produced smaller cakes. In contrast, with lysozyme foaming was drastically depressed whereas cake volume improved considerably.

The detrimental effect of ovomucin on volume has been attributed to its excessive insolubilization at the air-albumen interface leading to formation of more stable foams but with less expansive characteristics (MacDonnell et al., 1955). Examination at the microscopic level confirmed that without lysozyme the viscous ovomucin fraction concentrated at the film surface. It appeared that ovomucin contribution

Fig. 25. Scanning electron micrographs of foams differing in lysozyme content. Other proteins levels: ovomucin 2.5%, globulins 3%, ovomucoid 5%, conalbumin 6%, ovalbumin 77.5-83.5%.

a) and b) 0% lysozyme

c) and d) 6% lysozyme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to foaminess was primarily related to its ability to increase the solution viscosity, and thus facilitated film formation. However, ovomucin could not maintain or produce a cohesive membranous layer, which indicated the protein itself is unable to participate in intermolecular associations. Hence, the overall effect of ovomucin on the process of foam formation does not lend support to the observations of MacDonnell et al. (1955) since considerably more unstable films were formed. In addition, the fact that the cake prepared with the protein solution containing a high level of ovomucin and no lysozyme expanded normally and then collapsed during baking, suggested ovomucin also affected the heat coagulative process of the protein film.

The poor foaming characteristics of solutions with lysozyme and ovomucin were consistent with the observations of Garibaldi et al. (1968) in studies of ovomucin-lysozyme interaction. These authors pointed out that the formation of the complex depressed egg-white foaminess. Kato et al. (1975) indicated the carbohydrate units of ovomucin were involved in electrostatic associations with lysozyme. These evidences suggest that the viscous nature of ovomucin is altered in the interaction phenomenon. The appearance of the foam prepared with the solution containing ovomucin and lysozyme confirmed this observation.

Examination of Gels

When heated, proteins denature and usually association of the unfolded polypeptides into a three-dimensional network ensues. The extent of unfolding is dependent on the physicochemical characteristics of the proteins and environmental factors that exert an influence in these characteristics.

Figures 26 and 27 show scanning electron micrographs of selected albumen protein gels. At the lowest magnification (Figure 26) lysozyme, conalbumin, ovalbumin, and control gels were similar in appearance, displaying a disorganized aggregation of the polypeptide chains. Globulins gel was remarkably different and showed a definite orientation of the cross-linked polypeptides into multiple parallel membranes which were interconnected by numerous projections.

The possible causes for the differences observed in gel strength and percentage drainage of the various gels were not visually apparent at this level of magnification. The slightly better ability of the globulins gel to hold water in the interstices over that of conalbumin and ovalbumin gels could have been the result of the different orientation of the polypeptides in the gel matrix.

At the next level of magnification (Figure 27) the disorganized arrangement of the polypeptide aggregates in lysozyme, conalbumin, ovalbumin, and control gels appeared grape-like clusters. Moreover, differences in cluster size were noticeable in the various gels. Lysozyme gel (micrograph A)

Fig. 26. Scanning electron micrographs of albumen protein gels.

a) Lysozyme

b) Globulins

c) Conalbumin

d) Ovalbumin

e) Control

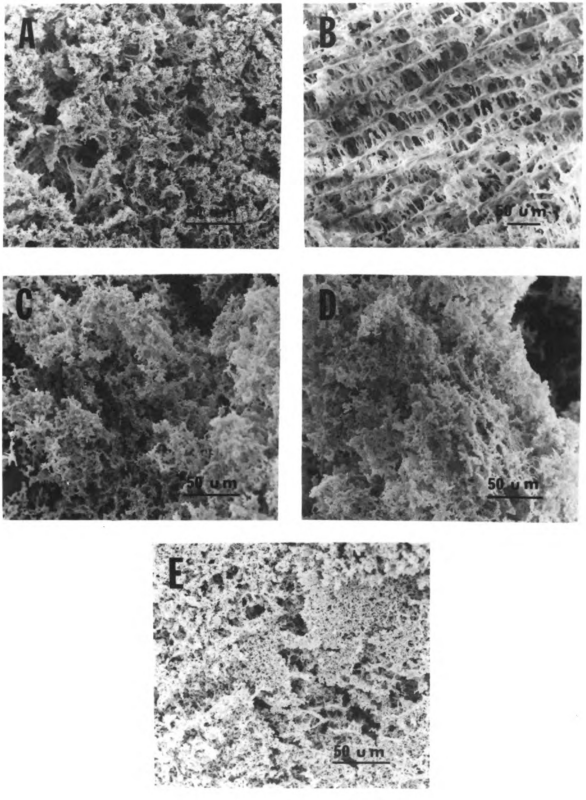
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Fig. 27. Scanning electron micrographs of albumen protein gels.

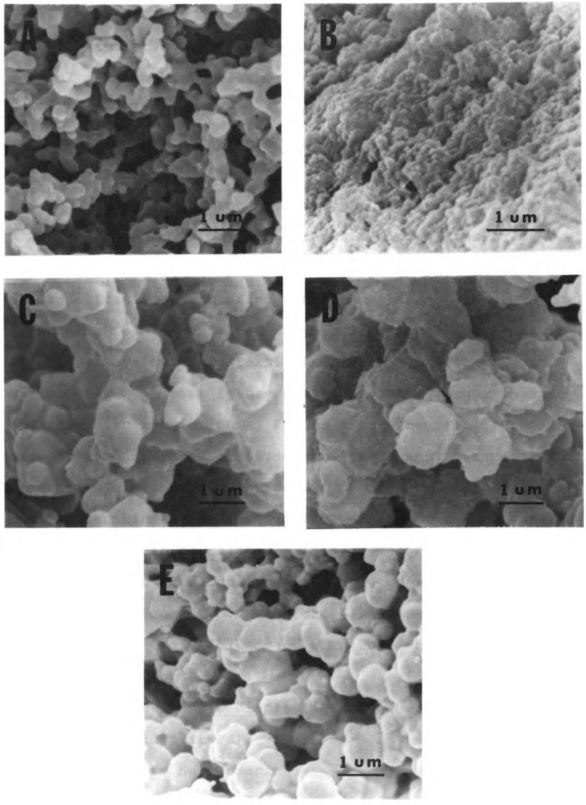
a) Lysozyme

b) Globulins

c) Conalbumin

d) Ovalbumin

e) Control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was characterized by a finer gel network of considerable small aggregates. Conalbumin and ovalbumin gels were roughly similar and showed mostly large conglomerates. The control gel exhibited clusters of intermediate size range, whereas globulins gel had the smallest polypeptide aggregates very tightly associated in the membrane-like arrangement.

