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OVALBUMIN THERMAL GELATION: PREDICTION OF GEL STRENGTH AS INFLUENCED BY SELECTED FACTORS

By

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ABSTRACT

OVALBUMIN THERMAL GELATION: PREDICTION OF GEL STRENGTH AS INFLUENCED BY SELECTED FACTORS

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A mathematical model was used to predict high temperature, low concentration ovalbumin gelation at pH 6. Apparent viscosity was utilized to evaluate the gelation process and was assumed to be a measure of gel strength. Activation energy of the denaturation/ gelation (E_a) reaction was found to be equal (38,000 cal/mole) for ovalbumin gelation at pH 6, 7 and with addition of 3% NaCl or a sulfhydryl blocking agent (N-ethylmaleimide). The conalbumin gelation reaction had the same E_a. Gelation of lysozyme, globulin and binary mixtures of protein with ovalbumin possessed E_a's equal to 50,000 cal/mole.

Model parameters were calculated from apparent viscosity and temperature-time histories of experimental data generated from ovalbumin gelation at 85, 90 and 95°C. These parameters were then used to successfully predict ovalbumin gelation at the three temperatures and at 3, 5 and 7% protein. Water holding capacity (WHC) was determined on selected ovalbumin gels. For gels prepared at 90°C, WHC was also successfully predicted using the model parameters. For all variables studied, model parameters were calculated.

As temperature of processing was increased, gel strength or WHC were obtained at a faster rate. Gel strength and WHC increased as pH increased from 6 to 7.

Also, as protein concentration increased, gel strength at these pH's increased. This was true for WHC at pH 6 but not at pH 7. Addition of 3% NaCl enhanced gel strength of ovalbumin while addition of NEM inhibited gelation due to a dramatic decrease in free SH content.

The four egg albumen proteins capable of thermal gelation, ovalbumin, conalbumin, globulins and lysozyme, were isolated and gel strengths compared at pH 6. The gel strengths of the proteins were rated as follows: conalbumin > lysozyme ~ globulins > ovalbumin. Binary mixtures of these proteins with ovalbumin were prepared which resulted in a decrease in gel strength in comparison to gels prepared from the individual proteins.

Two scanning electron microscopy (SEM) methods were used to evaluate gel ultrastructure: 1) postfixation with osmium-thiocarbohydrazide-osmium, ethanol and critical point drying (OTO/CPD) and 2) low temperature scanning electron microscopy. Using both methods allowed for more accurate ultrastructure interpretation of the low protein concentration, high moisture gels. Observed differences in ultrastructure supported differences found in functional properties.

DEDICATION

This thesis is dedicated to my husband Bruce and daughters, Marnie and Brittany for their constant love and support which enabled me to complete this dissertation.

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INTRODUCTION

Eggs are an important ingredient in prepared and processed foods because of their ability to fulfill a multitude of functional roles. They contribute to structure, binding, emulsification, foaming, appearance and flavor of foods. Their excellent nutritional value has long been recognized; however, recent dietary concerns about cholesterol has decreased whole egg and yolk usage in foods. Egg white (albumen), which is composed primarily of protein and water, provides excellent functional properties and is desirable from a nutritional standpoint. Currently, 30 million pounds are utilized in the US annually (Creamer et al., 1988).

One very important property of many food proteins such as egg albumen is their ability to form a heat set gel. This property often dictates the success or failure of cooked food products. Gelation involves the creation of a three-dimensional network at optimum ranges of pH, ionic strength, time and temperature of processing (Baldwin, 1986). This provides textural characteristics to foods, and through careful manipulation of the above factors, contributes to an optimum product. Frozen storage of cooked whole egg and egg albumen causes texture alteration of heat-set gels. These problems have been corrected with some success using chemical and enzymatic protein modification techniques.

Many investigators have attempted to elucidate the reactions involved in egg albumen gelation. Because egg albumen proteins differ in denaturation temperatures (Cunningham and Lineweaver, 1965), each may provide different contributions to thermal gelation. With exception of Johnson and Zabik (1981), few have examined the gelation properties of isolated albumen proteins as they relate to food applications.

The primary purpose of this study was to test the ability of a modified mathematical model (Lever, 1988; Morgan et al., 1989) to predict gelation characteristics of purified egg ovalbumin. Mathematical models have been used to predict thermal gelation in bovine plasma suspensions (Harper et al., 1978). Morgan et al. (1989) suggested a mathematical model to facilitate comparison between constant and variable time-temperature processes along with effects of variable heating rates, protein concentration and added ingredients. They studied thermal denaturation/gelation of soy doughs during extrusion.

The mathematical model of Morgan et al. (1989) was modified by Lever (1988) to predict the gelation characteristics of low concentration, high moisture isolated beef actomyosin. This modified mathematical model was then used to predict ovalbumin gelation in this current study using apparent viscosity measurements as a relative measure of gel strength.

Ovalbumin is the major protein in egg albumen

constituting 54% of the total protein. Along with conalbumin, lysozyme and globulin, ovalbumin's heat gelation abilities contribute to food texture (Johnson and Zabik, 1981). Ovalbumin was chosen as the protein to be used to develop the basic model around because of its relatively high purity after ammonium sulfate precipitation isolation and due to its high concentration in egg albumen. While there have been several studies which have investigated the gelation behavior of ovalbumin, none have attempted to apply a mathematical model to predict thermal gelation of the pure protein in combination with other purified egg albumen proteins.

Two further objectives of this study were to determine activation energies of ovalbumin denaturation/gelation to compare, isolated egg albumien protein gelation characteristics using calculated mathematical model parameters. Polymer-solvent (solution pH, sodium chloride or N-ethylmaleimide addition) and polymer-polymer interaction (lysozyme, globulin, and conalbumin) effects on ovalbumin gelation were evaluated by changes in E_a and or variations in basic model parameters.

Water holding capacity of ovalbumin gels was determined as a second means to evaluate gel characteristics. The free sulfhydryl and total sulfhydryl-disulfide contents of ovalbumin gels were also determined. This was done in order to estimate sulfhydryl and disulfide bonding changes as affected by process time and temperature, shifts in pH, as

well as sodium chloride and N-ethymaleimide (sulfhydryl blocking agent) addition.

The final objective was to use scanning electron microscopy (SEM) to visually evaluate ultrastructure of egg protein gels. When used in conjunction with other objective means of texture evaluation SEM can be a very powerful tool. Microscopic examination of the gel ultrastructure was performed using two scanning electron microscopic evaluation techniques. Structural differences between the egg proteins gels were noted and attempts made to correlate these to maximum apparent viscosity values.

LITERATURE REVIEW

Understanding the chemical composition and physicochemical properties of whole egg, egg yolk, and egg albumen and resultant changes during processing and storage is necessary to obtain optimum functional characteristics in egg containing products. The proximate analysis of whole egg, yolk and albumen is presented in Table 1. Valdehra and Nath (1973) detailed the chemical composition of avian eggs.

Table 1. Composition of albumen, yolk, and whole egg. (Powrie and Nakai, 1986)

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

The egg shell and membranes function as a barriers against microbiological spoilage and protect the developing embryo from physical damage. This is vital to quality maintenance of the egg yolk and albumen. Structure and composition of the chicken egg shell and egg membranes were described in detail by Powrie and Nakai (1986).

Biochemical composition of the major components of the

egg white and yolk is markedly different. This stems from the diverse roles each plays in the development of the chick. The yolk primarily functions as a food source while the albumen functions as a protective barrier. Greater than 50% of egg yolk solids are lipids. Yolk lipids are complex mixtures of glycoproteins, phosphoglycoproteins, lipoproteins and phosphoglycolipoproteins (Feeney, 1964).

In contrast, egg albumen solids are comprised primarily of proteins. Egg albumen protein composition is presented in Table 2. The lipids in egg white are found at about a 0.2% level with more than half the lipids being protein bound (Sato et al., 1973). Carbohydrates in the yolk and albumen are found in both the free and protein bound form. The major component of the uncombined albumen carbohydrate is glucose (0.4%) while glycoproteins contain mannose and galactose (0.5%). Free yolk carbohydrates are present at 0.7%. Protein bound yolk carbohydrates are found at 0.3% and are primarily composed of mannose-glucosamine polysaccharides (Powrie and Nakai, 1986).

Egg Albumen Proteins

Many research studies have been conducted to elucidate structure and physicochemical characteristics of egg albumen and the unique functional properties of the protein. Egg albumen can be readily separated into its component proteins. As many as 40 proteins have been found in egg albumen,

Table 2. Proteins in egg albumen.
(Powrie and Nakai, 1986)

Protein	Amount of albumen (%)	pI[1]	Molecular weight	Characteristics
Ovalbumin	54	4.5	45,000	Phospho-glycoprotein
Ovotransferrin	12	6.1-6.6	76,000	Binds metallic ions
Ovomucoid	11	4.6	28,000	Inhibits trypsin
Ovomucin	3.5	4.5-5.0	5.5-8.3 x 10^6	Sialoprotein
Lysozyme	3.4	10.7	14,300	Lyzes bacteria
Globulin				
G2	4.0?	5.5	3.0-4.5 x 10^4	--------
G3	4.0?	4.8	"	--------
Ovoinhibiter	1.5	5.1	49,000	Inhibits serine proteases
Ovoglycoprotein	1.0	3.9	24,400	Sialoprotein
Ovoflavoprotein	0.8	4.0	32,000	Binds ribo-flavin
Ovomacroglobulin	0.5	4.5	7.6-9.0 X 10^6	Strongly anti-genic
Avidin	0.05	10	68,300	Binds biotin

[1] Isoelectric point

however, most have not been isolated and only six have been found at concentrations of 1.0% or more (Vadehra and Nath, 1973).

Several electrophoretic techniques have been employed to separate albumen proteins. Moving boundary electrophoresis has been used to separate albumen into seven major peaks (Longsworth et al., 1940; Forsythe and Foster, 1949). Using zone electrophoresis, Evans and Bandemer (1956) separated the following proteins: ovalbumin, ovomucoid, ovoglobulin, conalbumin and lysozyme. Starch gel electrophoresis was used by several researchers to resolve 19 separated protein bands with great success (Lush, 1961; Steven, 1961; Feeney et al., 1963). Disc gel electrophoresis was used to fractionate egg albumen into 12 distinct bands (Chang et al., 1970). Polyacrylamide gel electrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) used in conjunction with carboxy methyl cellulose (CMC) chromatography were employed by Galyean and Laney (1980) to separate albumen proteins. Several fractions previously thought to contain pure protein were found to be heterogeneous.

Immunological procedures, ion exchange separation techniques and several other fractionation procedures were presented by Valdehra and Nath (1973) as ways to separate and purify albumen proteins.

Ovalbumin

The predominant egg white protein, ovalbumin, is approximately 54% of the total protein weight and was first crystallized by Neuberger (1938). Ovalbumin can be classified as a heterogeneous phosphoglycoprotein, with a molecular mass of 45,000 daltons. Three fractions differing in phosphorus content have been isolated electrophoretically: A_1 with 2 phosphates per molecule which are attached to serine (Nisbet et al, 1981), A_2 with 1 phosphate per molecule and A_3 containing no phosphate groups. The isoelectric point for ovalbumin is pH 4.6 (Powrie and Nakai, 1986).

Conformation of heated ovalbumin in solution was reported by Painter and Koenig (1976) to be intramolecular B-sheets. The carbohydrate fraction of ovalbumin composes 3.5% of the molecule and is heterogenic in nature. It consists mostly of the two hexoses, mannose and hexosamine.

Of all the egg albumen proteins, ovalbumin is the sole protein to possess free thiol groups. There has been some discrepancy concerning the number of thiol groups in ovalbumin due to inconsistencies in published results (Cecil and McPhee, 1959). Numbers of free thiols reported ranged from 2.2 to 4.8. Variation in thiol group reactivity to differing thiol reagents was responsible for some of the confusion. Fothergill and Fothergill (1970) established that ovalbumin possesses 4 thiol groups and 1 disulfide group by experimenting with and without reducing agents.

Currently, this is generally accepted as correct. These investigators further identified the C-terminal sequence of ovalbumin as -Cys-Val-Ser-Pro and the location of one end of the disulfide bond in this C-terminal peptide. Nisbet et al. (1981) determined the complete amino acid sequence of the ovalbumin molecule. The amino acid profiles of five proteins in egg white, at concentrations of 1% or more, are listed in Table 3. The ovalbumin molecule consists of about 50% hydrophobic amino acid residues.

Prolonged storage of ovalbumin especially at higher temperatures produces a variant form called S-ovalbumin (Smith and Back, 1965). The major difference in this form appears to be the greater heat stability of S-ovalbumin. The transformation mechanism of ovalbumin to S-ovalbumin has not been elucidated. No gross structural differences between these two molecules has yet been discerned. However, Kato et al. (1986) detected differences in covalent structure between these two molecules. Deamidation of asparagine or glutamine in ovalbumin resulted from the conversion to S-ovalbumin. It was suggested by Nakamura and Ishamuru (1981) that S-ovalbumin may have a more compact configuration and increased surface hydrophobicity. Also, a small difference in surface charge between ovalbumin and the S- variant was detected by Nakamura and Ishamura (1981). It has been hypothesized by some that the conversion to S-ovalbumin may be due to sulfhydryl-disulfide interchange reactions. However, Webster and Thompson (1980)

Table 3. Amino acid composition of egg albumen proteins.

Amino Acid	Ovomucin[1] (moles/ 10^5g)	Lysozyme[2] (residues/ mol)	Ovomucoid[3] (residues/ mol)	Conalbumin[4] (residues/ mol)	Ovalbumin[5] (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
Trypotophan	10.9	6	----	12.7	3
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[6]
Methionine	17.7	2	1.8	13.1	15
IMINO					
Proline	67.9	2	7.2	28.9	16

[1]-Robinson and Monsey (1972)
[2]-Jolles et al. (1963)
[3]-Davis et al. (1971)
[4]-Wenn and Williams (1968)
[5]-Fothergill and Fothergill (1970)
[6]-4 Sulfhydryls, 1 Disulfide

demonstrated that thiol-disulfide interchange reactions did not mediate S-ovalbumin formation.

A second ovalbumin variant is plakalbumin, a protease modified ovalbumin. The following factors have affected plakalbumin formation: enzyme type, pH, enzyme concentration, and storage temperature (Kilara and Sharkasi, 1984).

Ovalbumin can be denatured when exposed to vigorous shaking, denaturing agents, absorption at surfaces and heat. Thermal effects on egg albumen proteins will be discussed in detail in the Egg Albumen Thermal Gelation section.

Conalbumin (Ovotransferin)

Conalbumin was detected in egg albumen in 1900 (Osborne and Campbell, 1900) but did not gain prominence until its antibacterial function and iron binding capabilities were discovered (Alderton et al., 1946; Shade and Caroline, 1944). The specific function of conalbumin in the egg white is thought to be related to its antimicrobial property and its ability to inhibit lipid oxidation. This protein is about 13% of the total egg white protein and has been isolated by precipitation with ammonium sulfate and ion exchange chromatography (Warner and Weber, 1951; Azari and Baugh, 1967). More recently separation using immobilized metal affinity chromatography has been used (Mashikhi and Nakai, 1987).

Some important chemical properties of conalbumin are

presented in Table 2. This glycoprotein contains approximately 0.8% hexose and 1.4% hexosamine but lacks phosphorus and free sulfhydryl groups (Powrie and Nakai, 1986). Electrophoretic techniques have shown conalbumin to consist of two fractions at a 4:1 ratio (Feeney et al., 1963; Wenn and Williams, 1968). The amino acid content of conalbumin is presented in Table 3.

Conalbumin exists as a single polypeptide (Bezkorovainy et al., 1968; Green and Feeney, 1968) which has the ability to strongly bind with metal ions such as iron, cobalt, copper, zinc and aluminum. The molecule possesses two iron binding sites which are not equivalent (Donovan and Ross, 1975). Conalbumin's metal binding ability imparts greater heat stability to the molecule and is important in the heat pasteurization of egg albumen. In conalbumin isolated from native egg albumen, two iron atoms per molecule of protein were found bound (Aisen et al., 1966; Wenn and Williams, 1968). Iron binding occurred via two nitrogen and three phenolic groups located on aromatic amino acids (Windle et al., 1963; Malmstrom et al., 1963). Heat lability of conalbumin will be discussed later in the Egg Albumen Thermal Gelation section.

Globulin Fractions

Ovomacroglobulin, G1 or lysozyme, G2 and G3 comprise the globulin fraction and range from 11.3-13.0% of the total egg white protein. Ovomacroglobulin is considered a minor

egg white protein (0.8%) which has been separated from the other proteins using starch electrophoresis. It has a high molecular mass, 7.6-9.0 x 10^6 daltons and is strongly immunogenic (Miller and Feeney, 1966). According to Donovan et al. (1969), ovomacroglobulin possesses a sphere-like shape and very small alpha helix formation. Isoelectic pH values range from 4.5-4.7 (Miller and Feeney, 1966).

Unfortunately G2 and G3 have been poorly characterized to date. These proteins were isolated from other egg albumen proteins using ammonium sulfate precipitation (MacDonnell et al., 1955) and separated from each other using starch gel electrophoresis (Feeney et al., 1963) and CMC chromatography (Nakamura et al., 1980). Isoelectric points were reported to be 5.5 for G2 and 4.8 for G3 by Baker (1968). Very little other information is available pertaining to the biochemical characteristics of these globulin proteins.

Fractions G2 and G3 comprise about 8% of the total egg white protein and are important because of their excellent foaming ability (MacDonnell et al., 1955; Johnson and Zabik, 1981). These proteins and lysozyme demonstrate the ability to form thermal gels which will be discussed in a later section.

Lysozyme is an extremely well characterized protein widely distributed in nature and found in concentrations of 3-4% of the total egg white protein. It has an ellipsoidal shape with a distinct cleft on one side and possesses a

conformation as determined by Blake et al. (1966b). Fairly small amounts of alpha helix were found on the main chain while several long stretches were found with irregular conformations, some of which were B-pleated sheets. Lysozyme had its complete amino acid sequence determined by Canfield (1963). Acidic and basic side chains, along with terminal groups were located on the surface of the protein molecule, while hydrophobic amino acid chains were oriented towards the interior of the molecule. Lysozyme was found to contain 4 disulfide bonds but lacked free thiol groups. A list of the 129 amino acid residues found in lysozyme is presented in Table 3. The isoelectric point of lysozyme is the highest of the egg white proteins (pH 10.7) while the molecular weight is one of the smallest (14,300 daltons) (Sophianopoulos et al., 1962).

The lytic action of lysozyme on bacterial cell walls has been well established. It has the ability to hydrolyze the B(1-4) linkages between N-acetyl-neuraminic acid and N-acetylglucosamine (Berger and Weiser, 1959).

Isolation of lysozyme has been accomplished using several techniques such as 5% NaCl precipitation at pH 9.5 (Tyler and Geake, 1958). Various column chromatographic procedures have also been used: Bio-Rex 70, Amberlite XE-64, Amberlite CG-50, CMC, apatite gel, and affinity chromatography (Tallan and Stein, 1953; Stevens and Bergstron, 1967; Ahvenainen et al., 1980; Rhodes et al., 1958; Tiselius et at. 1956; Imoto et al., 1969). More recently, Durance and

Nakai (1988) developed a single column cation exchange procedure which simultaneously recovered lysozyme and avidin as separate peaks with good recovery and purity.