The scanning electron micrographs of albumen protein mixture gels are shown in Figure 28. Micrographs A and B illustrate lysozyme-globulins combination gel at two levels of magnification. Globulins exhibited the same trend in the orientation of the unfolded polypeptides with occasional inclusion of lysozyme clusters. At higher magnification it was clearly seen that lysozyme clusters were larger in size (compare with micrograph A, Figure 27). The pebbly appearance of some clusters resulted from the association of lysozyme and globulins polypeptides.

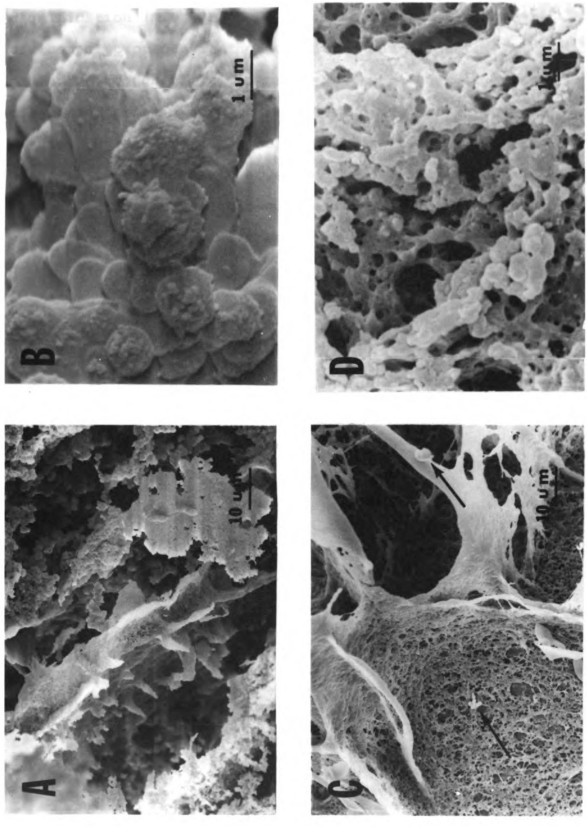
The striking difference in size of the lysozyme aggregates appeared to be related to its premature denaturation in the presence of globulins. McKenzie et al. (1963) pointed out that gelation may occur before a large number of polypeptide chains are unfolded and when this happens a coarser gel network forms.

Micrographs C and D display the gel prepared with 5 proteins (ovomucoid omitted). There were several protein strands which appeared to be ovomucin held by aggregates of polypeptides (micrograph C). A few large clusters of denatured

Fig. 28. Scanning electron micrographs of gels prepared with protein mixtures.

a) and b) Lysozyme-Globulins

c) and d) Ovomucin-Lysozyme-Globulins-Conalbumin-Ovalbumin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C
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proteins (indicated by arrows) were noticeable. At higher magnification (micrograph D) the disorganized arrangement of the polypeptides in the gel matrix was evident. It seemed as though globulins predominated in the gel. The coagulated protein appeared to be binding, and in some instances, enveloping the other protein aggregates. These were relatively small in size and the overall appearance of the gel matrix suggested a stronger structure. This observation was consistent with gel strength determination.

SUMMARY AND CONCLUSIONS

The primary objective of this study was to determine the functional properties of various albumen proteins and specific protein-protein interactions in two food systems. Foamability was evaluated in an angel food cake system and coagulability in a custard model system. Ovomucin, lysozyme, globulins, ovomucoid, conalbumin, and ovalbumin were isolated from egg white. They were further tested individually and in combinations in these food systems. Combinations of three levels of each protein determined with a mixtures experimental design were studied in the cake system. The effects of the protein-protein interactions in this system were analyzed through response surface methodology to designate protein levels for optimization of cake parameters. Textural characteristics of selected foams were studied in transmission and scanning electron microscopes, whereas selected coagulums were examined in a scanning electron microscope.

Evaluation of foaming properties of individual protein solutions revealed that globulins solution had very good air incorporation capacity and produced a large cake of excellent textural characteristics. Ovalbumin solution exhibited poor foaming ability and formed a foam after a

relatively long whip time. The cake had a large volume but was coarse textured with thick air cell walls. Conalbumin and lysozyme solutions showed very poor foamability, whereas ovomucin and ovomucoid had none.

In interactions studies, inclusion of both ovomucin and globulins at levels of 0.00, 1.25, and 2.50%, and 0, 3, and 13%, respectively, significantly improved foaminess of the solutions. However, cake volume progressively decreased, showing a strong significant negative correlation with foamability. Addition of lysozyme to these mixtures at levels of 0, 3, and 6% significantly depressed foaming power of the solutions, resulting in considerable improvement of cake volume. Conalbumin, ovomucoid, and ovalbumin showed no significant influence on foamability and angel food cake volume.

Tenderness and compressibility measurements reflected the degree of aeration of the products. More tender cakes were associated with larger cakes and low compressibility values.

In determining the sulfhydryl content of foams it was found that whipping reduced the number of SH groups. This implied that formation of the cohesive film at the air-solution interface involved inter- and/or intramolecular sulfhydryl-disulfide interchange.

Multiple regression analysis revealed the protein mixtures were significantly affecting viscosity, foaming index, volume, and cake tenderness. Partial positive effects on

viscosity were associated with ovomucin and ovalbumin, whereas conalbumin and the interaction terms of ovomucin with the other 5 proteins reduced viscosity. Foaming index was partially positively affected by ovomucin, and interaction of ovomucin with globulins. Interactions of ovomucin with ovalbumin, lysozyme, ovomucoid, and conalbumin had a negative effect on foaminess. Partial positive effects on cake volume were observed for ovomucin, lysozyme, and ovalbumin. Interactions of ovomucin with ovalbumin, globulins, and ovomucoid, as well as lysozyme with globulins, ovomucoid, conalbumin, and ovalbumin, significantly reduced cake volume. Cake tenderness was reduced by conalbumin, ovalbumin, and the interaction of ovomucin with lysozyme. Ovomucoid and conalbumin, conalbumin and lysozyme, as well as conalbumin and ovalbumin interaction variables produced more tender cakes.

Surface response methodology was used to evaluate protein solution foaming index and cake volume responses as a function of ovomucin, lysozyme, and globulins levels. It was found that a target angel food cake could be prepared with ovomucin, lysozyme, and globulins levels ranging from 0.2-1.0%, 0.0-1.8%, and 12.2-14.8%, respectively.

Studies of gelation properties of the albumen proteins showed that conalbumin was the least heat stable protein with a denaturation temperature of 57.3°C. Globulins and ovalbumin ranked second with denaturation transition temperatures of 72.0 and 71.5°C, respectively. Lysozyme denatured at

81.5°C while ovomucin and ovomucoid showed no coagulation abilities. Lysozyme produced the strongest gel, followed by globulins, ovalbumin, and conalbumin.

In the double combinations studies it was found that aggregation of polypeptides occurred near the denaturation transition temperature of the least heat stable protein. Therefore, in combinations of lysozyme, globulins, and ovalbumin with conalbumin, denaturation was apparent at 58.3, 57.8, and 58.1°C, respectively. Ovomucoid consistently increased the coagulation temperature ranges of globulins, conalbumin, and ovalbumin, and prevented coagulation of lysozyme. Gel strength varied according with the proteins present.

The combinations of 5 or all proteins resulted in subtler changes of temperature during coagulation. The destabilizing effects of conalbumin on the other proteins were still apparent. The control mixture exhibited two distinct coagulation temperature ranges of 61.5-62.5°C and 73.0-71.0°C.

Transmission electron microscopy studies of foams confirmed the presence of a layer of cross-linked polypeptides at the surface of the film enveloping an air inclusion. Scanning electron microscopic examination of a whipped solution with high levels of ovomucin and without lysozyme showed that the protein concentrated at the film surface and considerably decreased foam stability. The membrane appeared less cohesive in nature and draped fluidly over

the torn edges. Inclusion of lysozyme seemed to improve the overall foam appearance. The effects of lysozyme on foaminess in the presence of ovomucin appeared to be associated with formation of ovomucin-lysozyme complex. This complex seemed to alter the viscous nature of ovomucin.

In the scanning electron microscopic investigations of selected coagulums it was found that lysozyme, conalbumin, and ovalbumin polypeptides aggregated in grape-like clusters of variable size. The control mixture gel also exhibited the same pattern. Globulins polypeptides appeared to tightly associate in membrane-like arrangements and showed excellent binding abilities. Smaller cluster sizes seemed to parallel gel firmness.

The results of this study indicated that complex protein-protein interactions influenced the overall functional properties of the various proteins. Readiness of foam formation was associated with the highly viscous ovomucin fraction, whereas stability was dependent on formation of a cohesive membraneous layer of partially cross-linked polypeptides at the film surface. Low cake volumes resulted from unstable foams and lack of heat coagulative properties of the membrane. Globulins alone could produce an excellent angel food cake. Lysozyme and globulins, and combinations of lysozyme with globulins, produced the firmest gels. Changes in denaturation temperatures were associated with the destabilizing action of more heat unstable proteins.

SUGGESTIONS FOR FURTHER RESEARCH

The observations and findings associated with the present investigation raised a multitude of questions relevant to protein functionality:

1. Studies of foaming properties of the various albumen proteins should be elaborated further under different environmental conditions. Observations of maximum foam stability attainment in the neighborhood of the isoelectric point of the protein and the variable behavior of proteins at the extremes of pH values have been reported.

2. Similarly, the effects of salts and pH on the coagulation abilities of the proteins would warrant further investigation.

3. The contribution of hydrogen bonds, hydrophobic and electrostatic interactions to protein-protein interactions in association of polypeptides should be evaluated with specific chemical reagents. Urea, guanidine HCl and sodium dodecyl sulfate are known to disrupt hydrogen, hydrophobic and ionic bonds. The contribution of sulfhydryl-disulfide interchange reactions could be determined with N-ethylmaleimide, a sulfhydryl blocking reagent.

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APPENDICES

APPENDIX A

Experimental Design

The procedure for deriving the treatment levels combinations of the various proteins used for foamability studies was to first find all possible two level treatment combinations (low and high) of 5 proteins and leaving 1 protein level in blank. This step generated $K \cdot 2^{K-1}$ possible combinations, or $6 \cdot 2^{6-1} = 192$ combinations for each protein. Next, the protein level left in blank was filled with a level necessarily within its constraints, which would make the sum of all protein levels equal to 100. Hence, leaving ovalbumin in blank:

	OVN	LYS	GLOB	OVD	CON	OVB	Total
1	0.0	0	3	5	6	86.0	100
2	0.0	0	3	5	21	———	⋮
3	0.0	0	3	18	6	———	⋮
4	0.0	0	3	18	21	———	⋮
5	0.0	0	13	5	6	———	⋮
6	0.0	0	13	5	21	———	⋮
7	0.0	0	13	18	6	———	⋮
8	0.0	0	13	18	21	———	⋮
9	0.0	6	3	5	6	———	⋮
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
16	0.0	6	13	18	21	———	⋮
17	2.5	6	3	5	6	———	⋮
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
32	2.5	6	13	18	21	39.5	100

This procedure was repeated leaving the other protein levels in blank, i.e., $X_1X_2X_3X_4-X_6$, $X_1X_2X_3-X_5,X_6$, $X_1X_2-X_4X_5X_6$, $X_1-X_3X_4X_5X_6$, and $-X_2X_3X_4X_5X_6$. However, since ovalbumin (X_6) levels were set to 30 (low) and 89 (high) there were no possible combinations allowable at these levels. As it can be seen in the above calculations, the minimum and maximum possible levels for ovalbumin were 39.5 and 86.0, respectively. Therefore the other combinations were automatically excluded from the design. A total of 32 vertices were generated following this procedure. The centroids of the 3-dimensional face were found by averaging the vertices treatment levels with any two constant protein levels. Therefore, vertices 1 to 8, with ovomucin and lysozyme showing equal levels, were averaged; vertices 1 to 4 with ovomucin and globulins displaying equal levels, and so on. The fifth dimensional face represented the average of all treatment levels (vertices and 3-dimensional face centroids).