Lysozyme has been found to readily bind with other proteins such as conalbumin, ovalbumin, ovomucin and bovine plasma albumen (Steiner, 1953; Ehrenpreis and Warner, 1956; Forsythe and Foster, 1950; Nichol and Winzor, 1964; Hawthorne, 1950). These complexes are thought to be a result of electrostatic binding and may influence the functional characteristics of egg albumen.

Ovomucin

Ovomucin is a very large aggregated glycoprotein (720,000 daltons) which constitutes approximately 2% of the egg white protein. It is thought to contribute to the viscous, gel-like character of the thick egg white. As the pH is increased, the viscosity of ovomucin increases until pH 8.2 where a gel is formed (Vadehra and Nath, 1973). Twisted ovomucin fibers are believed to comprise the chalaziferous layer of the egg. Only slight differences were found in the polymerization state of the basic units making up ovomucin and chalaza (Itoh et al., 1987).

Ovomucin has been readily isolated by dilution with water at pH less than 6.0 or by dialysis against water at pH 6-8. Procedures for isolation and separation of ovomucin were summarized by Vadehra and Nath (1973).

Since its antihemaglutination abilities against viruses

was discovered (Sugihara et al. 1955), interest in ovomucin's biochemical characteristics has increased. Ovomucin is believed to exist as extended protein chains connected by disulfide links (Gottschalk et al., 1972) which would help explain the high disulfide content of the molecule. Little or no alpha helixes were detected in the strands while extended B or random coil conformation were found (Donovan et al., 1970). The amino acid composition of ovomucin is presented in Table 3. Acidic and basic amino acid residues were found at a ratio of 2:1 with 260 hydrophobic residues.

As high as 33% of ovomucin is made up of carbohydrate which is comprised primarily of hexose, hexosamine and sialic acid (Powrie and Nakai, 1986). Two components of ovomucin have been separated (Robinson and Monsey 1971; Kato and Sato, 1971). One is designated alpha-ovomucin or carbohydrate-poor and the second as beta-ovomucin or carbohydrate-rich.

Ovomucin readily complexes with lysozyme and other egg white proteins (Cotterill and Winter, 1955 and Kato et al., 1976). While ovomucin is considered to play an important role in the thinning of thick egg white during storage there have been conflicting reports on whether the dissociation or association of the ovomucin lysozyme complex is the cause of this phenomena. Recently, Hayakawa et al. (1983) theorized that the viscous, gel-like character of the thick white is caused by alpha-ovomucin-lysozyme complexes surrounded by

beta-ovomucin. Beta-ovomucin gradually dissociates from the ovomucin complex with increased alkalinity during storage which results in the egg albumen thinning. Alpha-ovomucin-lysozme complex then becomes insoluble (Kato et al., 1979).

Ovomucin is considered to be heat stable. When exposed to 90°C for 2 hours at pH's between 7.1 and 9.4, optical density or viscosity of ovomucin solutions were not altered (Powrie and Nakai, 1986).

Ovomucoid

Ovomucoid is a protein which represents 12% of the total egg white protein and possesses a high carbohydrate content of 25%. It is extremely heat and acid stable and demonstrates antitryptic behavior (Lineweaver and Murray, 1947). Isolation of the protein has been reviewed by Vadelhra and Nath (1973). The purified protein has demonstrated extreme heterogeneity which has been attributed to variations in isoelectric points (3.83-4.41), sialic acid and carbohydrate content. Its molecular weight is approximately 28,000 and the protein's amino acid composition is presented in Table 3. Ovomucoid has been reported to exist as a single chain with 22% helical sections and random coils (Ikeda et al., 1968). There are 8 disulfide linkages in each chain which helps explain its high heat resistance at pH's less than 8.0 (Beeley, 1971 and Beeley and McAirns, 1972). Heating at 100°C for 45 min could not totally eliminate the immunoreactivity of ovomuc-

oid to human IgE antibody (Jainzin et al., 1986). Shimada and Matsushita (1980b) found that ovomucoid did not form a precipitate even at its isoelectric point.

Other Minor Proteins

Several other proteins and enzymes are found in egg white but are not usually considered important to the functional characteristics because they are at levels less than 1% of the total protein. Among these proteins are flavoprotein (35,000 daltons) whose purpose is to bind all of the riboflavin found in the egg white. It has been separated into two fractions with 7 and 8 phosphate groups per mole (Rhodes et al., 1959). Another inhibitor in addition to ovomucoid and ovomucin is ovoinhibitor, which possesses at least three separate inhibitory sites for trypsin, chymotrypsin and elastase (Gertler and Feinstein, 1971). A heat resistant papain-ficin inhibitor was first purified by Fossum and Whitaker (1968) and was found to have a molecular weight of 12,700.

Avidin is known for its ability to bind biotin. It possesses a molecular weight of 53,000 daltons and an alkaline pI of 9.5. Green (1963) reported extensively on the properties and chemistry of avidin binding sites. Ovoglycoprotein is a homogeneous glycoprotein which was first isolated in 1962 by Ketterer and found to contain large amounts of hexose and glucosamine.

Egg Albumen Thermal Gelation

The importance of proteins to the food scientist is related to their nutritional significance and functionality which often controls the appearance, flavor, texture and overall acceptability of a food. When egg proteins are added as ingredients in thermally processed food formulations, they interact with, support and bind other ingredients within their gel structure thus contributing to the texture of the food product. Knowledge of the different factors which affect protein performance in a food must be understood in order to avoid undesirable changes during preparation or processing. This is a difficult task because of the complexity of food systems in which proteins can interact with each other and many other macro and micronutrient. An extensive review of the functional properties of foods was presented by Kinsella (1976).

Gelation Theory

Protein gelation is an important functional characteristic which reflects on the textural aspects of foods. It is considered to be the orderly aggregation of proteins into a three-dimensional matrix (Hermansson, 1979). Gelation has also been defined as a phenomenon controlled by electrostatic repulsion between molecules occurring between conditions of protein aggregation and nonaggregation (Kilara and Sharkasi, 1984; Hegg et al., 1979). This infers that

alterations in pH, salt type, ionic strength, or added chemicals may affect molecular electrostatic attraction or repulsion, and therefore gelation.

Discussion of thermal effects on proteins involves definition of some of commonly used terms. Confusion has resulted from the difference in connotation in the meaning of denaturation by scientists. The definition of denaturation used in this discussion is: the process in which the spacial arrangement or conformation of protein polypeptide chains are altered from their typical native state to a more disordered state (Kauzmann, 1959), without disruption of covalent bonds (Scheraga, 1961). Unfolding of the protein structure and change in physical characteristics are caused by the protein-solvent interactions of denaturation. Protein denaturation can be caused by several factors such as heating, freezing, high pressure, sonication, pH, and exposure to chemical denaturation agents (Kinsella, 1976). Aggregation occurs when proteins interact with each other forming high molecular weight polymers.

The term coagulation is often used when discussing heat effects on proteins and refers to protein denaturation (Hermansson, 1979) followed by random aggregation. Polymer-polymer complexes form more readily than polymer-solvent interactions (Schmidt et al., 1981). Loss of protein solubility accompanies protein coagulation. Often the term coagulation and gelation are used interchangeably when referring to egg albumen protein heat setting (Baldwin,

1986). Thermally induced gelation involves polymer-polymer and polymer-solvent interactions in conjunction with balanced electrostatic forces to form a three dimensional gel network (Schmidt et al., 1981). This discussion will be concerned with thermal denaturation followed by aggregation and gelation of protein.

In gel formation, the same type of bonds which are responsible for maintaining the three dimensional configuration of proteins i.e. hydrogen, electrostatic, hydrophobic and sulfhydryl-disulfide bonds, can also be involved in the protein polymer-polymer and polymer-solvent interactions (Kinsella, 1976; Schmidt et al., 1981; Jaenicke, 1967). However, the type of interactions which stabilize the gel may vary depending on the type of protein (Schmidt, et al., 1981).

A two step model was developed by Ferry (1948) to explain the mechanism of protein thermal gelation:

		(1)		(2)
Native protein	--->	denatured protein	--->	aggregated protein

In the first step unfolding of the protein molecules occurs (denaturation) followed by association of the long chains into a matrix of aggregated protein or gel. According to Ferry (1948) the correct ratio of attractive to repulsive forces was necessary to achieve a well-formed gel structure. Hermansson (1979) elaborated on this gelation mechanism,

explaining that the rate of the reaction affects the degree of gel network order and resultant gel character. Conditions favoring the denaturation step (1) over protein network formation (2) resulted in a finer gel structure. The slower aggregation allowed time for a well developed network to form which was characterized by good water holding capability, good elasticity and a less opaque appearance. Rapid aggregation resulted in course network formation, excessive gel syneresis and an opaque appearance.

A modification of the proposed gelation mechanism was suggested by Shimada and Matsushita (1980a). In the first step of the reaction, the denaturation of the native protein was thought to involve disulfide bond formation and exposure of hydrophobic amino acid residues. The denatured protein was believed to proceed to a coagulum or an aggregate depending upon various factors such as protein concentration, molecular weight, heating time, etc. The term coagulum referred to thermoirreversible gels and aggregate meant an opaque precipitate. Heating during the coagulum or aggregate step resulted in sulfhydryl-disulfide cross-linking reactions producing a gel matrix. Shimada and Matsushita (1980a) further theorized that hydrophobic bonding which is promoted by high temperatures, is important to the structure of the gel network or coagulum formed. A proper balance between electrostatic charges is important to form a homogeneous coagulum with good water holding capabilities.

A modification of the sequence of events occurring in the thermocoagulation of egg white proteins was proposed by Ma and Holme (1982):

N <---------> D ----> A ----> G

where N = native monomer, D = denatured monomer, A = aggregated protein or polymer, and G = gel or coagulated protein.

Hermansson (1986) stated that the kinetics of the gelation reaction dictates the resultant gel structure and its properties. A new method for continuous measurement of thermal gelation of egg white which provided kinetic data was developed by Gossett et al. (1983b). In addition, kinetic terms useful to describe gelation were presented by Gossett et al. (1984).

Factors Affecting Egg Albumen Gelation

Eggs or egg products are heated for the purpose of cooking or extending their storage life (dehydration, pasteurization). This discussion will focus on the factors which influence thermal gelation of egg albumen and the four major albumen proteins found to form gels: ovalbumin, conalbumin, lysozyme and globulins (G1, G2) (Johnson and Zabik, 1981). Unless otherwise noted, egg albumen was obtained from fresh eggs less than or equal to 48 hours old.

Effect of heat. The coagulation reaction between albumen proteins and water was observed as early as 1910 by Chick

and Martin. The appearance of the egg albumen changed from a clear, translucent fluid to an opaque solid at 62-65°C. Firmness of the egg gel increased as cook time and temperature increased. Barmore (1936) demonstrated increased tensile strength in egg albumen heated between 77.5 and 100°C. Beveridge et al.(1980) also found increased firmness when they used a shear press to examine the gel strength of heat-induced albumen coagulum. Samples were frozen and then thawed prior to heating at 77, 80, 85 and 90°C. Gels obtained at 77 and 80°C were significantly more tender than those heated at temperatures higher than 80°C for 60 minutes. Beveridge et al. (1980) suggested that an additional reaction caused the toughening which occurred at the higher temperatures.

A nondestructive temperature-controlled thermal scanning rigidity monitor (TSRM) was used by Montejano et al. (1984) to measure a rigidity modulus (G) and mechanical energy damping of egg albumen during heating from 5 to 90°C. Low rigidity values (<0.1 kPa) were observed from 5 to 60°C because samples remained fluid. A rapid increase in rigidity (stiffness) occurred from 71 to 83°C and a steep increase in rigidity occurred between 80 and 82°C. This agreed with the earier results of Beveridge et al. (1980).

Response surface methodology was used by Holt et al. (1984) to examine the rheological parameters of viscosity, gel strength and elasticity and their effect on thermocoagulation of egg albumen. Protein sols (8%) adjusted

from 0 to 0.1 M NaCl concentrations with pH ranges of 6.4 to 9.6, were heated from 66 to 90°C. Elasticity and gel strength were greatest at 85.2°C with a pH of 9.0 and the addition of 0.08 M NaCl. Temperature had the greatest effect of all of the rheological parameters evaluated.

Effect of pH. Changes in pH of albumen can have a dramatic effect on stability, and therefore, aggregation or gelation (Lewis, 1926; McPherson et al., 1945). Methods used to evaluate the reaction gave conflicting results. Slosberg et al. (1948) reported greater egg albumen heat stability at pH 6.5 than at 8.5 using whip time and angel cake volume to evaluate protein heat stability. Seideman et al. (1963) concurred, using gelation scores as a stability criteria. However, an earlier study by Barmore (1936) showed that as the pH decreased, the coagulation temperature was lowered. This agreed with the results of Seideman et al. (1963) who used optical density values as criterion for coagulation. It was thought that lower stability occurred at pH's corresponding to the isoelectric points of individual egg white proteins.

Nonthermal gelation of egg white obtained from eggs stored up to three days and then adjusted to acidic pH's was studied by Cunningham and Cotterill (1964). As pH decreased from 8.0 to 4.0, viscosity also decreased. At pH less than 4.0 the viscosity began to increase with cloudy, opaque gels forming at pH 2.2 and less. Nonthermal gelation of egg

albumen adjusted to alkaline pH with 3 N NaOH (pH 11.3-12.7) was also observed by Cunningham and Cotterill (1962). Viscosity increased rapidly until a translucent gel resulted from exposure of egg white to pH's greater than 11.9. However, this type of gelation was of limited practical use since the pH's of most food systems are acidic or neutral. The dependence of egg albumen coagulation on pH was also studied by Shimada and Matsushita (1980a) using turbidity and gel hardness as a measure of coagulation. Turbidity increased as pH decreased from pH 11 to 8 where it remained constant. However, a gel began to form at pH 9.5, with peak strength at pH 8.5 while gel strength decreased as pH 7 was approached.

The effect of pH (5.5-9.0) on egg gel firmness was examined by Beveridge et al. (1980). Minimum gel strength was observed at pH 7.0. The authors explained that in the alkaline region, sulphydryl-disulfide interchange reactions may be the major mechanism responsible for gel strength. As pH decreases to pH 6, these reactions are inhibited because the concentration of S^- are restricted. The increase in gel strength as pH is lowered from 7 is the result of decreased protein solubility which thereby increases protein in the gel network and thus increases gel strength.

Texture of heat-induced egg white gels was evaluated by Woodward and Cotterill (1985) using texture profile analysis and determination of expressed serum. While gels increased in hardness with increasing temperature and time, the rate

varied with pH. Greatest gel strengths were at pH 5. Longer heating times were necessary for gels adjusted from pH 6 to 8 in order to form firm gels (>1 kg). Generally, expressed serum and gel hardness were inversely proportional when heated at 75 to 90°C. Expressed serum levels were lowest for gels at pH 8.

Preparation of heat induced transparent gels from egg albumen was attempted by Kitabatake et al. (1988) in hopes of generating new opportunities for use of egg white as an ingredient. This was successfully accomplished by 1) reducing the ionic strength to a low level by dilution or dialysis, 2) removing a slight precipitate formed and 3) heating at acidic pH's (2-4).

Effect of salt. Removal of salt from egg albumen will inhibit gelation while too high of a salt concentration will cause curdling. The effects of salt on the coagulation (gelation) of egg albumen has been studied by many researchers. The reaction rate of protein aggregation in relation to the denaturation rate reflects on the quality of the egg.

Turbidity and gel hardness evaluation were the methods used to evaluate salt effects on thermocoagulation of commercially prepared egg albumen at concentrations of 2.8% (Shimada and Matsushita, 1981). Increased turbidity and gel hardness were detected in samples containing 0.3 M NaCl, versus no salt, when adjusted to alkaline pH's. Increasing concentration of anions resulted in a shift in turbidity

from the isoelectric point to alkaline pH's. Gel hardness was affected by anions and cations in the following order: SO_4 > Cl> Br> I> and SCN; Ca >> Li > Na ~ Cs. Shimada and Matsushita (1981) theorized that one of the functions of salt was to decrease the amount of protein bound water. Ions which had greater effect on gel strength and turbidity shifts dehydrated the protein more. Protein molecules would then bind more readily to each other, thermocoagulation would then occur over a greater pH range. Beveridge et al. (1980) studied the effect of salt addition on heat induced albumen coagulation. Of the salts ($FeCl_3$, $CuSO_4$, $AlCl_3$) added to 5% egg albumen protein heated at 90oC for 7 minutes (pH 9) only $FeCl_3$ addition caused an increase in gel strength. While metal ions stabilized conalbumin aggregation, metallic cations could have caused oxidation of SH to SS, thus, resulting in increased gel strength.

Sodium chloride was added to egg white gels prior to heating by Woodward and Cotterill (1986). They found that salt decreased gel strength at pH 5, 8 and 9 while it increased hardness at pH 6 and 7, at salt concentrations up to 0.32 M. Expressed serum values were reduced at pH 7 when 3.2 M NaCl was added and at pH 5, 6 and 8 when the gels contained 1.0 M NaCl. This resulted in a stronger water holding capacity (WHC) of the gel network. Addition of low salt levels (<0.03M) initially lowered expressed serum values (stronger WHC) for samples adjusted to pH 9. Expressed serum values gradually increased again when 1.0 M

NaCl was added.

Effect of protein concentration and sucrose addition. Dilution of egg albumen increased the temperature of coagulation (Baldwin, 1986) and caused an exponential decrease in shear force of the gel (Beveridge et al., 1980). Nakamura et al. (1978), varied the concentration of ovalbumin heated at 80°C from 0.12 to 1.2% causing almost 100% protein aggregation, while heating at 75°C resulted in 80% aggregation. When samples were heated at 70°C, no aggregation occurred unless concentrations of 1% protein or higher were used.

The effect of protein concentration on coagubility of egg albumen heated at 80°C for 15 minutes was evaluated by Shimada and Matsushita (1980a). Low protein concentrations (< 1.0%) produced gels having turbidity measurements very close to those near the isoelectric point. At pH ranges which were conducive for gelation, increased gel hardness resulted when concentration (7-12%) was increased. Shimada and Marsushita (1980b) looked at the relationship between thermocoagulation of proteins, molecular weight and amino acid composition of several proteins including egg albumen and conalbumin. It was concluded that protein concentration dependency for coagubility required that the protein should have a high molecular weight (> 60,000 daltons) and a high concentration of hydrophobic groups. Coagulation of protein having low molecular weight demonstrated no dependence on protein concentration even when high concentration of hydrophobic groups (> 34%) were present.

At an earlier date optical density and gel hardness scores were used by Seideman et al. (1963) to evaluate thermocoagulation of egg albumen sucrose mixtures as a function of pH. Egg albumen obtained from eggs up to 48 hours old was frozen and then thawed when needed. Sucrose was added to the egg albumen at 10 or 50% levels. Addition of sucrose at either concentration and especially at adjusted pH's above 8.5, increased the temperature required for coagulation. Increasing the sucrose concentration in custards reduced gel strength because of the protein dilution (Wang et al., 1974).