APPENDIX B

Observed and Predicted Values

Table 32. Observed and Predicted[a] Values of Protein Mixtures Physical Parameters.

Treatment[b]						Viscosity		Surface Tension		Foaming Index	
OVN	LYS	GLOB	OVD	CON	OVB	Observed	Predicted	Observed	Predicted	Observed	Predicted
						cps x g/cm^3		Dynes/cm		cm^3/g/min	
L	L	L	L	L	H	1.76	1.73	50.90	48.71	0.99	0.85
L	L	L	L	H	H	1.69	1.68	49.40	49.54	0.93	0.07
L	L	L	H	L	H	1.86	1.82	48.30	49.06	0.67	0.00
L	L	L	H	H	N	1.73	1.73	49.50	49.55	0.75	0.00
L	L	H	L	L	H	1.65	1.79	49.50	48.21	2.10	0.29
L	L	H	L	H	N	1.95	1.86	49.60	49.20	1.46	0.75
L	L	H	H	L	N	1.83	1.79	48.80	48.38	1.34	0.00
L	L	H	H	H	L	1.72	1.83	48.40	48.90	1.61	0.00
L	H	L	L	L	H	1.63	1.72	50.30	48.25	1.16	1.99
L	H	L	L	H	N-H	1.64	1.64	48.80	48.74	1.06	0.75
L	H	L	H	L	N-H	1.75	1.76	47.30	48.26	0.80	1.71
L	H	L	H	H	N-L	1.73	1.64	48.80	48.34	1.19	0.34
L	H	H	L	L	H	1.91	1.74	46.20	46.52	4.12	3.97
L	H	H	L	H	N	1.71	1.77	47.50	47.12	3.22	3.84
L	H	H	H	L	N	1.62	1.69	46.00	46.35	3.36	4.16
L	H	H	H	H	L	1.69	1.69	47.80	46.36	3.40	3.47
H	L	L	L	L	H	2.20	2.24	47.50	48.48	5.55	6.37
H	L	L	L	H	N-H	2.26	2.27	49.30	48.79	6.05	7.36
H	L	L	H	L	H	2.26	2.35	49.00	48.13	9.45	10.25
H	L	L	H	H	N	2.46	2.34	48.60	48.31	10.67	11.22
H	L	H	L	L	H	2.37	2.31	47.50	48.21	21.83	23.08
H	L	H	L	H	N	2.48	2.46	49.90	48.83	27.43	25.26
H	L	H	H	L	N	2.43	2.33	47.50	47.81	29.63	27.44
H	L	H	H	H	L	2.28	2.44	47.10	48.27	29.42	29.23
H	H	L	L	L	H	2.41	2.22	49.40	49.77	1.89	2.04
H	H	L	L	H	N	2.12	2.21	49.80	49.82	1.84	2.55
H	H	L	H	L	N-H	2.23	2.28	48.70	49.16	1.27	2.00
H	H	L	H	H	N-L	2.12	2.23	48.20	49.06	1.23	2.27
H	H	H	L	L	N-H	2.04	2.24	48.60	48.33	1.76	2.26

Table 32--Continued

Treatment[b]						Viscosity		Surface Tension		Foaming Index	
OVN	LYS	GLOB	OVD	CON	OVB	Observed	Predicted	Observed	Predicted	Observed	Predicted
						cps x g/cm^3		Dynes/cm		cm^3/g/min	
H	H	H	L	H	N-L	2.38	2.35	49.00	48.67	2.24	3.81
H	H	H	H	L	N	2.21	2.21	47.90	47.70	3.48	2.68
H	H	H	H	H	L	2.47	2.29	46.10	47.89	4.26	3.53
L	L	N	N	N	N-H	1.91	1.84	49.70	49.60	1.37	2.07
L	H	N	N	N	N	1.71	1.76	49.30	47.71	1.28	4.66
H	L	N	N	N	N-H	2.35	2.40	49.90	48.68	29.26	17.42
H	H	N	N	N	N	2.26	2.31	47.80	48.66	2.87	2.65
L	N	L	N	N	N-H	1.66	1.77	48.00	48.37	0.88	1.72
L	N	H	N	N	N	1.75	1.82	45.50	47.34	2.63	3.33
H	N	L	N	N	N-H	2.48	2.32	49.60	48.57	4.49	1.17
H	N	H	N	N	N	2.34	2.38	48.50	47.94	9.15	10.50
L	N	N	L	N	H	1.84	1.79	46.20	48.00	0.99	1.33
L	N	N	H	N	N	1.77	1.79	46.50	48.08	2.31	1.29
H	N	N	L	N	N-H	2.46	2.33	49.50	48.91	6.72	6.74
H	N	N	H	N	N	2.36	2.35	50.30	48.52	6.32	9.24
L	N	N	N	L	H	1.75	1.69	46.70	47.18	1.89	0.73
L	N	N	N	H	N	1.74	1.67	48.20	47.90	1.91	0.50
H	N	N	N	L	H	2.14	2.21	48.30	47.73	4.90	7.74
N	L	L	N	N	H	1.95	1.99	47.60	48.21	2.82	2.95
N	L	H	N	N	N	2.28	2.07	49.70	47.86	3.77	10.74
N	H	L	N	N	N-H	1.93	1.93	46.80	47.91	1.15	0.00
N	H	H	N	N	N-L	1.94	1.97	46.00	46.33	2.79	0.67
N	L	N	L	N	H	1.86	2.01	44.60	48.24	3.89	5.32
N	L	N	H	N	N	2.09	2.04	48.30	48.16	3.22	8.50
N	H	N	L	N	N-H	1.96	1.95	46.00	47.74	1.85	0.00
N	H	N	H	N	N-L	1.98	1.94	50.00	47.31	1.34	1.11
N	L	N	N	L	H	1.86	1.90	46.00	47.42	3.58	5.09
N	L	N	N	H	N	1.93	1.93	47.80	47.83	3.36	8.15

Table 32--Continued

Treatment[b]						Viscosity		Surface Tension		Foaming Index	
OVN	LYS	GLOB	OVD	CON	OVB	Observed	Predicted	Observed	Predicted	Observed	Predicted
						cps x g/cm^3		Dynes/cm		cm^3/g/min	
N	H	N	N	L	N-H	1.95	1.84	46.80	46.91	1.21	0.00
N	L	N	N	H	N	1.85	1.94	48.90	47.28	3.18	1.75
Control						2.02	2.04	46.70	47.27	3.08	2.60

[a]Based on regression analysis.

[b]OVN = ovomucin, LYS = lysozyme, GLOB = globulins, OVD = ovomucoid, CON = conalbumin, OVB = ovalbumin. L = low, N = normal, H = high. Numerical values for low, normal, and high levels are listed in Table 9.

Table 33. Observed and Predicted[a] Values of Angel Food Cake Parameters.

Treatment[b]						Volume		Tenderness		Compressibility	
OVN	LYS	GLOB	OVD	CON	OVB	Observed	Predicted	Observed	Predicted	Observed	Predicted
						cm^3		work/g[c]		work/cm[c]	
L	L	L	L	L	H	294	282	3.444	3.311	0.763	0.821
L	L	L	L	H	H	271	272	3.195	3.663	0.794	0.903
L	L	L	H	L	H	274	281	3.353	3.428	0.783	0.707
L	L	L	H	H	N	270	267	3.135	3.356	0.570	0.647
L	L	H	L	L	H	295	302	3.141	3.133	0.848	0.715
L	L	H	L	H	N	290	294	3.439	3.751	0.932	0.779
L	L	H	H	L	N	302	299	3.302	3.075	0.598	0.656
L	L	H	H	H	L	273	284	3.203	3.184	0.523	0.547
L	H	L	L	L	H	294	297	3.643	3.412	0.829	0.674
L	H	L	L	H	N-H	284	290	3.412	3.248	0.963	0.782
L	H	L	H	L	N-H	300	298	3.992	3.723	0.667	0.758
L	H	L	H	H	N-L	275	287	3.292	3.120	0.640	0.707
L	H	H	L	L	H	297	292	2.938	3.004	0.533	0.586
L	H	H	L	H	N	294	286	3.226	3.082	0.645	0.664
L	H	H	H	L	N	296	290	2.946	3.112	0.597	0.724
L	H	H	H	H	L	281	275	2.689	2.641	0.600	0.604
H	L	L	L	L	H	217	232	3.500	3.631	0.955	0.926
H	L	L	L	H	N-H	240	232	4.531	3.881	1.300	1.048
H	L	L	H	L	H	206	208	3.373	3.383	0.841	0.976
H	L	L	H	H	N	191	204	3.030	3.203	1.174	0.949
H	L	H	L	L	H	251	237	3.468	3.428	0.850	0.938
H	L	H	L	H	N	232	238	3.661	3.934	0.776	1.036
H	L	H	H	L	N	217	210	3.077	2.994	1.101	1.043
H	L	H	H	H	L	208	203	2.845	2.975	0.905	0.959
H	H	L	L	L	H	312	302	4.004	4.203	0.542	0.573
H	H	L	L	H	N	311	305	3.385	3.936	0.632	0.718
H	H	L	H	L	N-H	296	280	4.650	4.146	0.801	0.821
H	H	L	H	H	N-L	286	277	3.581	3.433	0.581	0.798

Table 33--Continued

Treatment[b]						Volume		Tenderness		Compressibility	
OVN	LYS	GLOB	OVD	CON	OVB	Observed	Predicted	Observed	Predicted	Observed	Predicted
						cm^3		work/g[c]		work/cm^3[c]	
H	H	H	L	L	N-H	288	282	4.046	3.767	0.674	0.603
H	H	H	L	H	N-H	290	286	3.668	3.729	0.936	0.711
H	H	H	H	L	N	245	256	3.573	3.493	0.723	0.905
H	H	H	H	H	L	250	249	2.928	2.886	0.721	0.803
L	L	N	N	N	N-H	290	301	3.574	3.266	0.771	0.660
L	H	N	N	N	N	295	309	2.761	3.033	0.668	0.636
H	L	N	N	N	N-H	223	220	2.980	3.340	0.881	0.970
H	H	N	N	N	N	288	280	3.886	3.575	0.546	0.737
L	N	L	N	N	N-H	294	277	2.824	3.105	0.739	0.802
L	N	H	N	N	N	287	285	2.707	2.851	0.716	0.727
H	N	L	N	N	N-H	230	242	3.352	3.427	0.950	0.918
H	N	H	N	N	N	211	234	3.047	3.135	1.410	0.959
L	N	N	L	N	H	282	279	2.959	3.062	0.739	0.959
L	N	N	H	N	N	280	276	2.890	2.928	0.899	0.907
H	N	N	L	N	N-H	236	252	3.765	3.554	0.978	1.072
H	N	N	H	N	N	208	225	2.958	3.046	1.207	1.181
L	N	N	N	L	H	289	296	3.483	3.422	0.670	0.653
L	N	N	N	H	N	290	287	3.968	3.544	0.745	0.662
H	N	N	N	L	H	247	241	3.462	3.785	1.011	0.838
N	L	L	N	N	H	243	231	3.419	3.492	0.892	0.857
N	L	H	N	N	N	257	248	3.548	3.346	0.968	0.814
N	H	L	N	N	N-H	257	278	3.728	3.611	0.674	0.722
N	H	H	N	N	N-L	258	270	3.319	3.207	0.422	0.691
N	L	N	L	N	H	238	242	3.969	3.654	0.995	1.051
N	L	N	H	N	N	245	235	3.611	3.296	0.803	0.975
N	H	N	L	N	N-H	265	274	3.602	3.478	0.679	0.826
N	H	N	H	N	N-L	272	269	2.369	3.291	1.872	0.940