Disulfide bond formation. The importance of cross-linking of proteins by disulfide bond formation to form heat induced gel networks has been studied by many investigators (Cunningham and Cotterill, 1962; Seideman et al., 1963; Beveridge et al., 1980). Jensen (1959) observed that protein denaturation leading to aggregation involving sulfhydryl-disulfide reactions was the most often studied of all examples of this type of bonding. Cecil and McPhee (1959) proposed a mechanism by which gel matrices were formed:

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

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

This sulfhydryl-disulfide interchange reaction involved side-by-side association of protein molecules which were produced by sulfhydryl (SH) group initiation of a chain reaction of protein molecules. After initiation, no further

decrease in SH groups occurred because a mercaptide (S^-) group formed for every disulfide (SS) produced (Buttkus, 1974). Buttkus compared the fluctuation of SH values in rabbit myosin and actomyosin preparations by several different investigators. The erratic results may have been due to the oxidation of SH groups and cleavage of SS groups by metals (Cecil and McPhee, 1959). Both Buttkus (1974) and Holme (1963) suggested that sulfhydryl-disulfide interchange reactions were of major significance in thermocoagulation of egg proteins.

Effects of thermal treatment on SH levels in egg albumen was examined by Beveridge and Arntfield (1979). Results showed that heating of samples adjusted to pH 8 and heated to 90°C initially increased exposed SH groups which then decreased gradually with time. Increased temperature accentuated the effect. Decreased numbers of SH groups were thought to be caused by oxidation of SH groups to SS groups or other compounds. The amount of dissolved oxygen in the sample and oxygen in the head space may have affected the firmness of the coagulum due to possible oxidation of SH to SS. No differences were observed in SH levels when samples were heated at pH 4, 5, or 6. Sulfhydryl levels were lower as the alkalinity of the samples was increased up to pH 10. At higher pH's a rise in SH groups occurred due to alkaline hydrolysis of the SS groups after 20 minutes of heating.

Since polymers of egg albumen formed by heating could easily be converted to monomers when reduced, it was

suggested by Ma and Holme (1982) that sulfhydryl-disulfide interchange reaction played a role in gelation. Many environmental factors such as pH, ionic strength and heating conditions affected the gel formation. High net charge prevented gelling and hydropohobic interaction was found necessary for gel formation.

Thiol (SH) dependent gelation occurred at temperatures lower than those required for thermal gelation (Hirose et al., 1986). The reaction was greatly affected by pH with strong gel strength maxima (0.2 kg-wt) at pH 4 and 12. Since gelation also occurred due to nonspecific gelation at pH 2 and 12 without thiol addition, thiol dependent gelation was investigated at pH 8. At this pH a hard gel (0.05-0.09 kg-wt) was produced. As a result of this study, gelation was found to be inhibited by saturating the iron-binding sites of conalbumin. A gel which was formed at pH 7~9 was centrifuged and a sample of the pellet was analyzed using polyacrylamide gel electrophoresis (PAGE). When compared to the nontreated albumen control, the gel pellet showed an almost complete disappearance of the conalbumin band. This suggested that conalbumin was the major protein component in the thiol dependent gel.

Heat Gelation of Individual Egg White Proteins.

Heat denaturation of egg white and its component proteins at pH 7.0 was carried out by Donovan et al. (1975) using differential scanning calorimetry. Two major

endotherms, at 65 and 84°C, were caused by the denaturation of conalbumin and ovalbumin, respectively.

As egg albumen is heated at a moderate rate, the sample temperature levels off or slightly drops during coagulation. This is considered an endothermic reaction. These time-temperature relationships for gels prepared with isolated albumen proteins were used as a means to detect coagulation temperatures by Johnson and Zabik (1981) using a custard model system which replaced yolk proteins with egg albumen proteins and milk with a salt solution containing a similar ionic strength (0.165) to milk. Total protein was 2.6% in the model system. Coagulation temperatures for lysozyme, globulins (G1, G2), conalbumin (iron free) and ovalbumin were 81.5, 72.0, 57.3 and 71.5°C, respectively. Temperatures at which whole egg proteins lost their electrophoretic mobility when heated were reported by Woodward and Cotterill (1983). Coagulation temperatures for 10% weight/weight (wt/wt) albumen proteins were as follows: 78°C for conalbumin, 81°C for G2, 63°C for G3, 75°C for ovalbumin and 63°C for lysozyme.

In developing a pasteurization method for egg albumen, Cunningham and Lineweaver (1965) demonstrated the practical importance of knowing the heat sensitivities of individual egg albumen proteins. The effect of heating on the four proteins (ovalbumin, conalbumin, globulin (G2, G3) and lysozyme found to form heat coagulable gels in a custard model system by Johnson and Zabik (1981) will be discussed.

Ovalbumin. Heat stability of ovalbumin was shown to be pH dependent. Greatest stability was demonstrated at pH 6.5 to 7.0 in an early study by Lewis (1926) and later at pH 6 to 10 by Hegg et al. (1979). At sufficiently high concentrations and temperatures, ovalbumin forms firm gels (Holme, 1963). The effect of neutral salts on ovalbumin (4.4%) thermal aggregation and denaturation was also examined by Hegg et al. (1979). Addition of 170 mM (1%) NaCl at had no effect on ovalbumin's denaturation temperature which varied with pH (62-77°C). $CaCl_2$ (17 mM) addition decreased the denaturation temperature slightly at pH's from 6 to 11 and was more efficient than NaCl at promoting thermal aggregation. In a salt free environment, the majority of thermal aggregation temperatures occurred 4-7°C below denaturation temperatures found with salt addition and only at pH's close to the isoelectric point. Thus, the number of net charges on the molecule determined the aggregation behavior of ovalbumin. This was later supported by Nakamura et al. (1978). Gelation occurred at or above the denaturation temperature when conditions were not favorable for protein aggregation. Gelling occurred in intermediate ranges of net charge repulsion, not high enough to cause solubilization and not low enough to cause precipitation.

Controlling electrostatic forces was suggested by Egelandsdal (1980) as the prime means of influencing ovalbumin (8.2%) gelation. At low ionic strengths, gel

strength maxima were found at pH 3.5 and 7.0 which were on both sides of the isoelectric point. Maximum rigidity was greater for the sample at pH 3.5 than at 7 (600 verses 280 g/cm). Addition of increasing amounts of NaCl (0.003 M, 0.03, 0.3 M) caused a shift in the gelation maxima, even farther away from the isoelectric point. Addition of salt appeared to function by electrostatic shielding of the repulsive fixed charges. Therefore, increasing ionic strength will either inhibit or promote gel rigidity depending upon the pH. In summary, the as the pH rose or fell away from the isoelectric point, increased salt concentrations tended to promote gel rigidity up to a maximum concentration. The NaCl concentrations of 0.3 M inhibited ovalbumin gelation at pH's from 2 to 8.

Properties of heat-set, highly concentrated ovalbumin (10-35%) gels were very dependent upon pH, with samples at pH 10 demonstrating homogeneous, strong and fairly clear gels (Van Kleef, 1986). Gel heat set at pH 5, were heterogeneous and weak. Ionic strength had a less dramatic effect on the gel compared to the infuence of solution pH. However, the addition of 3% NaCl did shift the storage shear modulus (G') peak height away from the isoelectric point although not as much as previously demonstrated with gel hardness values (Egelandsdal, 1980). Using tensile experiments, Van Kleef (1986) showed breaking stress values for samples adjusted to pH 10 and heat-set at 100°C for 20 minutes were about 30 times that of samples adjusted to pH 5

and heated. Deformation at break was about 2 times larger. Addition of 3 % NaCl (wt/wt) caused a shift in the G´ to higher pH´s. A G´ maxima occurred without salt at pH 4 and with salt at pH 5. In addition, the combined effect of pH and temperature on conformational changes of the ovalbumin molecule (1% w/w solution) were evaluated by Van Kleef (1986) using high-resolution H-NMR. Ovalbumin was found to unfold completely prior to network formation when heated at a high pH value (pH 10) to temperatures up to 90°C. Only partial unfolding occurred prior to aggregation at pH 5 when ovalbumin samples were heated in the same manner. By increasing the protein concentration to 15% w/w, overall the mobility of the protons was decreased. However, the same trend with respect to pH and temperature and the resultant unfolding of the molecule was observed.

Turbidity and hardness of thermally induced ovalbumin (80°C, 5%) gels were examined by Hatta et al. (1986). Two pH gel maxima were demonstrated which was consistent with results shown previously by Eglandsdal (1980). Transparent gels were found over a narrow pH and ionic strength range where the greatest coagulum strength was demonstrated. Hatta et al. (1986) explained that increased ionic strength may lower electrostatic repulsive forces due to a shielding effect. Best gel structures were formed by a balance of hydrophobic and electrorepulsive forces. Hydrophobic areas in the ovalbumin structure were exposed by the heating process. Strong attractive (hydrophobic) forces resulted in

a turbid suspension. When the attractive forces were lowered, a turbid gel was formed. Decreasing attractive forces even further, resulted in formation of a transparent gel. When repulsive forces were greater than attractive forces, a solution was formed.

The roles of hydrophobicity, net charge and sulfhydryl groups in gel formation were evaluated by Hayakaw and Nakai (1985). Coagulation (random aggregation) and gel strength were determined on 0.5 and 5% ovalbumin samples adjusted to pH's from 5.5-8.0 in 0.05 M NaCl and heated to 75, 85 and 95°C. They concluded that hydrophobicity and net charge affected coagulability while hydrophobicity and SH groups influenced gel strength. These results supported the earlier conclusions of Voutsinas et al. (1983).

Kitabatake and Doi (1985) formed transparent gels using limited proteolysis by pepsin at pH 4 with ovalbumin concentrations of 3 to 7% weight/volume (wt/v), and heating at 98°C for 3 minutes. In order to form transparent ovalbumin heat-set gels at higher ionic strengths (>0.20 mM NaCl) than previously found (Hatta et al., 1986), Kitabatake et al. (1987) attempted a new gel preparation method. Transparent solutions were made from 5% ovalbumin wt/v, adjusted to pH 7 and heated at 80°C for 1 hour without salt addition. By adding 0.2 M NaCl and reheating the solution a transparent gel was formed. Heating of the ovalbumin without salt resulted in the formation of a soluble aggregate composed of monomers and oligomers connected by SS

bridges. Sulfhydryl groups decreased slightly with heating time.

Only a few observations have been made dealing with the thermal gelation of combinations of ovalbumin with other egg white proteins. The gelation properties of isolated albumen proteins alone and in combination with each other were studied by Johnson and Zabik (1981) in a custard model system which contained salts at concentrations equivalent to those of milk. Gel strengths, for the four proteins (2.6%, pH 8.0) which coagulated when heated at 0.74°C/min, were as follows: lysozyme > globulins > ovalbumin > conalbumin. Percent drainage of the lysozyme gel was the smallest while that of conalbumin was the greatest, demonstrating poor water holding ability. Binary mixtures of the individual egg albumen proteins gelled near the coagulation temperature of the least heat stable protein. Firmest gels were formed from mixtures of lysozyme and globulins and gels with mixtures which did not contain ovalbumin or ovomucoid. The pH of the gels decreased during heating. Gel sulfhydryl contents were consistently lower than in the starting solution.

Prolonged storage of ovalbumin leads to conversion to a more heat stable form, S-ovalbumin. Differential scanning calorimetry (DSC) was used by Donovan and Mapes (1976) to distinguish between samples of ovalbumin and its S variant at concentrations of 0.7-1.4% wt/v. When samples were adjusted to pH 9, the ovalbumin denaturation temperature was

84.5°C while that of S-ovalbumin was 92.5°C. Freeze-dried preparations stored 12 years (-12°C) and 8 years (2°C) demonstrated only partial conversion to an intermediate ovalbumin. When ovalbumin samples were adjusted to pH's from 2.5 to 7.5, two peak gel rigidity heights were found on each side of the isoelectric point of ovalbumin. Maximum heights were less for the S-ovalbumin than the ovalbumin. Increased S-ovalbumin content was previously found by Egelandsdal (1980) to reduce gel rigidity.

The cross links in thermocoagulated ovalbumin gels treated with 6 M urea, were found to be disulfide bridges (Van Kleef et al., 1978). Gel mechanical properties were approximately that of an ideal rubber. Using the classical theory of rubber elasticity, the number of cross-links per ovalbumin molecule were calculated and agreed with the number of SH groups found in ovalbumin (Fothergill and Fothergill, 1970). Van Kleef et al. (1978), thus, concluded that polymer physics could be used to relate the chemical structure of ovalbumin to physical properties of the gel.

Conalbumin. The heat sensitivity of conalbumin was discovered by Osborne and Campbell in 1900. Iron bound conalbumin was found to be more heat stable than metal free conalbumin (Azari and Feeney, 1958). Conalbumin stability at acid pH's was lower than at neutral pH's due to the dissociation of metal from the conalbumin metal complex (Kline et al., 1953). Stability of conalbumin in alkaline

pH regions was demonstrated by Seideman et al. (1963). Electrophoretic mobility of heat denatured conalbumin was altered at pH 7 to 7.5, but not at pH 8.5. As concentrations were increased from 3 X 10^{-4} to 32 X 10^{-4} M Fe, conalbumin became more stable (Cunningham and Lineweaver, 1965). Numerous cations were evaluated for their stabilizing effect on conalbumin in heat pasteurization. Best results were obtained with Fe^{+3}, Cu^{+2}, and Al^{+3}, at pH 7. The effect of Al^{+3} was most interesting because unlike the other cations, it did not result in any off color development and it is biologically inert. Cake volume and texture were improved by adding Al^{+3} to egg white prior to pasteurization. Though whipping time increased, addition of triethyl citrate effectively helped to alleviate the problem.

As early as 1937, a patent was issued involving use of sodium dodecyl sulfate (SDS) as an additive to egg albumen to increase protein heat stability (Vadhera and Nath, 1973). Much later, a study was conducted by Hegg et al. (1978) which involved addition of SDS to solutions of conalbumin to test its effect on protein thermal precipitation (Hegg et al., 1978). Addition of SDS to conalbumin solutions protected against protein precipitation at pH's from 6 to 8. Variations in heating rates allowed for precipitation of conalbumin to occur at higher temperatures than those required for completion of denaturation (as determined by DSC).

Johnson and Zabik (1981) demonstrated conalbumin gel formation in a custard model system with ionic strengths and salts types added equivalent to those found in milk. However, Hegg (1982) detected no gelation in salt free solutions.

Researchers Hirose, Oe and Doi from the Institute for Food Science at Kyoto University, Japan, have conducted several studies on low temperature protein gelation resulting from thiol addition. Since conalbumin contains 15 disulfide linkages (Williams et al., 1982) it was proposed that thiol-dependent gelation correlated to the cleavage of disulfide linkages. A thiol agent, 2-mercaptoethanol (2-ME), was added to conalbumin,(pH 8, 1.6%) and incubated at 35°C (Oe et al., 1986). SS bonds were broken which resulted in an increased SH content, along with a concurrent increase in hydrophobicity. Circular dichroism results showed that cleavage of SS bonds occurred. The tertiary structure was altered though the secondary structure did not change. Hirose et al. (1986) suggested that most of the nine SS bonds which are cleaved by 2-ME in the absence of iron, are located near ion-binding sites and are important for conalbumin gelation. These investigators theorized that in gelation the first step involves conformational changes caused by cleavage of the SS bonds. Hydrophobic groups are exposed as this change in conformation occurs, causing polymerization through intermolecular hydrophobic interaction of the protein molecules (Oe et al., 1986).

Oe et al. (1987) examined the effect of pH and salt type on thiol (2-ME) dependent conalbumin (1.6%) gelation occuring when samples were heated at 35°C for 24 hours. Greatest gel hardness resulted in samples with a pH of 8.0 and 8.8, in sodium phosphate buffer and tris-HCl buffer, respectively. In general, as the heating temperature increased up to 40 or 50°C, for samples in phosphate and tris-HCl, respectively, the gel strength increased. As ionic strength increased, the gel hardness increased, with di-and tri-valent anions having greater effect on gel strength than monovalent anions. It was suggested that anions bind at the iron binding sites and may cause retardation of the SS cleavage in conalbumin. Also, it has been found that denaturation should proceed faster than aggregation to form optimum gels (Hermansson, 1978; Gossett et al, 1984).

Lysozyme. Lysozyme has demonstrated great enzymatic heat stability in the acidic pH ranges (Meyer et al., 1936; Smolelis and Hartsell, 1952). Lysozyme loss of solubility was examined by Beychok and Warner (1959). Results showed maximum stability when solutions were heated from 85 to 95°C and adjusted to pH 5.5. A rapid decrease in stability occurred at higher pH's.

Ten percent of the lysozyme in egg white was inactivated at pH 7, when heated to 62.5°C, while 95% was destroyed pH 9 (Cunningham and Lineweaver, 1965). No

lysozyme inactivation occurred when samples were heated to 62.5°C in phosphate buffer, pH 9. However, 10% was lost when the temperature was increased to 65°C. Lysozyme was found to be 50 times more sensitive in egg white than in phosphate buffer. No explanation was given for this effect.

Lysozyme gels were prepared by Johnson and Zabik (1981) in a custard model system containing the equivalent salts and ionic strengths of milk. No lysozyme (4.4%) gelation was observed by Hegg (1982) in a salt free solution. Gelation of 5% lysozyme solutions heated at 80°C was evaluated by Hayakawa and Nakamura (1986) at varying pH's, ionic strength and concentrations of dithiothreitol (DTT). Maximum breaking strength was found when solutions containing 50 mM NaCl and 15 mM DTT were adjusted to pH 7.2 or 7.8, and held for 15.8 minutes. When no DTT was added no lysozyme gel was observed, agreeing with the earlier results of Hegg (1962). Hayakawa and Nakamura (1986) suggested that external contamination or artificial reduction of the SS bonds may have caused the lysozyme gelation in the study conducted by Johnson and Zabik (1981). Small amounts of salt were necessary for gel formation while excessive levels inhibited gelation due to interference with hydrophobic interaction of proteins and protein-ligand binding in the system (Damodaran and Kinsella, 1981; Melander and Horvath, 1977).

Lysozyme has the ability to combine with other proteins to form complexes. Aggregation between lysozyme and heat-

denatured ovalbumin was examined by Matsudomi et al. (1987). Increasing amounts of lysozyme were added to 0.1% ovalbumin in phosphate buffer, at pH 7.6, and 0.1 ionic strength. Samples were heated to 75°C. The molar ratio of lysozyme to ovalbumin was 1.5 in the resultant aggregates and thought to be caused by electrostatic interaction. Matsudomi et al. (1987) found that addition of NaCl inhibited aggregation and sulfhydryl bonding was not involved in the aggregation reaction. Lysozyme readily interacted with the unfolded ovalbumin in the monomeric and polymerized oligomeric states to form insoluble aggregates. They concluded that thermocoagulation of egg white was facilitated by protein interactions such as that between lysozyme and ovalbumin.

Globulins. Very little information has been presented on the heat sensitivity of the G2 and G3 globulin fractions. Cunningham and Lineweaver (1965) concluded that the globulins were heat stable at pH 6 after heating for 60 minutes at 58-61°C. Johnson and Zabik (1986) examined the thermocoagulation of the globulin proteins, finding that the coagulation temperature was 72°C. Gel strength of globulins was second only to lysozyme and greater than ovalbumin and conalbumin, at pH 8, 2.6% protein.