Table 33--Continued

Treatment[b]						Volume		Tenderness		Compressibility	
OVN	LYS	GLOB	OVD	CON	OVB	Observed	Predicted	Observed	Predicted	Observed	Predicted
						cm^3		work/g[c]		work/cm[c]	
N	L	N	N	L	H	235	250	3.398	3.738	0.778	0.733
N	L	N	N	H	N	251	245	4.238	3.979	0.712	0.753
N	H	N	N	L	N-H	270	283	3.482	4.149	0.732	0.591
N	L	L	N	H	N	285	251	3.597	3.517	0.558	0.902
Control						272	250	4.047	3.513	0.705	0.899

[a]Based on regression analysis.

[b]OVN = ovomucin, LYS = lysozyme, GLOB = globulins, OVD = ovomucoid, CON = conalbumin, OVB = ovalbumin. L = low, N = normal, H = high. Numerical values for low, normal, and high levels are listed in Table 9.

[c]Work = lb x cm.

APPENDIX C

Comparison of Time-Temperature Relationships of Albumen Proteins with Their Double Combinations

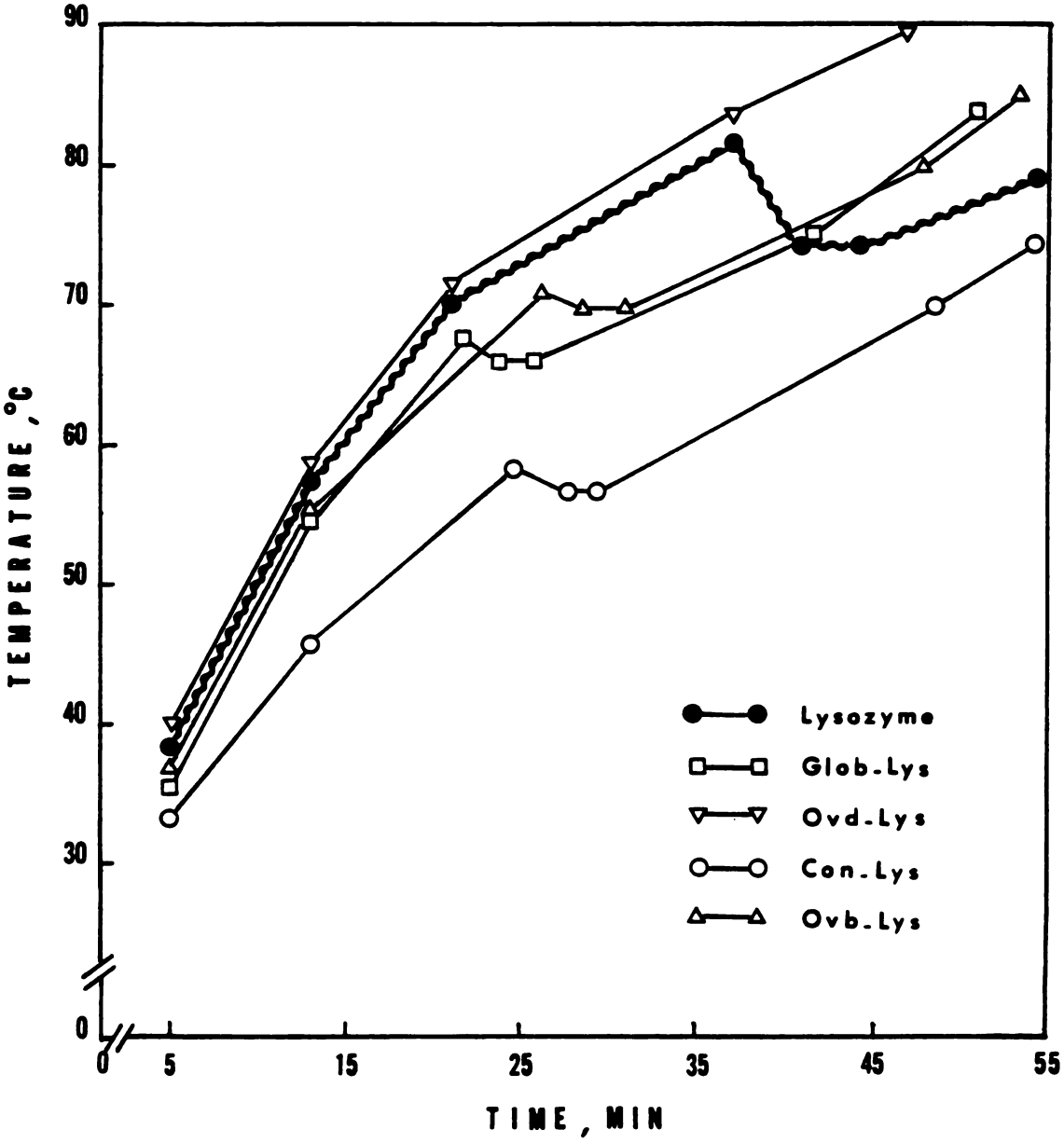

Fig. 29. Changes in time-temperature curve of lysozyme solution with the addition of globulins (Glob), ovomucoid (Ovd), conalbumin (Con), and ovalbumin (Ovb). Heating rate = 0.74°C/min., protein concentration = 1.27%, ionic strength = 0.275, pH = 8.0.