Methods of Gelation Evaluation

Physical methods have often been used to evaluate protein gel character. Two major types of physical tests

used to evaluate gel characteristics are small-strain testing and structural breakdown testing (Hamann, 1978). Small-strain testing of gels is a nondestructive method to produce continuous or intermittent rheological data using a specific time and temperature process.

The purpose of small strain rigidity testing is to monitor physical changes in gels which correlate to molecular changes. Hamann (1987) reviewed the small strain rigidity testing methods and classified them into two modes of monitoring parameters: 1) constant temperature and 2) varying temperature. Changes over time at a constant temperature using numerous samples and temperatures, provide rate constants and activation energies. These correlate with kinetic rate constants for chemical reactions which have been suggested to cause rheological changes. The other means of monitoring the sol-to-gel transformation is temperature scanning with continuous observation of changes. An advantage of this technique is that the temperature program used can approach that used during commercial processing (Hamann, 1987).

The results from structural breakdown testing can correlate to textural properties such as gel strength or deformation. Gel strength can be determined by removing samples from a heating medium at different time intervals and performing compression tests on the gels. While several instruments have been used for this, the Instron universal testing machine (UTM) adapted by Bourne et al. (1966), is

most commonly used for compression testing. A special type of compression test was developed by Szczesniak and associates (Szczesniak, 1963; Szczaeniak, and Hall, 1975), called texture profile analysis (TPA). Instrons or texturometers (General Foods) have been adapted to run TPA tests (Larmond, 1975). Force-distance (deformation) curves obtained were related to textural properties of hardness, springiness, and cohesiveness of egg protein gels (Shimada and Matsushita, 1980a; Hirose et al., 1986; Woodward and Cotterill, 1986).

A puncture test measures the force required to push a probe or punch into a food (Bourne, 1965). A probe can be attached to the Instron UTM and surface yield point of a gel can be used as an indication of hardness (Gossett et al., 1984). Using this technique, the temperature influence on maximum egg white gel strength was shown to vary with pH by Dunkerley and Hayes (1980). Puncture force methods using the Instron UTM or other similar texture measuring instruments have been used by several investigators (Johnson and Zabik, 1981; Hayakawa and Nakai, 1985; Hayakawa and Nakamura, 1986).

Back extrusion is a technique which utilizes a solid rod attached to an Instron (UTM). The rod penetrates into a sample contained in a cup or tube. As the rod penetrates the sample, displaced material moves opposite to the plunger. Protein suspensions (Harper et al., 1978) and gels (Hickson et al.,1980) were evaluated using this technique to

obtain the viscosity index which is a reflection of gel strength, puncture force and apparent elasticity. Back extrusion was used by Holt et al. (1984) to study the effects of temperature, pH and NaCl concentration on egg albumen rheological parameters. Temperature had the greatest effect on viscosity, gel strength, and elasticity.

Steffe and Osorio (1987) expanded the definition of back extrusion to include the use of a hollow rod in a cylindrical container. This minimized material displacement during back extrusion and increased sample surface area. Low cost, speed, and readily available equipment are advantages of back extrusion. Force curves generated by this technique were similar to texture profile curves.

A nondestructive, small-strain testing instrument called a thermal scanning rigidity monitor was developed by Burgarella et al. (1985). Mixtures of egg white, fish proteins and whey protein concentrate were examined. The proteins were found to gel independently from one another. Mixtures had less gel strength together than predicted from their demonstrated gelling ability alone, probably due to a dilution effect.

A capillary extrusion apparatus was fitted to an Instron UTM by Gossett et al. (1983b) to examine the apparent viscosity and yield force of thermocoagulated egg albumen gels. Gel strength increased with rising temperature and pH (7-11).

Measurement of protein solubility is another method

used to monitor thermal gelation of proteins. Solubility is thought to be related to other functional properties and is be used to monitor functionality (Kakalis and Regenstein, 1986). Numerous investigators have used turbidity as a measure of protein stability and an indicator of protein thermal gelation (Cunningham and Cotterill, 1962; Seideman, 1963; Shimada and Matsushita, 1980b, 1981; Kitabateake and Doi, 1985; Hatta et al., 1986; Oe et al., 1986, 1987; Matsudomi, et al., 1987).

Separation of aggregated or coagulated protein by centrifugation has also been used to follow the coagulation process. Hegg et al. (1979) used this technique with ovalbumin and found that the protein demonstrated maximum heat stability between pH 6-10. The pH range of aggregation was extended by addition of calcium and sodium salts. Aggregation and gelation of conalbumin and lysozyme were also evaluated by Hegg (1982). The weight of the aggregated protein was measured and dry matter was calculated using a gravimetric technique.

Electrophoresis can also be used to follow disappearance and appearance of protein bands which are due to alterations in heat treated proteins. Several investigators have used this technique to study thermally induced protein polymerization (Chang et al., 1970; Shimada and Matsushita, 1980a; Matsuda et al., 1981; Ma and Holme, 1982; Nakamura and Matsuda, 1983; Wantanabe et al., 1985).

Change in protein structure resulting from heat

treatment has been followed using several techniques. Denaturation of egg proteins has been followed using DSC. Chemically modifying egg white did not cause extensive unfolding or denaturation of the proteins (Ma and Poste, 1986). Circular dichroism (Matsuda et al.,1981b; Oe et al., 1986) and changes in optical rotation of molecules (Ma and Holme, 1982) have been used to indicate changes in egg albumen protein conformation.

Nuclear magnetic resonance (NMR) has also been used to study changes in gel structure and formation (Lambelet et al., 1988). During network formation the mobility of water changes. This mobility controls proton relaxation times (T_1) and T1 has been determined using NMR techniques. A relation between water proton transverse relaxation data and 1) egg protein viscosity and 2) rheological properties of gels was observed (Lambelet et al., 1988). Protein denaturation, which was accompanied by decreased protein chain mobility and changes in net mobility of bound water molecules, was thought to be responsible (Oakes, 1976). Goldsmith and Toledo (1985) studied egg gelation using NMR and found it useful in monitoring thermophysical transition of proteins. The value T_1 was highly correlated with gel strength.

EVALUATION OF EGG ALBUMEN GEL MICROSTRUCTURE

Scanning electron microscopy (SEM) has been widely used to elucidate the ultrastructure of a wide variety of foods: meats, vegetables and fruits, protein gel and dough systems. The water content of some globular heat-set protein gels, such as egg albumen, soy proteins or serum albumin, is high (> 90%). To view these gel structures using electron microscopy, preparative techniques must be performed with care to minimize artifacts and misinterpretation of ultrastructure (Hermansson and Buchheim, 1981). Davis and Gordon (1984) reviewed microstructural analytical techniques used to characterize protein and carbohydrate gel systems and summarized recent food application examples of SEM.

Several investigators have studied heat-induced protein gel formation by examining gel texture and microstructure. Freeze-drying techniques were used by early investigators to remove water from heat set gels prior to using SEM. Gel strength was shown to be related to egg albumen coagulum ultrastructure of egg albumen coagulum (Beveridge et al., 1980) using a freeze drying technique. Short time, low temperature (78°C, 9 min) heating of albumen resulted in gels of fine grain cellular structure. Increased heating time (40 minutes) increased the coarseness of the gel and cell wall thickness. High temperature gelation (93°C, 40 minutes) produced a gel with some loss of cellular structure and development of condensed protein strands in the coagul-

um. Johnson and Zabik (1981) examined individual egg albumin protein gelation using a custard model system (pH 8). Freeze-drying was used to prepare samples for SEM examination. They found that small cluster sizes appeared to parallel gel firmness.

Thermally induced, highly concentrated (10-35%) ovalbumin gels were prepared, fixed in glutaraldehyde (GA) and freeze dried prior to SEM evaluation (Van Kleef, 1986). Gels prepared in water and adjusted to pH 10, and in 6 M urea, exhibited similar ultrastructure. They had a cellular structure with fibrillar, sheet-like protein interconnected. The rheological characteristics of these gels were also similar. Transmission electron microscopic evaluation of gels using a thin sectioning preparative technique, showed a homogeneous structure made up of polymer networks. Ice crystals formed during the freezing step gave rise to the cellular structure found in the SEM samples. The ovalbumin gels prepared at pH 5 had a granular structure with clumps of aggregated protein. This heterogeneous structure was also shown with transmission electron microscopy (TEM). Similar results were reported by Heertje and van Kleef (1986) when the microstructure and rheology of ovalbumin gels were examined. The same sample preparation was used for both TEM and SEM. Gels prepared at pH 5 produced lower breaking stress and deformation of break than the pH 10 and 6M urea gels. These gels demonstrated network formation of tiny protein filaments when viewed by TEM. When freeze-

drying was used to prepared the specimen for SEM examination, the protein filaments concentrated into dense protein regions and were no longer visible. Ice crystal were thought to cause this structural change which resulted in a cellular structure of the pH 10 samples and ovalbumin gel in 6 M urea. Heertje and van Kleef (1986) found glutaraldehyde effective in fixing the structure of the original gel; glutaraldehyde counteracting any swelling which urea might induce.

Fixation of protein gel structure followed by critical point drying (CPD) is a technique used to avoid structural alterations caused by freezing. Soy protein gels were fixed in 2% OsO_4 dehydrated in ethanol and critical point dried by Hermansson and Buchheim (1981) in order to examine gel ultrastructure using SEM. SEM micrographs of gels prepared using a CPD method showed a more compact coarse gel structure. Another sample was frozen and examined in a sample holder cooled with liquid N_2 in order to test for artifact formation. A coarse gel made up of a network-like distribution of protein was thought to be the result of ice crystal formation. A fine, detailed structure was found by using TEM to examine ultrastructure. Some interesting observations were made by Hermansson and Buchheim (1981) concerning fixation techniques. Fixation with glutaraldehyde prior to freezing did not prevent freeze artifacts. Gels became brittle and greater alteration in microstructure resulted than in unfixed samples. Addition of cryoprotectants such

as glycerol prevented freeze artifacts but caused considerable swelling in gels prepared in water. Also, addition of glutaraldehyde caused some protein aggregation while addition of OsO_4 did not. They concluded that when viewing the ultrastructure of gels containing high water contents, more than one technique should be used to insure correct interpretation of results.

Structures of various types of polyacrylamide, agarose and alginate gels were examined by Colombo and Spath (1981) using SEM. A rapid, chemical dehydration technique and a freeze-drying technique were compared. Freeze dried gels' microstructure exhibited pore sizes which were much larger than predicted. Proteins with smaller sizes than the pores of the freeze-dried gels have been routinely separated on the polyacrylamide gels. It appeared that freezing and sublimation of water enlarged pore size. Chemical dehydration followed by CPD produced greater shrinkage than freeze drying. Also, some apparent structure alteration occurred. Larger pores were found connecting the center and outer borders of gels prepared in this manner.

Preparatory techniques used in the SEM evaluation of egg proteins were evaluated by Woodward and Cotterill (1985). Freeze drying produced artifacts due to ice crystal formation. Gels fixed with glutaraldehyde alone shrank 50% during CPD. Further fixation using osmium tetroxide and urnanyl acetate or thiocarbohydrzide decreased shrinkage. Woodward and Cotterill (1986) studied changes in egg white

gel microstructure resulting from temperature, pH, protein and NaCl concentration. They used glutaraldehyde fixation followed by post fixation with the osmium-tannic acid-uranyl acetate (OTU). Results showed that gel strength corresponded to specific gel structure. Egg white gels at low pH demonstrated poor water binding and gel strength and a correspondingly coarse, open structure. Fine, uniform gel systems had excellent gel strength.

Rheological parameters and ultrastructure of heat set native and modified egg white gels were studied by Montejano, et al. (1984). Fixation using glutaraldehyde, cryofracturing, ethanol dehydration and CPD were used to prepare samples for SEM evaluation. Native egg white produced ultrastructures which were sponge-like networks while chemically modified egg white had microstructures with dense proteins structures surrounded by large open pores. The ultrastructure of thiol induced low temperature conalbumin gels (pH 8) were evaluated by glutaraldehyde fixation, ethanol dehydration and CPD (Oe et al., 1987). Gel structures formed in sodium phosphate buffers were sponge-like, uniform networks with fine strands composed of a number of particles. Gels formed in tris-HCl buffer had coarse networks and thick protein strands. Significantly higher gel strength was found in gels prepared in phosphate buffer. Using TEM to evaluate conalbumin gels in tris-HCl buffer, Oe et al.(1986) found rod-like structures after 20 minutes incubation at 35°C. Gels demonstrating branched aggregates

were produced after incubation for 32 minutes.

Cryo or low temperature scanning electron microscopy (LTSEM) has been used by some investigators to evaluate the ultrastructure of protein gels. Early studies had been done on modified scanning electron microscopes (Davis and Gordon, 1978). More recently cryo systems have become commercially available, equipped with excellent vacuum and temperature controls which minimize frost contamination. The LTSEM technique has used low temperature ($\sim$ -180°C) to freeze and maintain specimens during fracturing, etching ($\sim$-65°C), sample coating and viewing.

Examples of LTSEM use for specimen preparation and ultrastructure observation was presented by Wilson and Robards (1984). Applications to food were studied by Sargent (1988). This technique avoided shrinkage which accompanied freeze drying or CPD sample preparation methods. However, Davis and Gordon (1984) claimed that freezing 10% collagen gels prepared in water, for LTSEM evaluation, produced an irregular gel network not detected by freezing in a cryoprotectant (30% sucrose).

Soybean protein and whey protein concentrate gelation were examined by relating rheological parameters to gel microstructure by Sone et al. (1983). Well-defined network structures were obtained using LTSEM. Sodium sulfate modified whey protein concentrate (WPC) gels exhibited dense microstructure. This correlated with higher elastic modulus (1-2.2 vs 1-1.2 dyne/cm^2 x 10^{-5}) and water holding capacity

(93.1 vs 91.7 %) than the WPC gels in $CaCl_2$.

MATHEMATICAL MODELING OF PROTEIN GELATION

A mathematical model was developed to predict the effect of protein concentration on the rheological parameters of bovine serum albumen gelation (Harper et al., 1978). Gel strength prediction of thermally induced gelation of soy protein isolates and concentrates (Morgan et al., 1989) was accomplished by developing a generalized mathematical model. The model developed by Morgan et al. (1989) predicted the effect of temperature-time history (TTH) on apparent viscosity (Y) for a given shear rate, protein concentration and solution pH. One basis for the model was found in polymer rheolgy. Apparent viscosity (Y) was assumed to be a relative measure of gel strength. Increased apparent viscosity was assumed to occur from increased polymer-polymer interactions which resulted in increased polymer molecular weight as gelation proceeded. Both Harper (1978) and Morgan et al. (1989) assumed that protein denaturation followed a "pseudo" first order reaction. This meant that while the reactions were complex, an assumption of a first order reaction simplified the mathematical model and still accurately predicted the results. In the model, concentration of the reactive polymer species (protein network) remained constant while disappearace of the monomer species (protein) could be predicted when pseudo first-order

polymerization theory was assumed (Morgan et al.,1989). This model was used to predict the heat-induced gel strength of chicken myofibrillar protein (Smith et al. 1988). The effect of concentration, time-temperature history (TTH), and pH on protein gelation was evaluated. Denaturation activation energy of 20 kcal/mole and a reaction threshold temperature of 30°C were calculated. Maximum viscosity occurred at pH 6 for the chicken myofibrillar protein gels.

A modification of the model used by Smith et al. (1988) was used by Lever (1988) to predict heat-induced beef myofibrillar protein gel strength. Lever presented an extensive review of the model development. This model was used to predict gelation and change in water holding capacity as a function of TTH and protein concentration. Denaturation activation energy for this gelation reaction was identical to that found by Smith et al. (1988).

MATERIALS AND METHODS

The experimental procedure is divided into four sections. The first section consists of the fractionation procedures used to isolate ovalbumin and the three proteins whose interactions with ovalbumin were studied. The second section describes methods used to induce gel formation and its subsequent evaluation in addition to proximate analyses of the proteins. Section three contains a description of the mathematical model which was evaluated for application to ovalbumin gelation. Also, techniques utilized to find mathematical parameters capable of predicting ovalbumin gelation under varying experimental conditions are also provided in this section. And finally, the scanning electron microscopic (SEM) procedures used to evaluate the ultrastructure of ovalbumin heat-set gels are presented in the last section.

Protein Isolation and Purification

The chemicals used in this study were ACS reagent grade. SDS-PAGE standards used to check the purity of isolated proteins were low molecular weight standards (10,000-100,000 daltons) obtained from Bio-rad Laboratories (lot 32569).

Source of Eggs

Unfertilized large eggs were obtained from Single Comb White Leghorn hens and delivered to the laboratory from a local farm within approximately 24 hours after laying. Yolks and whites were immediately separated as described in Figure 1.

Egg Albumen Preparation

Upon receiving the eggs, the shells were thoroughly washed under cold running tap water. The shells were then broken, yolks were separated from the whites and the chalaziferous material was removed with tweezers. The separated egg albumen was kept on ice during this step. After 1,500 grams (g) were weighed out (one batch,~50 eggs), whites were poured into a Sears Instablend high speed blender attached to a variable autotransformer (model 3PN-1010) and blended slowly for 1 minute (min) at setting 22 and 30 seconds (s) at setting 30. Aluminum foil was placed onto the surface of the albumen and along the sides of the blender to limit air incorporation during low speed blending. After homogenization, the albumen was dialyzed against deionized, distilled water (DDW) for approximately 72 hours (hr) with frequent changes of water at 2-4°C. Water insoluble protein precipitate was centrifuged out at 10,000 g, for 8 minutes (4°C) in a Damon/IEC B-20A centrifuge, model 3444. This removed the water insoluble proteins which interfered in the globulin isolation. The

protein from the supernatant was used to isolate the egg albumen proteins using the procedures as presented in Figure 1.

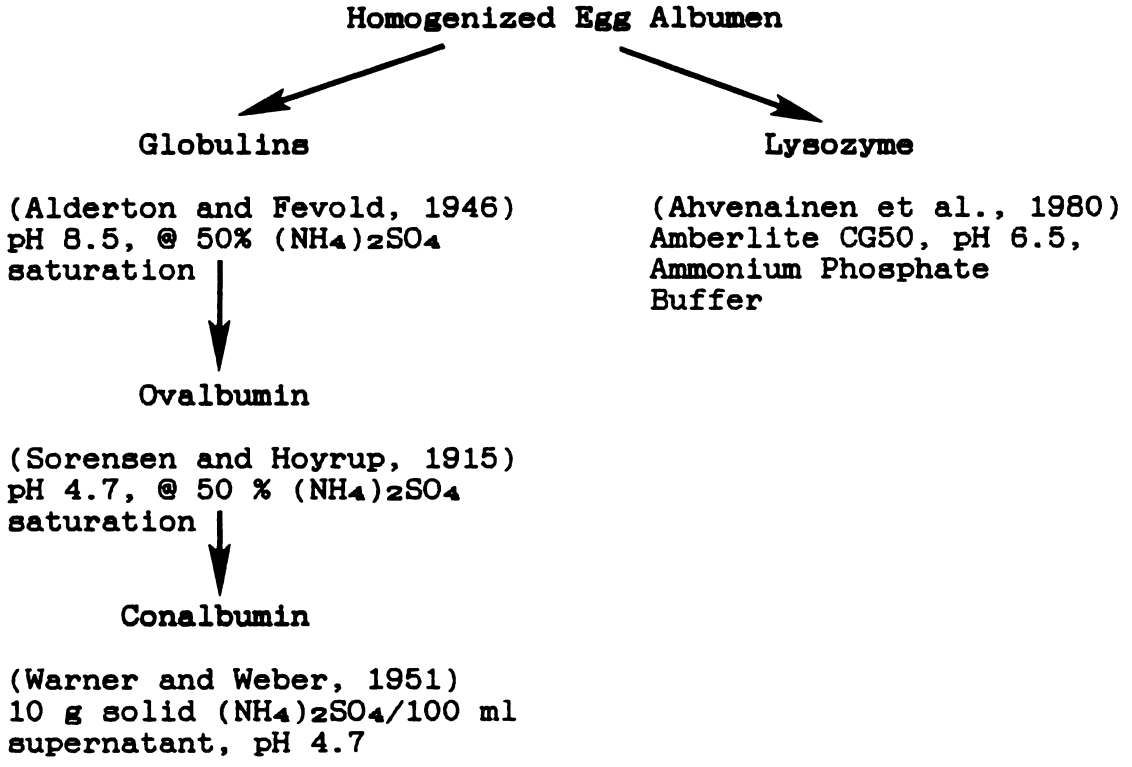


Figure 1. Flow diagram of egg albumen protein isolation.

Several batches (1,500 g albumen) of each protein were prepared and then combined after freeze-drying to generate a sufficient amount of protein to be used in experiments. Globulin, ovalbumin and lysozyme were isolated from the same batch using combinations of pH and ammonium sulfate precipitation separation procedures while only lysozyme was separated from other batches using an ion exchange chromato-

graphic procedure. Unless otherwise noted, all samples were placed on ice or in a cold room (4°C) during isolation or dialysis. The following four sections describe the fractionation procedures in detail.

Globulins

A modification of the procedure described by MacDonnell et al. (1955) was used to isolate the globulin fraction (G2, G3). Approximately 1,500 g of egg albumen were dialyzed against DDW and separated from the water insoluble protein. An equal volume of cold (4°C), saturated ammonium sulfate was added to the supernatant. The pH was adjusted to 8.5 by slowly adding 4 N KOH drop wise with constant stirring. The sample was mixed for 30 min and then centrifuged for 8-10 min, at 1,500 x G, 4°C. The precipitate (ppt) was redissolved in 600 ml DDW and an equal volume of saturated ammonium sulfate was added. The pH was readjusted to 8.5 to reprecipitate the protein. Centrifugation was again performed as previously described. Addition of DDW to the ppt, addition of saturated ammonium sulfate, pH adjustment and subsequent centrifugation was repeated three additional times. After the final centrifugation, the ppt was dissolved in 600 ml DDW and placed into dialysis membrane tubing (3.6 cm diameter) with a molecular weight cut off of 12-15,000. All dialysis tubing used in this study had been treated to remove impurities prior to use according to the procedure of McPhie (1971). Dialysis was carried out in 8-

10 liters of DDW with frequent changes until all ammonium sulfate was removed. This was verified by adding a few drops of barium chloride solution to the dialyzate which remained clear when all the sulfate ions had been removed. Finally, the sample was centrifuged to remove any ppt and the globulin solution was poured into several 8 inch round aluminum pans to a depth of one inch and kept frozen at-20°C until freeze-dried. Supernatant from the first two centrifugations was saved for ovalbumin and conalbumin fractionation.

Ovalbumin

The procedure used to isolate ovalbumin was taken from Sorensen and Hoyrup (1917). Combined supernatant saved from the globulin isolation was adjusted to pH 4.7 by drop wise addition of approximately 3.8 N sulfuric acid with constant stirring. The sample was held at 2-4°C for approximately 3-4 days to assure maximum ovalbumin precipitation from the supernatant. Centrifugation at 15,000 x G, 4°C for 5 min was necessary to separate the ppt out. The ppt was dissolved in 800 ml DDW and an equal volume of saturated ammonium sulfate (4°C) was added to reprecipitate the ovalbumin. The sample was stirred at 4°C for at least 15 hours and then centrifuged for 10 min as previously described. After addition of 800 ml of DDW to the ppt, reprecipitation with saturated ammonium sulfate, centrifugation and subsequent DDW addition to the ppt was

repeated three more times. After the final wash and centrifugation the ovalbumin protein fraction was dissolved in 500 ml DDW. Dialysis and freezing were performed as previously described. Supernatant from the first two centrifugations was saved for subsequent conalbumin fractionation.

Conalbumin

Isolation of conalbumin was based on the procedure of Warner and Weber (1951). One gram solid ammonium sulfate was added per 10 ml of supernatant saved from the ovalbumin isolation. This was mixed for two hours at room temperature and centrifuged at 15,000 x G for 8 min at 4°C. The ppt was dissolved in 600 ml DDW and dialyzed as described for globulin. The sample was then adjusted to pH 6.0 with approximately 4 N KOH and made 0.2 M NaCl by adding the appropriate amount of 1 M NaCl. The mixture was then adjusted to 20% ethanol by addition of 50% ethanol-0.02 M NaCl solution. After storage for 2-5 days at 2-4°C, the ppt was centrifuged out at 20,000 x G for 8 min at -10°C. Care was taken to keep the sample very cold to avoid redissolving of the ppt prior to centrifugation. The resultant ppt was redissolved in 500 ml DDW and the solution was made 20% ethanol-0.2 M NaCl as described previously. The ethanol precipitation procedure was repeated four more times. After the final wash, the solution was dialyzed at 2-4°C as described for the globulin fraction except that a dilute

silver nitrate solution was used to detect presence of chloride ions (white ppt). When no chloride was present, the solution was then dialyzed against a 0.01 M potassium citrate buffer, pH 4.7 for 24 hours (2-4°C) with frequent changes of buffer. Removal of iron from conalbumin was accomplished by using the procedure as described by Warner and Weber (1951). To remove the iron, protein fraction was mixed with Dowex-1, 1x8-50, chloride form. The resin was pretreated by 1) suspension in 10% NaCl, 2) stirring 15 min 3) removal of excess chloride ion with several washings of DDW and 4) a final wash in the citrate buffer. Dowex (10 g) was then added to the conalbumin fraction and stirred 30 min. This mixture was filtered through Whatman #4 filter paper and the resin was then rinsed three times with 10 ml of citrate buffer. The procedure was repeated two more times and dialyzed against DDW for 48 hr at 2-4°C with frequent changes of DDW. The solution was frozen as previously described until it was freeze-dried.

Lysozyme

The procedure of Ahvenainen et al. (1980) was used to isolate lysozyme from egg albumen. A weakly acidic cation exchange resin, Amberlite CG-50 (Sigma lot 124F-0198, wet mesh 100-200) was used to separate lysozyme from egg albumen. Approximately 50 g of CG-50 was allowed to equilibrate overnight in starting buffer, 0.05 M ammonium phosphate, pH 6.5. The fines were then removed and the

resin was added to 1,500 g egg albumen and stirred at room temperature for 30 min. This mixture was then filtered through a coarse filter (16.5 cm milk filter) and then washed extensively with starting buffer (~2 L). The resin was poured into a Pharmacia K-25x100 jacketed column (4°C) and packed. Ammonium phosphate buffer (0.1 M) was pumped through the column at approximately 30 ml per hr until the effluent absorbance at 280 nm (LKB ULTRASPEC II spectrophotometer) leveled off at ~0.200-0.400. Finally, 0.5 M ammonium phosphate buffer, pH 6.5, was pumped through the column to elute lysozyme. Samples were not collected until - 50 ml of buffer had passed through the column. About 7.5 ml effluent were collected in each tube using an ISCO (Instrumentation Specialties Company, model 326) Collector. Tubes from the ascending, peak and descending portion of the eluted protein peak were combined and purity was evaluated using SDS-PAGE (see following section). One protein band with the molecular weight of lysozyme was eluted on the descending protein peak. Therefore, on all subsequent fractionation procedures, these tubes were combined, dialyzed against DDW and frozen as previously described.

Freeze-Drying

The protein solutions which were frozen in 8 inch aluminum pans were covered with Whatman #1 filter paper (27 cm) and cheese cloth prior to freeze-drying. Three pans

were placed in a Virtis Unitrap II freeze-drier with a capacity of eight liters, and equipped with a clear drum (43.2 cm high x 30.5 cm). Drying was carried out at 4-6 x 10^{-2} Torr with trays at approximately 40-50°C for 48 hours. Samples were immediately placed in a desiccator until transferred and sealed into glass or plastic storage bottles and stored at -20°C.

Electrophoresis

Selected individual batches of purified protein and pooled batches were tested for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the modified procedure of Laemmli (1970). Stock solutions used were as follows 1) a stock gel solution of 37.5:1:150 acrylamide:N,N'-methylenebisacrylamide (BIS): H_2O wt/wt, 2) 1.5 M tris-hydrochloric acid buffer, pH 8.8, 3) 0.2 M ethylenedinitrilo tetraacetic acid disodium salt (EDTA) solution and 4) 10% SDS. Solutions 1-3 were stored at 4°C and solution 4 was stored at room temperature. Sample buffer consisted of 1% SDS, 0.05 M Tris-HCl, pH 6.72 and 0.05% mercaptoethanol and 0.001% bromophenol blue containing 10 ml of saturated sucrose solution (wt/v). This mixture was stored at -20°C in a plastic bottle.

The electrophoresis equipment used consisted of a Buchler Polyanalist, model 3-1750, connected to a Bio-rad power supply (model 500/200). Glass tubes (10 cm long x 0.5 cm I.D. x 0.8 cm O.D.) were soaked overnight in saturated

KOH in ethanol. Tubes were thoroughly rinsed in tap water, DDW and finally in a dilute photoflow solution (1/2000), drained and dried.

Gels (7.75% acrylamide) were prepared by combining 10 ml stock gel, 3 ml tris-HCl buffer, 0.3 ml each of the SDS and EDTA solutions, 11.3 ml of DDW, and 5% ammonium persulfate solution, freshly prepared. This mixture was degassed for approximately 5 min and 0.015 ml TEMED was added immediately prior to pipetting into the tubes to a 7 cm level. DDW was gently layered onto the surface of the gels and polymerization was allowed to take place within 30 min. Gels were covered with Parafilm and held at room temperature for 16-18 hr.

Chamber buffer consisted of 0.05 M tris-0.38 M glycine, pH 8.41, containing 10 ml of 10% SDS and 0.2 M EDTA. Approximately 3.0 mg of protein was placed into glass test tubes, dissolved in 2.5 ml of sample buffer (~1.2 mg/ml) and placed in a boiling water bath for 10 min. Samples were cooled on ice and approximately 10 µl were carefully placed with a microsyringe onto the surface of gels which were in the electrophoresis chamber submerged under chamber buffer. The lower buffer chamber was then filled two thirds full with chamber buffer and electrophoresis was run at 1 mA/tube for 3-4 hrs or until the tracking dye reached the bottom of the tube. Gels were removed by forcing water between the gel and the tube wall with a syringe while rotating the tube. Tracking dye was marked with a needle dipped in black

ink. Tubes were placed in 10% TCA for up to 1 hr and staining was accomplished by stirring in 0.03% wt/v coomassie brilliant blue (R-250) in 50% methanol and 7% acetic acid for 16-18 hr. Destaining was accomplished by stirring the gels in an electrophoresis diffusion destainer (Bio-rad) containing 5% methanol-7.5% acetic acid for 36-72 hr until protein bands were clearly visible. Protein purity was assessed using a densitometer (Shimadzu Dual-Wavelength Thin-Layer Chromato Scanner Model C5-930).

Methods of Analyses

Moisture

Moisture content of egg albumen used to fractionate the different proteins was determined using the AOAC method 17.006 (1984) with the exception that approximately two grams of liquid egg white were weighed out in duplicate. Samples were placed into a Hotpack vacuum oven, model 633, at 100°C and a vacuum of 26-28 inches Hg and dried to constant weight. The moisture percentage was calculated using the following equation:

$$\frac{\text{initial weight - final weight}}{\text{initial weight}} \times 100$$

Total Nitrogen

A micro-Kjeldahl procedure (AOAC, 1984, 17.008) was followed to determine total nitrogen. A Buchii automated nitrogen analyzer 322/342 equipped with a Epson HX-20

minicomputer was used. Approximately 0.5 g was taken from each batch of egg albumen which was used to prepare individual proteins. This amount was accurately weighed into a 100 ml straight sided digestion tube. Samples were run in triplicate. To each sample, 8 ml concentrated sulfuric acid and one Kjeltab MT catalyst tablet (Fisher) containing 3.5 g K_2SO_4/3.5 mg Se, were added. Digestion was started in a 1016 Digester (Tecator) at low heat (setting 1) and the settings were increased one setting per 15 min interval until samples boiled consistently without danger of sample loss. At this point the temperature was turned on high and heating continued until all samples were clear (@ 2.5-3 hr total heating time). The flasks were allowed to cool in the digestion device when digestion was completed. Cooled flasks were transferred to the automated nitrogen analyzer which had been prepared and preheated as suggested in the equipment manual. The unit controls were set at 1) 1.9 for DDW, 2) 2.1 for 30% NaOH and 3) 1.0 for distillation time. Sixty ml of 4% boric acid were added to the receiving vessel. Beginning pH was recorded and entered as the titration endpoint on the automatic titration device (Impulsomat/614). Water (DDW) and 30% NaOH were automatically added and distillation and titration were then completed automatically. The volume of 0.0963 N HCL added was displayed on the Dosimat/655. The following

calculations to determine percent nitrogen were performed:

$$\frac{(\text{sample-blank})\text{ml HCl} \times \text{normality HCL} \times 14\text{mg/mmole} \times 100}{\text{sample weight (mg)}}$$

A factor of 6.25 was used to convert the total nitrogen to percent protein.

Protein Thermal Coagulation

In this study, the proteins which were heat-set were one of the following or a combination of the following: ovalbumin, globulins, conalbumin (iron free) or lysozyme. Freeze-dried protein was allowed to equilibrate to room temperature, quickly ground into a fine powder and weighed. A small portion of 0.1 M phosphate buffer was added to make a paste with the protein which allowed for easier solubilization. When the effect of 3% NaCl (wt/v) or 20 mM N-ethylmaleimide (NEM) on ovalbumin gelation was evaluated, they were added to the buffer at this step. It was necessary to add NEM into heated phosphate buffer and to stir until it dissolved. For all samples, half the total volume of the buffered solution was then added as 0.1 M phosphate and any necessary adjustment to the sample pH was done by adding small amounts of 0.1 M NaOH or HCl. DDW was added to obtain a final concentration of 0.05 M phosphate. Gelation was evaluated in 0.05 M phosphate buffer, pH 6 or 7, and at concentrations 3, 5, or 7% wt/v. Samples were pipetted (4 ml) in 16/100 mm pyrex screw top tubes and

preheated at 50°C for 5 minutes in a circulating water bath, Exacal-EX 1000 (Neslab). Tubes were then processed at constant temperatures of 85, 90 or 95°C in a second circulating water bath. This bath was comprised of a heat and circulating control from a Haake D3 circulator placed into a styrofoam container, 19.5 x 25.5 x 18 cm deep lined with 2-3 layers of polyethylene. This allowed for the high temperature processing conditions to be accurately maintained without covering the bath. The total number of test tubes was kept constant so that any initial bath temperature reduction would be identical for every experiment. Blanks containing 4 ml of water were used when a smaller number of samples were needed. A constant level of water was kept (9,500 ml) for each experiment and water temperature was monitored with a thermocouple attached to a Honeywell Brown Electronik (potentiometer). During processing, the center of one sample's temperature was monitored with a thermocouple inserted into the center of the tube. Samples were removed in triplicate at varying intervals (30-3600 s) and quickly cooled in a ice-water bath for 5 minutes.

Gel Apparent Viscosity Determination

A description of gels for which apparent viscosity (gel strength) was performed is presented in Table 4 and 5. These samples were used to determine the basic model parameters using ovalbumin and to evaluate the effect of pH,

added chemicals, and isolated albumen protein on model parameters. After thermal gelation, all samples were held at 22°C for not more than two hours prior to examination of gel strength using the modified back extrusion apparent viscosity method of Harper et al. (1978). A flat bottomed stainless steel plunger (23 cm long with 0.733 cm diameter) was forced through the gel using an Instron Universal Testing Machine (model 4202, Canton, OH). The Instron was equipped with a 50 N (5 kg) load cell in conjunction with a microcomputer (Hewlett Packard 86B) and operated at 20 mm/min cross head speed and 25 mm travel distance.

Table 4. Experimental design for basic mathematical model experiments using normalized apparent viscosity data.[1]

Protein	Temperature° (°C)	Time (sec)	Concentration (% w/v)	pH
Ovalbumin	85	0-3600	3, 5, 7	6
	90	"	3, 5, 7	"
	95	"	3, 5, 7	"

[1]Apparent viscosity determinations were done in triplicate, using at least two replications.

Apparent viscosity (viscosity index) was calculated using a modification of the procedure of Hickson et al. (1982). Apparent viscosity was considered a measure of gel strength. A program was developed (Lever, 1988) to perform these calculations given the Instron operating variables as

Table 5. Experimental design for mathematical model experiments based on selected variables and using normalized apparent viscosity data for data collected at 0-3600 seconds.[1]

Protein	Temperature (°C)	Concentration (% w/v)	pH	Additive
		Effect of pH		
Ovalbumin	85	5	7	
	90	3,5	"	
		Effect of Additive		
Ovalbumin	85	5	6	NEM[2]
	90	3,5	"	
	85	5	"	3% NaCl
	90	3,5	"	
		Effect of Protein		
Globulins	85	5	6	
	90	3,5	"	
Lysozyme	85	5	"	
	90	3,5	"	
Conalbumin	85	5	"	
	90	3,5	"	
G/O[3]	85	5	"	
	90	3,5	"	
L/O[4]	85	5	"	
	90	3,5	"	
C/O[5]	85	5	"	
	90	3,5		

[1]Apparent viscosity determinations were done in triplicate, at least two replications
[2]N-ethylmaleimide, 20 mM
[3]globulin/ovalbumin, 1:1
[4]lysozyme/ovalbumin, 1:1
[5]conalbumin/ovalbumin, 1:1

listed previously. Distance (mm) and force (N) were read every 300 milliseconds by the program and these data were used to calculate the back-extrusion apparent viscosity. Area under the curve formed from the distance-force data were converted into a right triangle containing equivalent area and travel distance (Lp) to the original curve. The height of the triangle was then equivalent to the maximum or peak force (Fp) found by : Fp = 2 Area/Lp. The ratio of Fp to Lp times a constant (Hickson, 1982) was determined to be the apparent viscosity of the gel sample. All variables were replicated at least twice.