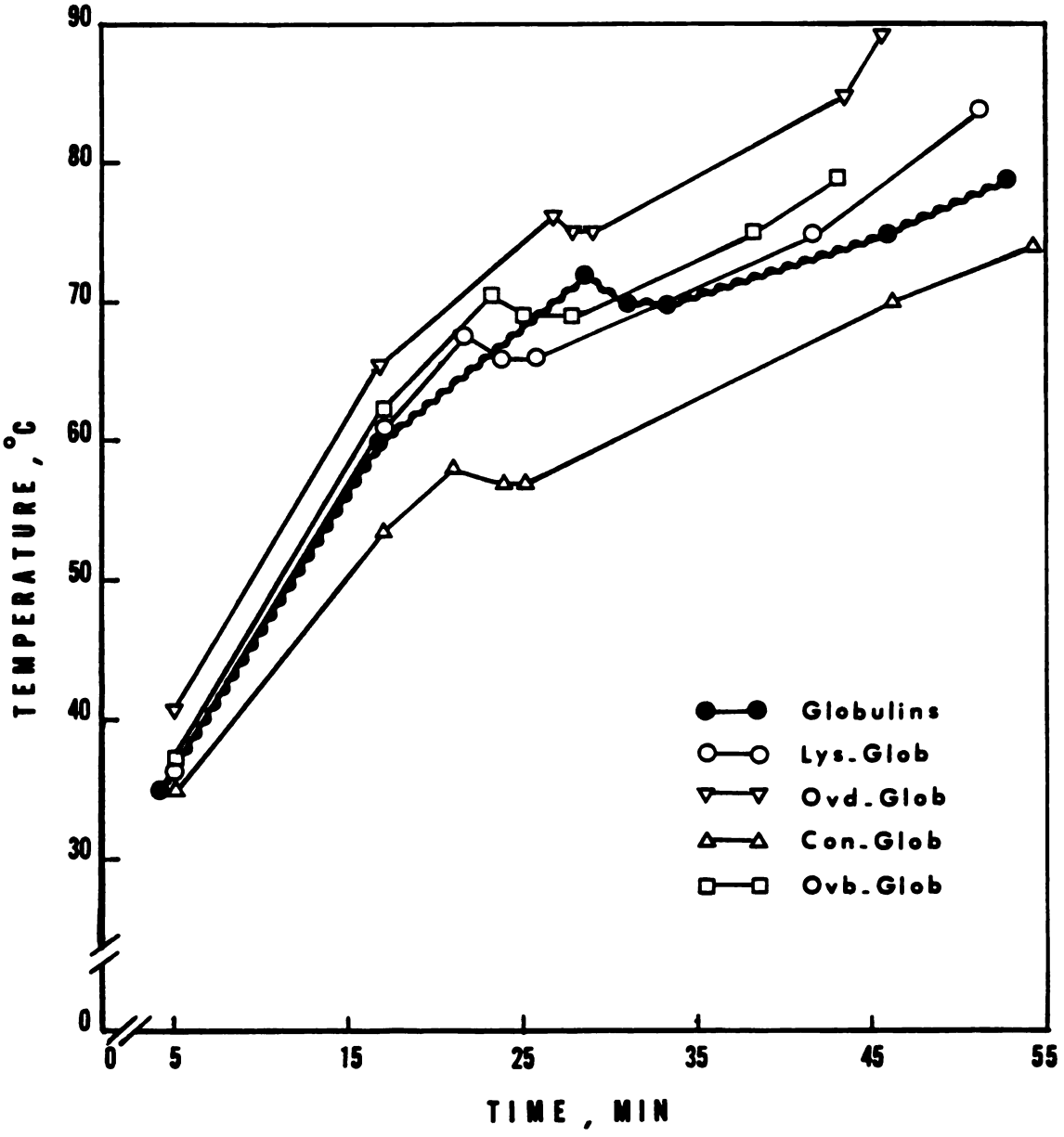

Fig. 30. Changes in time-temperature curve of globulins solution with the addition of lysozyme (Lys), ovomucoid (Ovd), conalbumin (Con), and ovalbumin (Ovb). Heating rate = 0.74°C/min., protein concentration - 1.27%, pH = 8.0, ionic strength = 0.275.