Sulfhydryl Groups

A description of the ovalbumin samples evaluated for free sulfhydryl (SH) and total disulfide sulfhydryl (SH-SS) content is presented in Table 6. Free sulfhydryl groups were determined by a modification of the method described by Ellman (1959). Approximately 3-5 mg of freeze-dried protein were dissolved in 5 ml 0.01 M sodium phosphate buffer, pH 8.0 containing 2% SDS and 0.4% EDTA (wt/v). Samples were boiled for 30 min and cooled to room temperature. To this solution 0.2 ml of a freshly prepared 0.01 M 5,5′- dithiobis -2- nitrobenzoic acid (DTNB) solution in 0.1 M sodium phosphate buffer, pH 7.0 was added. Color was allowed to develop for 1 hr and then the samples were filtered through Whatman #4 filter paper. Final volume for the assay was 6.2 ml and absorbance was measured at 412 nm in a LKB-Ultraspec

II. Two blanks were run parallel to samples as references. Concentrations of SH groups were calculated using the following formula:

$$\text{moles SH/ g protein} = \frac{\text{(sample-blank) absorbance x final volume}}{\text{extinction coefficient (13,600) x mg sample}} \times 10^6$$

Table 6 . Experimental design for total sulfhydryl-disulfide sulfhydryl and free sulfhydryl determination of ovalbumin gels heat-set for 0, 300 and 3,600 seconds.[1]

Temperature (°C)	Concentration (% w/v)	pH	Additive
85, 90, 95	5	6	
90	3, 5, 7	6	
90	5	7	
90	5	6	NEM[2]
90	5	6	3% NaCl

[1]Determinations were run in duplicate for each of two replications.
[2]N-ethymaleimide, 20 mM

Total Sulfhydryl - Disulfides

A modification of the method of Cavallini et al. (1966) was used to determine total SH-SS. Approximately 1.5 mg of freeze-dried sample was accurately weighed and dissolved in 1.0 ml of 0.05 M sodium phosphate buffer pH 7.4 containing 0.02 M EDTA. To this, 2 ml 1-octanol and 1 ml of freshly

prepared urea-sodium borohydride solution (10 g urea, 0.25 g $NaBH_4$ dissolved in 10 ml DDW) were added. Tubes were vortexed and incubated in a water bath at 40°C for 30 min. After cooling samples at room temperature (ca 10 min), 0.5 ml potassium phosphate-HCl buffer, pH 2.7 was added drop wise, wetting the walls of the tube to destroy all traces of $NaBH_4$. Acetone (1 ml) was added and mixed for 5 min to complete the $NaBH_4$ destruction. To this solution 0.2 ml of a freshly prepared 0.01 M DTNB in 0.1 M sodium phosphate buffer, pH 7.0 was added and mixed. Thirty minutes were allowed for color development. The solution was then filtered through Whatman #1 filter paper. Absorbance at 412 nm was read after diluting the sample with 4 ml DDW. Total volume for the assay was 9.7 ml and total SH-SS were calculated as follows:

$$\text{moles SH/ g protein} \times 10^5 = \frac{\text{(sample-blank) absorbance} \times \text{final volume}}{\text{extinction coefficient (13,600)} \times \text{mg sample}}$$

Water Holding Capacity

The water holding capacity (WHC) of ovalbumin gel was determined using a modification of the procedure described by Yasuda et al. (1986). A description of the samples on which WHC was determined is presented in Table 7. Two ml of 5% protein solution were pipetted into polyvinylchloride tubes with tops and then heated and cooled as described

previously. Samples were centrifuged at 10,000 x G for 15 min at 15°C. Tubes were inverted, liquid was decanted off and the insides of the tubes were wiped dry. Water holding capacity was calculated as :

$$\frac{\text{Weight of aggregate}}{\text{Total weight of unheated protein solution}} \times 100$$

Samples were run in duplicate and the determination was replicated twice.

Table 7. Experimental design for water holding capacity determinations of ovalbumin gels heated for 0-3,600 seconds.[1]

Temperature (°C)	Concentration (% w/v)	pH
85	3, 5, 7	6, 7
90	"	"
95	"	"

[1]Determinations were run in duplicate for each of two replications.

Statistical Analysis

Evaluation of apparent viscosity and determination of the mathematical model parameters required data analyses using linear and nonlinear regression. Linear regression

analysis was performed by the integrated graph-statistical program Plotit (Eisensmith, 1985) using the least squares method. The same program was used to perform nonlinear regression with the algorithm for least squares estimation of non-linear parameters developed by Marquardt (1963). Statistical analyses of water holding capacity, free SH, and total SH-SS data were performed using the MSTAT program (1985, version 3.01): Analysis of Variance and Tukey's Honestly Significant Difference Test.

Mathematical Model Development

A primary purpose of this study was to apply the mathematical model developed by Morgan et al. (1989) to ovalbumin thermal gelation in the absence of shear. Many theories and mathematical relationships have been developed for predicting plastic and similar material polymerization (Ferry, 1970; Shah and Darby, 1976). The mathematical model of Morgan et al. (1989) incorporated polymer melt rheology as a means of approximating viscometric behavior of denaturing proteins. However, drawing analogies between plastic polymers and protein relationships does pose some problems. Polymers undergo reversible melting and irreversible polymerization reactions during thermoplastic extrusion. Certain proteins can undergo irreversible denaturation with exposure to varying degrees of physical and or chemical treatments. Protein thermal denaturation or

gelation can occur with network entanglement and possible cross-bonding. While first or second order reactions are assumed for plastic monomer and polymers, heat-induced gelation of proteins such as ovalbumin is considered more complex. The mathematical model (Morgan et al. 1989) utilized in this study was based upon the assumption of "pseudo" first order protein denaturation (Harper et al., 1978). This assumes that the concentration of the polymer remains constant and the disappearance of the monomer can be predicted. While protein denaturation is more complex than a first-order reaction, a simple reaction can approximate the effects of denaturation on "average-overall-viscosity." Thus, the outcome of the thermal effect on the protein can be predicted. Apparent viscosity was assumed to be a relative measure of the strength of gels formed by heat driven denaturation and subsequent cooling (Hickson et al., 1980).

Temperature-Time History

Variable temperature-time histories were incorporated into the model to account for temperature increases from initial to final sample temperature over time for experimental or commercial processes. Based on the original work of Eyring and Stearn (1939), Morgan (1979) developed

the TTH function to account for this effect:

$$TTH = \int_0^t T(t)\, e^{\frac{-\Delta Ea}{RT(t)}} dt \qquad (1)$$

where E_a = activation energy of denaturation , R = the universal gas constant, and T(t) is the temperature-time profile above a minimum threshold temperature (T_d) at which denaturation occurs. (A list and description of terms for all equations is presented in Table A, Appendix). As T_d is exceeded, the protein molecules denature demonstrating changes in their size, shape and molecular weight. In proteins capable of thermal gelation, increases in apparent viscosity accompany these molecular changes caused by denaturation.

Temperature-time history (TTH) was calculated with a Hewlett Packard 86B microcomputer using a program developed by Lever (1988). The procedure was based on the transient heat transfer model for infinite cylinders (Parker et al., 1970) as presented by Smith et al. (1987). This model predicted temperature-time data for ten different radial positions within a test tube cross section using the initial, center and process temperature for each experiment. Ten equal area concentric rings within the cross section were selected based upon preselected radial positions. These temperature-time data were used to calculate TTH (Eq. 1) for each ring followed by calculation of a mass average

(overall) of all the ring TTH values. This technique was shown by Morgan (1979) to provide greater accuracy in accounting for variation in reaction rates within a sample due to temperature-time profiles than using center temperature-time data or mass average temperature-time data.

Temperature-time data were used to calculate TTH from the 5% ovalbumin gelation at 85, 90 and 95°C for the basic model samples described in Table 4. These TTH values were used for all remaining samples since heating conditions remained constant for all samples tested.

Activation Energy of Denaturation/Gelation.

Activation energy of denaturation/gelation, E_a, was initially determined using normalized apparent viscosity and TTH values for 5% ovalbumin at 85, 90 and 95°C. Samples described in Table 4 were referred to as the basic model. They were chosen to thoroughly determine E_a, and model parameters over a larger temperature and concentration range than was possible for all variables. The amount of time necessary to obtain adequate amounts of purified protein limited the number of variables studied. Therefore, the sample conditions as presented in Table 5 were used to study all variables other than the basic model. For these samples E_a's were determined using 85 and 90°C apparent viscosity (Y') data.

Ea was first estimated using Arrhenius kinetic theory. This predicts the reaction rate constant for a given

activation energy (E_a), temperature and time using the following relationship:

$$ki = k^{-E_a/RT} \quad (2)$$

for a selected time condition. Ea is determined by taking the natural log (ln) and rearrangement of both terms, and plotting the inverse of temperature (K) versus ln of time. The slope of the line was calculated by $-E_a$ divided by the gas constant (R = 1.987 cal/mole°K). Since apparent viscosity values were assumed to be a measure of the extent of ovalbumin gelation reaction, times to attain the same three normalized apparent viscosity values (Y′) at 85, 90 and 95°C heating temperatures were plotted. The Y′ values were arbitrarily chosen from the linear portion of the curve.

A second technique used to calculate E_a was based on the assumption that the normalized viscosity ratio Y′ (Eq. 3) is a function of temperature-time history (TTH) (Morgan, 1979). Use of TTH corrected for differences in sample temperature that occurred when the sample was placed into a constant temperature water bath until the sample reached the processing temperature. Combined apparent viscosity data versus calculated TTH for all three temperatures were plotted. Based on the estimated E_a, different E_a values were used in an iterative procedure to produce TTH values which when plotted versus Y′ values produced a maximum R^2. If the selected E_a was incorrect, a separate curve for each

temperature process was produced. This was indicated by a low R^2 value which resulted when the data for all temperatures were combined and plotted.

Generalized Model

Sample viscosities were measured via a modified back extrusion procedure (Harper, 1978; Hickson, 1982), under constant temperature and shear rate. Shear history during gelation was zero. Applying these conditions to the model of Morgan et al. (1989), the normalized viscosity function Y' was given by :

$$Y' = \frac{N - N_0}{N_0} = \frac{B[M_m M_c^o]^{\alpha}}{P_c} \cdot \left(1 - e^{-aTTH}\right)^{\alpha} \qquad (3)$$

Where B = a constant, M_m = molecular weight of the monomer, M_c = initial monomer concentration, P_c = reactive polymer species concentration, and TTH is defined in equation (1). The term "a" is related to the reaction rate constant for protein denaturation and cross-linking while alpha is a relative measure of molecular entanglement during viscometric shear. The terms "a" and alpha are two of the four parameters determined in this study to predict protein gelation. According to Morgan et al. (1989), it is not practical to quantify M_m, M_c and P_c for food proteins. But the ratio M_c/P_c, may be considered the "effective

concentration" (C) of the undenatured protein (monomer) (dry weight basis). Then Eq (3) becomes

$$Y' = B(M_m)C^{\alpha} \cdot (1 - e^{-aTTH})^{\alpha} \quad (4)$$

To simplify Eq.(4), A´ was defined as A´ = B(Mm) yielding:

$$Y' = A'C^{\alpha} \cdot (1 - e^{-aTTH})^{\alpha} \quad (5)$$

The term A´ is a measure of the effective molecular weight of the unfolded protein macromolecules (polymerized gel) (Lever, 1988; Morgan et al., 1989) and is the third parameter used in this study to predict protein gelation.

The fourth parameter is maximum normalized apparent viscosity, Y'_{∞}. This is calculated by assuming that an infinite heating time produces Y'_{∞} which allows for simplification of the mathematical model. It is the Y'_{∞} parameter which is assumed to correlate with the functional characteristic of sample gel strength. Further explanation of this parameter is found in the discussion section.

Determination of Model Parameters (a) and Alpha

Determinations for ovalbumin thermal gelation (a)-alpha parameters utilized 85, 90 and 95°C apparent viscosity data of the basic model variables (Table 4). For the other

variables, (Table 5), 90°C Y′ data were used to calculate these parameters. This temperature was used since apparent viscosity data were collected for two concentrations at 90°C.

The model parameter (a) employed in this study is related to the reaction rate constant for protein denaturation and cross-linking as presented by Lever (1988) and is identical to the k term in the generalized model used by Smith et al. (1988) to evaluate heat-induced chicken myofibrillar protein gel strength. In order to calculate (a), an iteration process was applied. Lever (1988) developed a computer routine to calculate the correct value of (a) using several values of Y′ and TTH based upon the following relationships:

$$\frac{Y_1'}{Y_2'} = \frac{B'(1 - e^{-aTTH_1})^{\alpha}}{B'(1 - e^{-aTTH_2})^{\alpha}} \qquad (6)$$

Equation 6 could also be written as:

$$\frac{Y_1'}{Y_2'} - \frac{B'(1 - e^{-aTTH_1})^{\alpha}}{B'(1 - e^{-aTTH_2})^{\alpha}} = M \qquad (7)$$

where M = 0 when the iterated value of (a) was correct. Y_1 and Y_2 are normalized apparent viscosity values at two thermal treatments (TTH), and B is the estimated parameter for $A'C^{\alpha}$ using the Plotit nonlinear regression algorithm (Marquardt, 1963).

Determination of Model Parameter A´

The A´ parameter is a measure of the effective molecular weight of the unfolded protein (polymerized gel) (Morgan et al., 1989). In order to determine the A´ parameter, a special case was considered where an infinite heating time produces a maximum normalized apparent viscosity (Y'_{∞}) (gel strength). This allowed the second term of the model to become one and Y'_{∞}, equal to the first term of the mathematical model $A'C^{\alpha}$:

$$Y' = A'C^{\alpha} \qquad (8)$$

where C = % protein (dry basis). The dry weight basis (dwb) protein contents corresponding to 3, 5 and 7% were used in the calculations and were as follows: 79, 86 and 89%, respectively. However, to simplify discussion, protein concentration will be referred to as wet weight basis (wwb).

Scanning Electron Microscopic Evaluation of Protein Gels

Two scanning electron microscopic (SEM) techniques were used in this study to examine the ultrastructure of protein gels. The first was the osmium-thiocarbohydrazide-osmium (OTO) postfixation technique followed by ethanol dehydration and critical point drying (CPD) utilized by Woodward and Cotterill (1985). The second method was a low temperature

SEM (LTSEM) technique which involved examination of fresh samples at very low temperatures and high vacuum.

All protein solutions were adjusted to the desired pH, prepared and heat processed at 85°C for 60 min as previously described.

OTO/CPD Method

After cooling, gelled samples were cut (less than 5mm^2) and fixed in 2% glutaraldehyde, 0.1 M sodium phosphate buffer, pH 7.0 for 4 hours, 22°C and held overnight at 4°C. Specimens were washed several times with distilled water between changes of each fixative. Postfixation (Woodward and Cotterill, 1985) was in 1% OsO_4, for 2 hrs, at 22°C followed by immersion in 1% thiocarbohydrazide for 30 min and then 1% OsO4 for 1 hour. After ethanol dehydration, samples were critical point dried (Balzers, model CPD010), mounted on aluminum stubs with silver paint and gold sputter coated using a EM Scope SC Sputtercoater for 8 min. Specimens were viewed on a JEOL JSM-35C scanning electron microscope, 15 kV, at a working distance of 15 mm, with the condenser lens set at 600.

Low Temperature Scanning Electron Microscopy (LTSEM)

Egg protein samples were evaluated on a EMscope SP-2000 Sputter-Cryo system used in conjunction with a JEOL JSM-35C SEM. A small fresh sample (less than 5 mm^2) was mounted on a copper stub and submerged into frozen nitrogen slush

(-196°C). The temperature was maintained at -190 to -160°C by liquid nitrogen and held under vacuum for the entire process unless otherwise noted. The sample was then transferred to the preparation chamber (< -160°C) and carefully fractured using a knife maintained at the same low temperature. The sample was moved to the cold stage of the SEM and etched for 3 min at -65°C. After the sample temperature was lowered to -160°C, it was transferred back into the preparation chamber and gold sputter-coated for 6 min. The final step involved reinserting the sample back to the cold stage of the microscope and viewing. The SEM settings were a 15kV beam, working distance of 39 mm and condenser lens setting of approximately 600.

RESULTS AND DISCUSSION

Sufficient amounts of protein were isolated from high quality egg albumen in order to investigate application of a mathematical model (Lever, 1988; Morgan et al., 1989) to thermal gelation of purified egg albumen proteins. The advantage of mathematical modeling is that once a model has been proven to apply, future outcomes can be predicted without generating the data. Assumptions can be made to simplify the model if predicted results have been proven accurate. Thus, the mathematical model of Morgan et al. (1989) as modified by Lever (1988) was used to determine model parameters and test its application to ovalbumin thermal gelation. Once the predictive ability has been established, the model parameter, maximum apparent viscosity or Y', can be used to evaluate the functional characteristics of protein gel strength. Interrelations between gel strength, gel ultrastructure, protein water-binding and gel sulfhydryl-disulfide contents can then be investigated.

Composition of Egg Albumen

Moisture and Protein

The average percent protein (10.37) in the twelve batches of egg albumen evaluated varied no more than 1.5% (Table 8) and agreed with data presented by Powrie and Nakai

(1986). Average moisture content of the albumen (88.11%) also fell within the range presented by Powrie and Nakai (1986) for egg albumen (87-89%). Since protein is the major constituent of egg albumen (Table 1), carbohydrate, lipid and ash contents were not determined.

Table 8. Moisture and protein content of starting material for several egg albumen batches.

Batch	Protein %	Moisture %	Batch	Protein %	Mositure %
1	10.68	88.03	7	9.43	88.37
2	10.15	87.94	8	10.53	88.54
3	10.49	87.61	9	10.62	88.20
4	10.92	88.24	10	10.03	87.46
5	10.64	88.30	11	9.68	87.94
6	10.38	88.12	12	10.86	88.54
Total Average	10.37	88.11			
Standard Deviation	±0.46	±0.34			

Electrophoresis of Egg Albumen Proteins

All batches of egg albumen used in the preparation of globulins, ovalbumin, and conalbumin were first dialyzed against DDW and then centrifuged. Water insoluble protein, ovomucin (Ball and Hoover, 1940), which could interfere with the isolation procedures was removed in this step. The starting material used for the preparation egg albumen tube

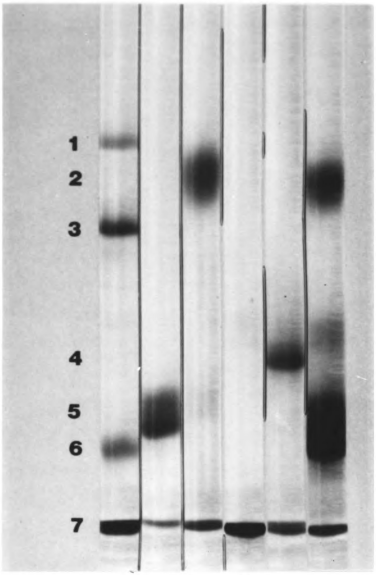
gel (Figure 2, E) was egg albumen from which ovomucin had been previously removed. Use of low molecular weight standards (14,400-92,500 daltons) allowed for molecular weight estimation of the egg white constituent proteins used in this study: lysozyme, ovalbumin, globulins, conalbumin (14,400, 41,500, 50,000 and 74,000 daltons, respectively).

Apparent viscosity (gel strength) experiments required large quantities of purified protein (2.5-5.0 g/experiment). Therefore, isolation procedures were chosen to provide batches with reasonable purity and percent recovery. When the purity of each isolated protein was checked using SDS-PAGE, one major protein band with only minor contamination was found (Figure 2). Individual batches of proteins were combined prior to use in the gelation studies. The purity of all combined batches was checked using the SDS-PAGE technique. Results of purity assessment using the Shimadzu densitometer were as follows: ovalbumin, 87%; conalbumin, 77%; globulins, 79% and lysozyme, 89%.

Application of the Mathematical Model to Ovalbumin Gelation

Since the thermal gelation of egg albumen is dependent on a minimum level of salt (Zabik, 1989) a simple, low ionic strength buffer (0.05 M phosphate) was chosen to evaluate gelation of ovalbumin and conalbumin, globulins and lysozyme. These proteins demonstrated the ability to heat-set when studied by Johnson and Zabik (1981) in a model

Figure 2. SDS-PAGE elecrophoretic determination of egg albumen protein tube gels. S = molecular weight (mw) standards, 1=phosphorylase B (97,400 mw); 3 = bovine serum albumen (66,200 mw); 6 = ovalbumen (42,699 mw); 7 = lysozyme (14,400 mw). Sample proteins: O = ovalbumen (5= 41,500 mw), C = conalbumen (2 = 74,000 mw), G = globulins (4 = 50,000 mw), L = lysozyme (7= 14,400 mw). E = egg albumen.


1
2
3
4
5
6
7
S O C L G E

system with salt composition similar to that of milk.

Ovalbumin clearly comprises the largest portion of the egg albumen. Of the albumen proteins capable of thermal gelation, it is most readily purified in large quantities. Therefore, it was chosen as the protein to apply the modified mathematical model proposed by Morgan et al. (1989) to a high moisture, low concentration thermal gelation. Ovalbumin was adjusted to pH 6 prior to thermal gelation. This was done in order to evaluate heat-set capability (binding) at a pH which is common in low acid foods. The effects of the following on ovalbumin gelation and resultant alteration in model parameters were then studied:
1) addition of chemical agents, 2) increasing pH to 7 and 3) interactions with other egg albumen proteins (globulins, lysozyme, conalbumin).

Donovan et al. (1975) reported the denaturation temperature of ovalbumin to be 84°C at pH 7. When evaluating egg albumen thermal gelation, Beveridge et al. (1980) and later, Holt et al. (1984) reported that a different reaction appeared to occur at temperatures above 80°C compared to lower temperature gelation. Therefore, in this study, temperature ranges of 85, 90 and 95°C were chosen to evaluate high temperature ovalbumin gelation.

Activation Energy Determination

Several investigators have used viscosity index or apparent viscosity as determined by a counter-flow back-

extrusion technique, as a measure of thermally induced gel strength (Harper et al., 1978; Hickson et al., 1980; Hickson et al., 1982). Therefore, normalized heat-induced apparent viscosity (Y′,Eq. 3) determined by using this technique was also assumed in these experiments to be a measure of protein gel strength. Five percent ovalbumin protein in a 0.05 M sodium phosphate buffer was adjusted to pH 6.0 and heated at 85, 90 and 95°C. Resultant experimental values of Y′ were found to be a function of time at the temperatures selected to study gelation when analyzed using a non-linear regression method (NLR)(Marquardt, 1963), model 17, contained in the graph-statistical program, Plotit (Eisensmith, 1985). Experimental data fit this model well, and had R^2 values of 0.97-0.98 for 85, 90 and 95°C heating treatments (Figure 3). A similar relationship between viscosity index (apparent viscosity) and time was found by Hickson et al. (1982) for beef plasma proteins and egg albumen.

Activation energy of the ovalbumin denaturation/ gelation reaction (E_a) was first determined using Arrhenius kinetic theory (Eq. 2) as described in the procedure section. The Y′-time relationship was used to find the times which corresponded to equal Y′ values at each of the three process temperatures studied. Three Y′ values were arbitrarily chosen in the linear ranges for the denaturation /gelation reaction (Figure 4). It was assumed that the temperature was constant throughout the heating process, and

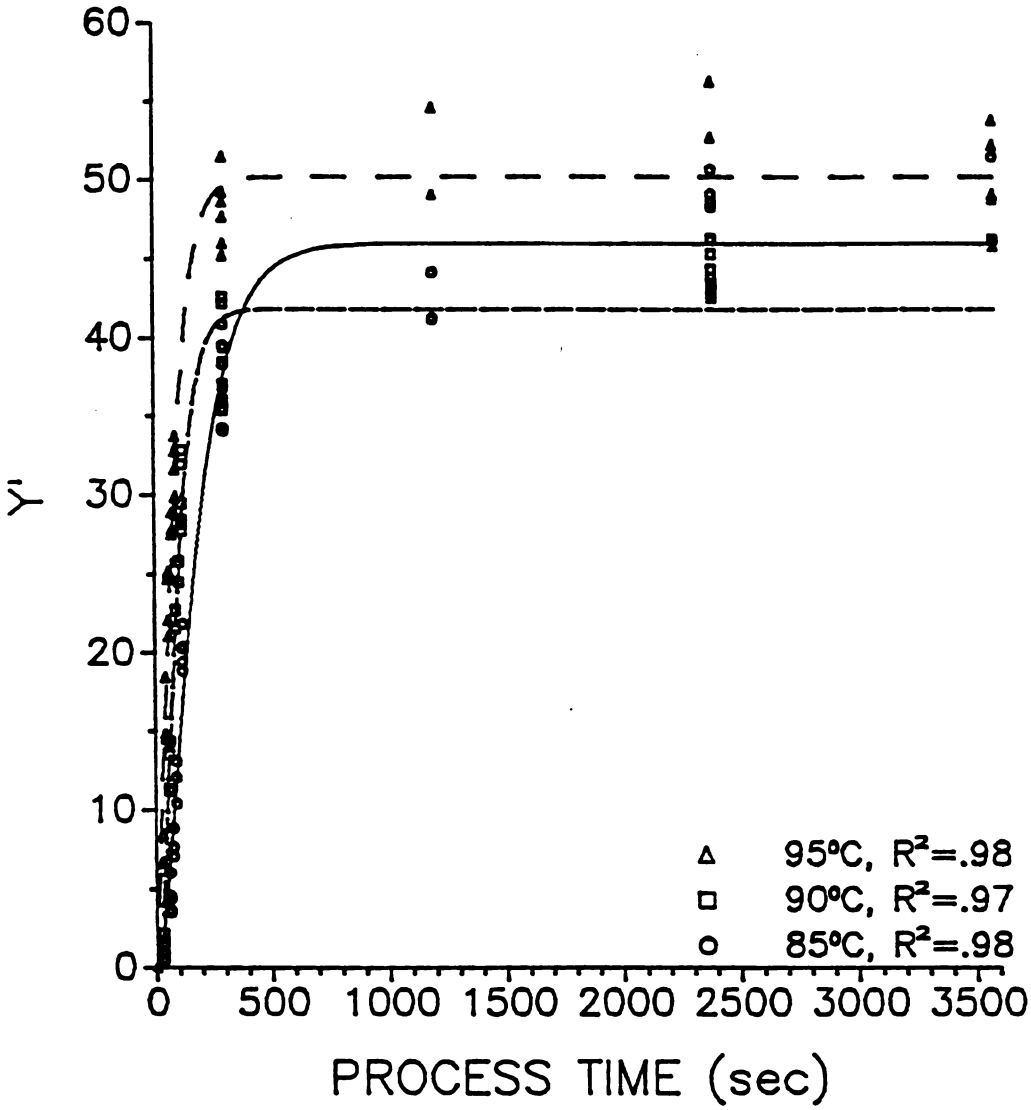


Figure 3. Back–extrusion normalized apparent viscosity of 5% ovalbumin, pH 6, 30–3600 seconds at three process temperatures.

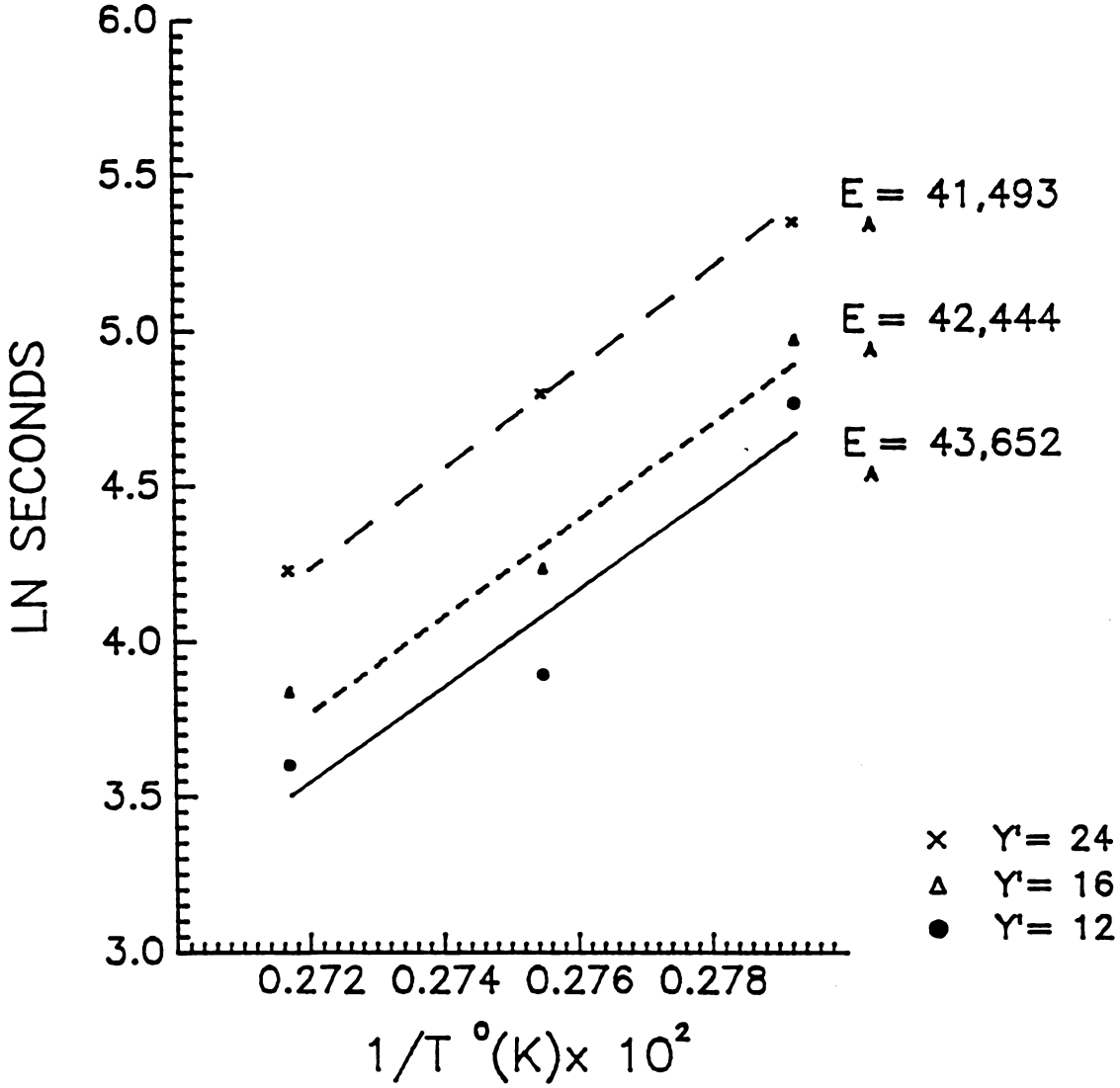


Figure 4. Estimated activation energy range for three temperatures using thermally induced normalized apparent viscosities (Y'). Ovalbumin gelation, pH 6, 5%.

the sample instantaneously reached the process temperature. The average E_a for denaturation/gelation was then found to be 42,530 cal/mole (Figure 4).

In early research conducted by Lewis (1926) a critical increment (activation energy) of non buffered ovalbumin heat denaturation was found to be 130,000 cal at 70.2°C. Chick and Martin (1910) reported that presence of neutral salts lowered the critical increment. Therefore, the low temperature and lack of buffer may explain the large difference in E_a in the study reported by Lewis (1926). Dwek and Navon (1972) estimated the E_a for denaturation of egg albumen to be 24,000 cal/mole and Beveridge et al. (1984) reported an E_a of 32,000 cal/mole for freeze-dried egg albumen from rate constants obtained at 85 and 90°C. Best agreement was found with the results of Goldsmith and Toledo (1985) who used NMR data and calculated activation energies for changes in egg albumen T1 (spin lattice relaxation times for protons of water) and gel strength to be 42,620 and 43,680 cal/mole, respectively. The same technique utilized in this study was used by Goldsmith and Toledo (1985) to calculate the E_a using Arrhenius kinetics and temperatures of 70, 75, 80 and 90°C. While ovalbumin is the major egg white protein (54%) and might be expected to dominate the gelation reaction, it is reasonable that there would be some difference in its E_a when compared to egg albumen.

Temperature measurement within ovalbumin gels using a

thermal couple centrally located in samples showed that it took approximately 210 seconds to reach equilibrium (bath) temperatures. A second method was used to determine E_a using temperature-time history (TTH) which more accurately described the processing conditions. Therefore, the previously determined value of E_a (42,530 cal/mole) was used as an estimate to begin calculation of E_a using this second method.

An assumption of the second technique was that Y´ values generated during the gelation reaction were a function of the temperature-time history of the thermal process (TTH). This technique (Eq. 1) corrected for any temperature differences within a sample and between the sample and the water bath.

Values of TTH were calculated as described in the procedure section using the estimated E_a value of 42,530 cal/mole (Eq. 1). Y´ values were plotted versus TTH or log TTH utilizing the same mathematical model (Plotit, NLR, model 17) used to generate curves of Y´ versus time. Similar shaped curves to those presented in Figure 3 resulted. The initial phase ovalbumin thermal gelation was shown to be a linear function TTH for 85, 90 and 95°C. It was this area of the three curves which was used to calculate the final E_a. By utilizing an iterative procedure, maximum R^2 was found using an E_a = 38,000 cal/mole (Figure 5) when experimental Y´ values generated from three process temperatures were plotted versus TTH put

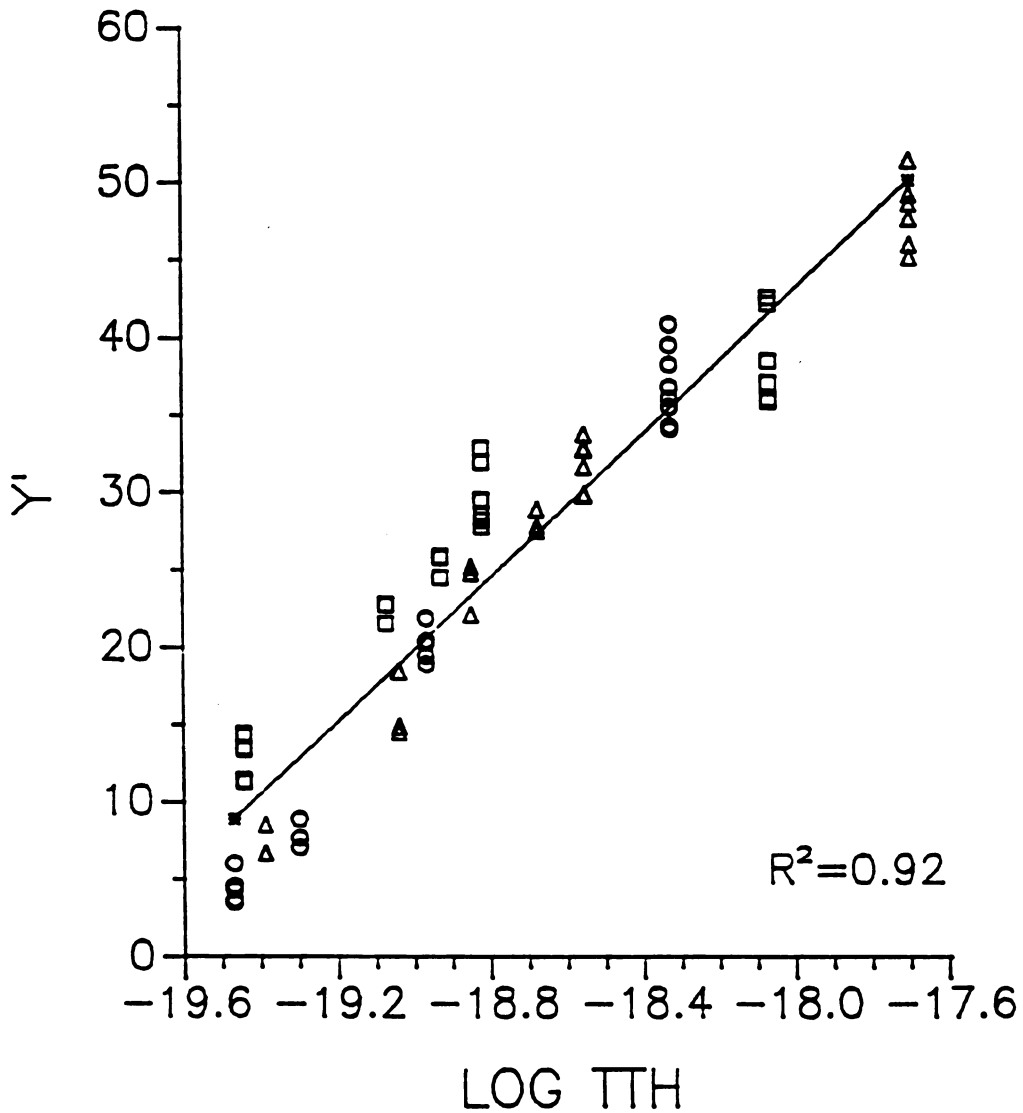


Figure 5. Correlation of Y' values and their estimated TTH for ovalbumin, pH 6 at 85, 90 and 95°C. Ea = 38,000 cal/mol.

values. Thus, the three temperature curves were merged into one curve. This E_a was closer to the value reported by Beveridge et al. (1984) for egg albumen gelation at 85 and 90°C (32,000 cal/mole) but further from the results of Goldsmith and Toledo (1985) (43,680 cal/mole). However, according to Trotman-Dickenson (1968) thermodynamic values obtained for pure ovalbumin may not be applicable to egg albumen protein because of the heterogeneous nature of these proteins. Thus, differences between E_a for egg albumen and individual egg albumen proteins could be expected.

The same procedure was used to determine the E_a for all variables studied at the 5% protein level and high temperature heating (85, 90, 95°C)(Table 9). Increasing the pH to 7, addition of 3% NaCl(0.52 M) and 20 mM NEM did not effect the E_a for ovalbumin thermal gelation (38,000 cal/mole). Conalbumin gelation also had an E_a of 38,000 cal/mole while the other two egg albumen proteins studied (globulin, lysozyme) produced E_a of 50,000 cal/mole. Mixtures of all three proteins and ovalbumin (1:1 ratio v/v) resulted in E_a's of 50,000 cal/mole for protein thermal gelation. Mixtures of ovalbumin with globulins and lysozyme had E_a's equal to the protein with the higher activation energy. However, combinations of ovalbumin and conalbumin also demonstrated an E_a of 50,000 cal/mole even though each alone had E_a of 38,000 cal/mole. This appeared to indicate interaction between these two proteins resulting in a higher E_a. Activation energies for thermal denaturation/gelation

of ovalbumin, conalbumin, globulins and lysozyme or binary mixtures of these have not been found by this author in the literature.