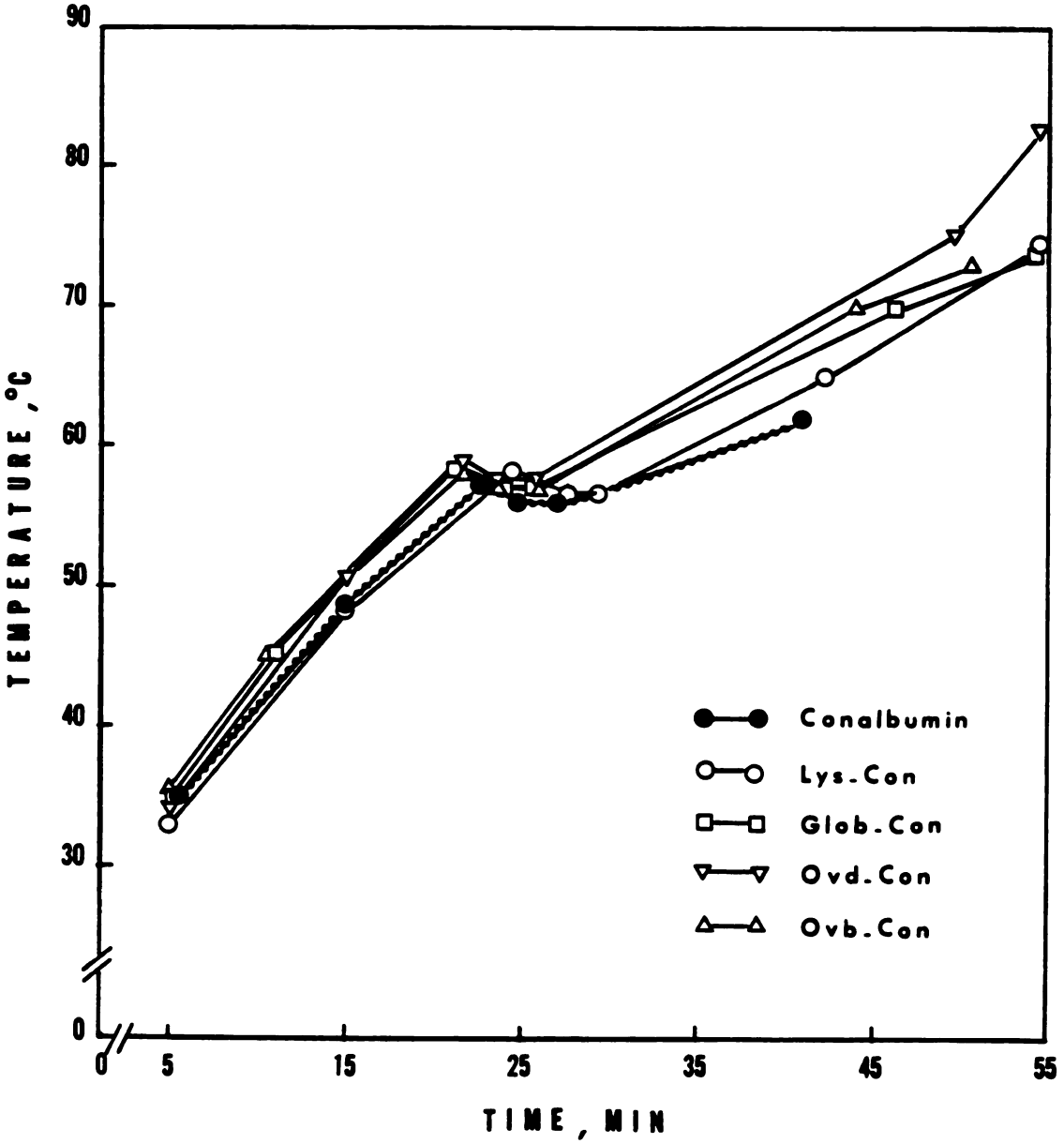

Fig. 31. Changes in time-temperature curve of conalbumin solution with the addition of lysozyme (Lys), globulins (Glob), ovomucoid (Ovd), and ovalbumin (Ovb). Conditions are the same as those in Fig. 29 .

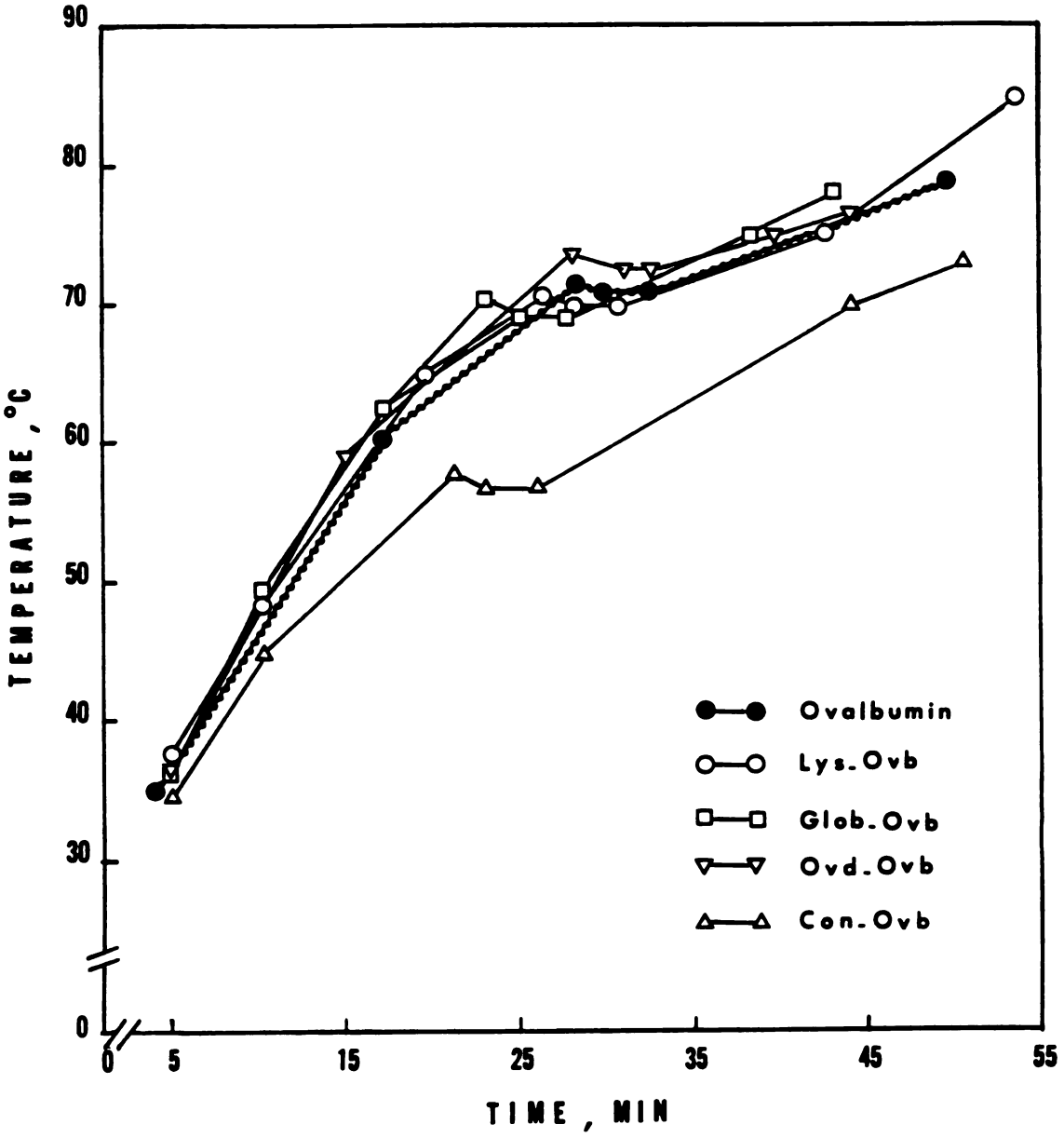

Fig. 32. Changes in time-temperature curve of ovalbumin solution with the addition of lysozyme (Lys), globulins (Glob), ovomucoid (Ovd), and conalbumin (Con). Conditions are the same as those in Fig. 29 .