Table 9. Calculated activation energies (E_a) for 5% ovalbumin, globulins, lysozyme and binary combinations of thermally induced gels.

Variable	pH	E_a (cal/mole)	Variable	pH	E_a (cal/mole)
Ovalbumin	6	38,000	Lysozyme	6	50,000
Ovalbumin, NaCl	7	38,000	Globulins	6	50,000
Ovalbumin, NEM	6	38,000	G/O[1]	6	50,000
Ovalbumin	6	38,000	L/O[2]	6	50,000
Conalbumin	6	38,000	C/O[3]	6	50,000

[1]globulin + ovalbumin, 1:1
[2]lysozyme + ovalbumin, 1:1
[3]conalbumin + ovalbumin, 1:1

Determination of Model Parameters (a) and Alpha

The model parameter (a) employed in this study is related to the reaction rate constant for protein denaturation and cross-linking as presented by Lever (1988). Experimental Y´ and TTH data inputted into Eq. (7) were used to define the relationship between (a) and alpha for a specific concentration. The term alpha refers to the relative measure of molecular entanglement during shear (Smith et al., 1988; Morgan et al., 1989). Selected values of alpha were chosen based upon ranges found in the

literature. Morgan et al. (1989) reported values of alpha to be approximately 1.0 and equal to or greater than 3.5 for low and high molecular weight polymers, respectively. In addition, Shah and Darby (1976) reviewed the literature and observed that high molecular weight polymers produced values of alpha ranging from 3.4 to 8.0. Therefore, in this study of ovalbumin gelation, a range of alpha was chosen to be the following: 0.5, 0.75, 1.0, 2.0, 3.0 and 8.0.

Values of (a) and corresponding alpha were found to have the exponential relationship demonstrated in Figure 6 when NLR (Marquardt, 1963), model 3 was performed using the statistical program Plotit (Eisensmith, 1985). Excellent correlation coefficients were obtained (R^2 = 0.99) with all variables tested. A similar relationship was shown by Lever (1988) in studying thermal gelation of myofibrillar beef proteins. Numerous predicted paired values of (a) and alpha (Figure 6) were calculated. These were then fitted into the mathematical model (model 17, Plotit) using normalized apparent viscosity and TTH values. The values of (a)-alpha selected were the pair which provided the highest correlation coefficient using an iterative procedure (Figure 7). The alpha and (a) selected for use in the basic model were determined based upon Y´ experimental data for 85, 90 and 95°C. Values of alpha and (a) for all other variables were calculated based upon experimental data obtained at 90°C. TTH data for all variables were calculated using the predetermined E_a as shown previously in Table 6 .

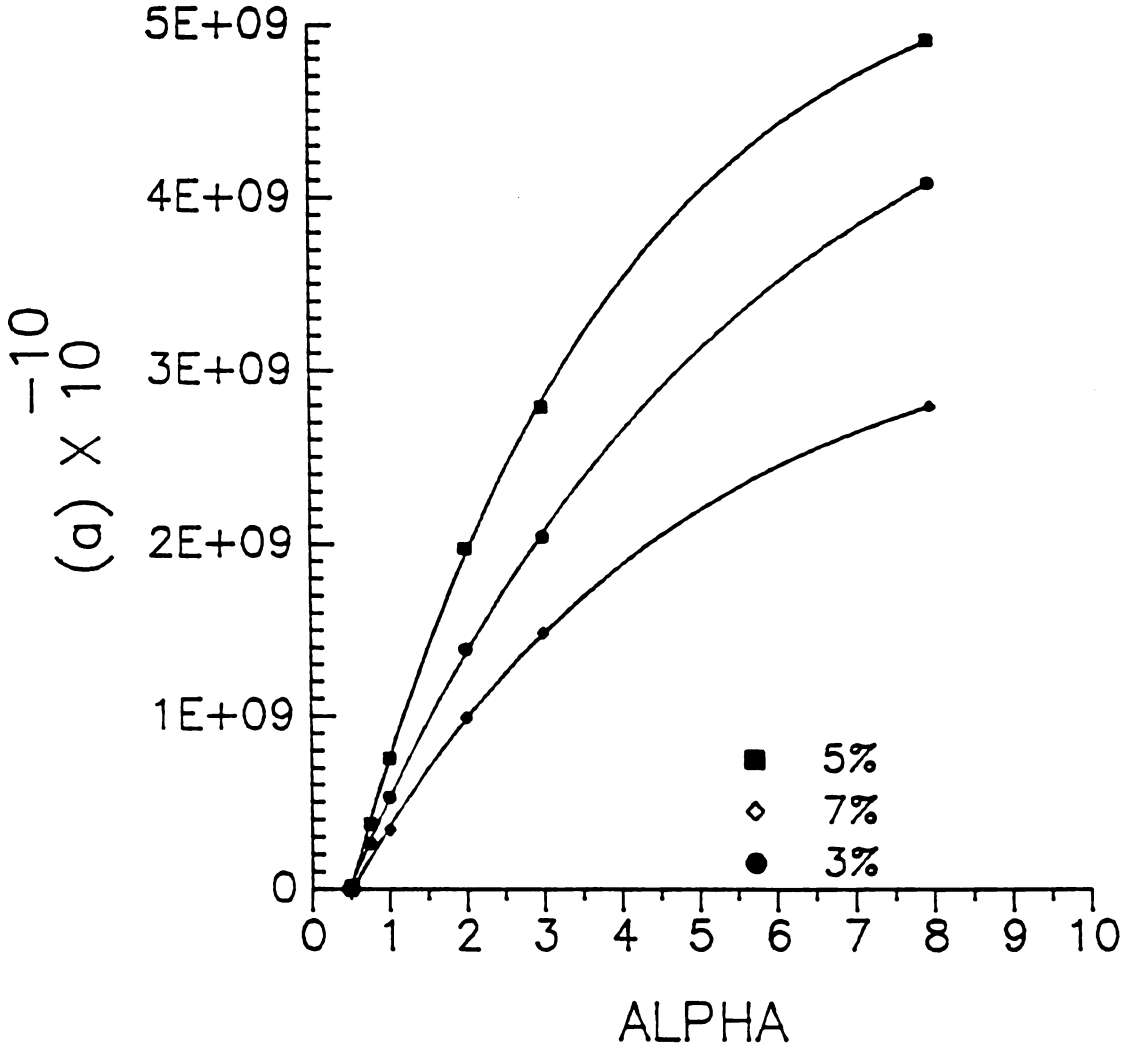


Figure 6. Correlated values of a and alpha based on Y' and alpha, E_a = 38,000 cal/mole, for ovalbumin pH 6.

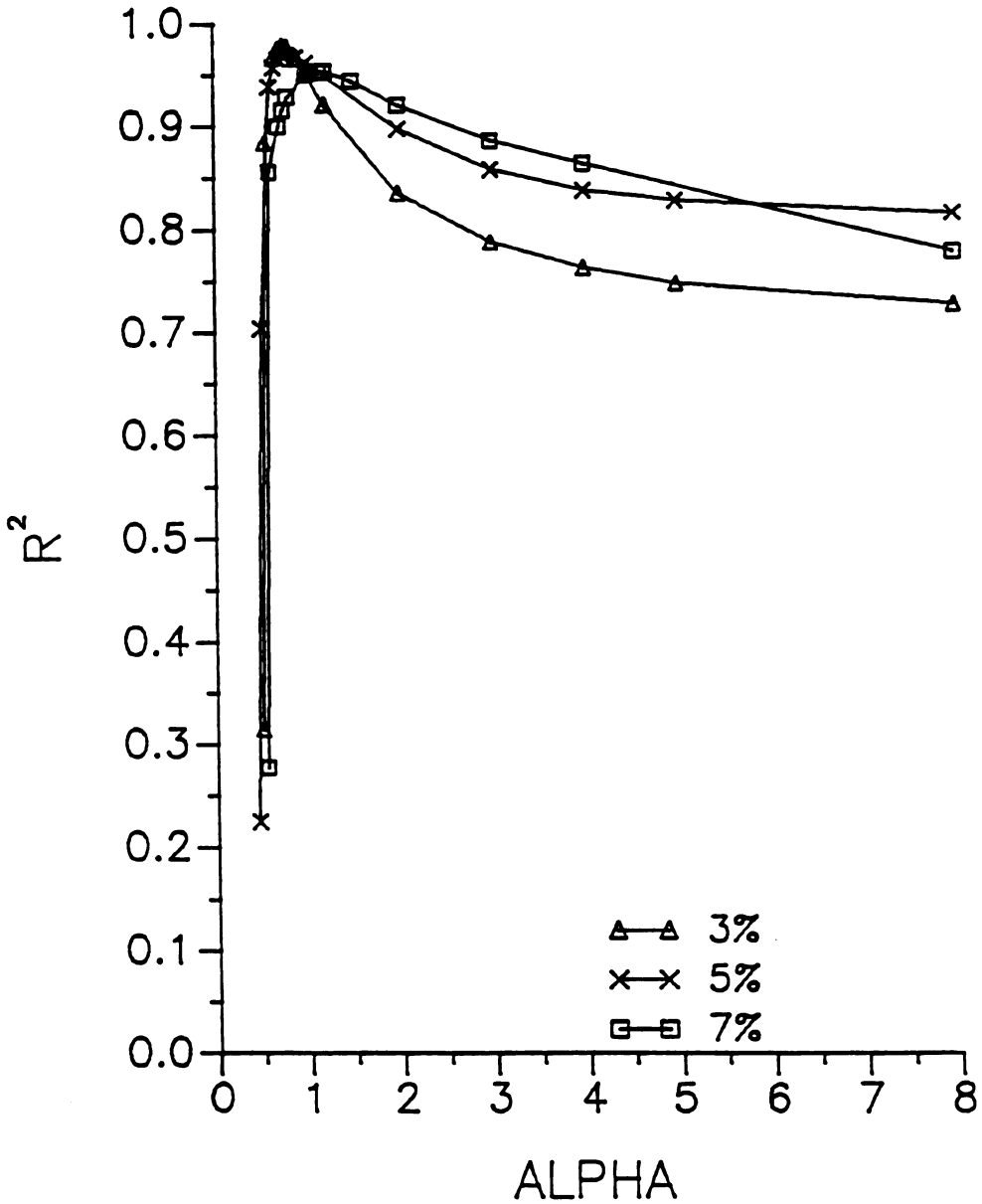


Figure 7. Values of R^2 for nonlinear regression of the mathematical model using fixed values of alpha and a, for three concentrations of ovalbumin adjusted to pH 6.

Data in Table 10 present (a)-alpha values for the following ovalbumin treatments at 3 and 5% protein levels heat-set at 90°C : 1) pH 6 and 7, 2) addition of 3% NaCl, and 3) addition of 20 mM NEM. In addition, Y′ values obtained from samples heat-set at 85 and 95°C and three protein levels (3, 5 and 7%) were used to calculate (a)-alpha values for the basic model. The reaction rate constant (a) obtained for the basic model variables increased slightly as concentration increased (0.425, 0.461, 0.618 E+18). However, these differences may not be statistically significant considering possible variations caused by experimental error and the small rate increases.

Table 10. Comparison of (a) and alpha model parameters for ovabumin gelation variables. Ea = 38,000 cal/mole.

Variable	Protein %	"a" (E+19)	alpha
Ovalbumin, pH 6[1]	3	0.43	0.75
	5	0.46	0.75
	7	0.62	1.00
Ovalbumin, pH 7[2]	3	4.20	2.50
	5	1.10	1.00
Ovalbumin, pH 6[2] 3% NaCl	3	1.20	1.60
	5	1.00	1.30
Ovalbumin, pH 6[2] NEM[3]	3	1.10	0.70
	5	1.30	1.00

[1]data from 85, 90 and 95°C [2]data from 90°C
[3]N-ethylmaleimide, 20 mM

The general trend expressed in Figure 6 was that (a) increased as alpha increased, with small variations in the magnitude of increase occurring as protein concentration varied. The alpha data for ovalbumin at pH 6 (Table 10) were the same (0.75) for 3 and 5% gelation while a slightly higher value occurred for 7% gelation (1.0). Values of alpha were very close to the theoretical value of 1.0 reported by Morgan et al. (1989) for low molecular weight polymers. It appeared that little, if any differences existed in the alpha or (a) parameter for ovalbumin thermal gelation at pH 6, 3, 5 or 7% protein concentration using combined experimental Y′ data for 85, 90 and 95°C.

The remainder of the variables listed in Table 10 were calculated using experimental Y′ data obtained from ovalbumin heat-set at 90°C. These data were used since concentrations for these samples (Table 5) were only varied at this temperature and therefore (a)-alpha could be compared. While (a) for these data were higher than the pH 6 data presented, they were never more than ten times greater at comparable concentrations. Also, (a) for these variables were close (1.0-1.3 E+19) with the exception of 4.2 E+19 for pH 7, 3% gelation. These data appeared to indicate a slightly slower reaction rate for gelation at pH 6 compared to rates of the other ovalbumin variables.

Values of alpha, 2.5 and 1.0 for 3 and 5 % ovalbumin for pH 7 variables (Table 10) demonstrated a trend similarly to that reported by Lever (1988). In his study, low

myofibrillar beef protein concentrations (25.2% dwb) produced high alpha values (8.0) which decreased rapidly and leveled off at approximately 0.7-1.0 as protein concentration was increased (30.4-45.2% dwb). At low concentrations, proteins may spread out extensively, not being constricted by the presence of other protein molecules in close proximity to one another. This might explain why an apparently larger degree of molecular entanglement occurs during shear at low concentrations which drops to approximately 1.0 when concentration is increased to a critical level. Ovalbumin gelation at pH 7 appeared to cause some protein molecule unfolding prior to thermal gelation as observed from the SEM data which will be discussed in a later section. According to Van Kleef (1986), the attractive forces which are present at pH's closer to the isoelectric point cause the heat denatured protein monomer to aggregate prior to unfolding. At pH's farther away from the isoelectric point, repulsive forces increase which results in the monomer unfolding prior to forming the protein network. This may explain why the alpha observed in 3% ovalbumin samples adjusted to pH 7 samples was higher than in samples adjusted to pH 6.

Addition of the sulfhydryl blocking agent NEM to 3 and 5% ovalbumin, produced alpha data (Table 10) approximately the same (0.7, 1.0) and close to the theoretical value of 1.0 as reported by Morgan et al. (1989). While addition of NaCl produced higher alpha values (1.6 and 1.3) than 1.0,

they were close for both concentrations of ovalbumin studied and still well below the values of 3.4-8.0 for high molecular weight polymers (Shah and Darby, 1976).

Table 11 presents the (a)-alpha values for the three other egg albumen proteins studied and binary mixtures of these proteins with ovalbumin. Samples with protein levels of 3 and 5% were adjusted to pH 6 prior to heat-setting at 90°C. Conalbumin was the only protein which had an E_a of 38,000 which was equal to that of ovalbumin. Values of (a) (reaction rate) and alpha at both conalbumin concentrations were equal to each other, and comparable to those of ovalbumin (0.2 E+19 and 0.75, for (a)-alpha, respectively). The remaining protein had an E_a of 50,000 which produced (a) values E+8 higher and therefore could not be compared to ovalbumin and conalbumin reaction rates. Different E_a's resulted in changing TTH values which affected the magnitude of the reaction rate constant (Eq. 1). Reaction rates for globulin were higher for 3% than 5% gelation (1.9 vs 0.37 E+26). However, lysozyme showed an opposite trend with reaction rates of 3% lower than 5% gelation (0.12 vs 0.99 E+26). This inconsistency may indicate no real differences in reaction rates for these proteins at the two concentrations studied. Binary mixtures of globulins/ ovalbumin (G/O) produced reaction rate constants in the same range as those of globulin and lysozyme, while mixtures of conalbumin / ovalbumin (C/O) were slightly greater (4.3 and 2.2 E+26) than either protein alone. The gelation ratio of

Table 11. Comparison of (a) and alpha model parameters for isolated egg albumen proteins and selected binary mixtures.

Variable	Protein %	"a"	alpha
Conalbumin[1]	3	0.21 E+19	0.75
	5	0.20 E+19	0.75
Lysozyme[2]	3	0.12 E+26	1.00
	5	0.99 E+26	0.60
Globulins[2]	3	1.90 E+26	1.40
	5	0.37 E+26	1.30
C/O[3]	3	4.32 E+26	1.00
	5	2.23 E+26	1.00
L/O[4]	3	17.60 E+26	5.00
	5	11.10 E+26	2.00
G/O[5]	3	0.75 E+26	0.45
	5	1.30 E+26	0.40

[1]Ea = 38,000 cal/mole
[2]Ea = 50,000 cal/mole
[3]Conalbumin + Ovalbumin, 1:1
[4]Lysozyme + Ovalbumin, 1:1
[5]Globulin + Ovalbumin, 1:1

lysozyme/ovalbumin (L/O) was approximately one order of magnitude greater, in comparison to the individual protein's gelation rates.

Examining alpha for the data in Table 11 (E_a = 50,000) shows very little or no difference between protein concentration with the exception of the L/O data. Overall, globulin alpha values were 0.4-0.8 larger than lysozyme alpha data suggesting greater molecular entanglement during shear for lysozyme. However, both proteins' alphas were close to the theoretical value of 1.0 for low molecular weight polymers, and therefore, it was not clear if real differences existed in this parameter. Protein mixtures of g/o resulted in approximately a 1.0 unit reduction in alpha values compared to gelation of globulin alone and 0.3-0.35 lower than ovalbumin gelation. Mixtures of C/O produced alpha values (1.0) close to those of ovalbumin and conalbumin alone.

The alpha values of L/O were very high compared to all other variables. The higher values for lower protein concentration (Lever, 1988) was also observed in these variables. An explanation for the higher alpha's may be found in the ability of lysozyme to readily bind with other proteins (Forsythe and Foster, 1950; Hawthorne, 1950; Steiner, 1953; Ehrenpreis and Warner, 1956). Aggregation between lysozyme and heat-denatured ovalbumin was examined by Matsudomi et al. (1987). They concluded that lysozyme interacted electrostatically with fully unfolded monomeric

ovalbumin (0.1%) during aggregation as evaluated by turbidity measurements. It is possible that similar interaction could occur at higher protein concentrations capable of thermal gelation. SEM microstructures of L/O gels which will be presented in a later section, appeared to support this possibility.

Determination of Model Parameter A´and Y´∞

The A´ parameter is a measure of the effective molecular weight of the unfolded protein (polymerized gel) (Morgan et al., 1989). Y'_{∞} was calculated using the alpha and (a) values presented in Table 10 and 11 by inputting them into the mathematical model (Equation 5) using Y´ and corresponding TTH data and the Plotit nonlinear regression algorithm, model 17 (Marquardt, 1963). A´ values were then calculated (Eq. 8) and are presented along with maximum apparent viscosity values (Y'_{∞}) in Table 12.

For all variables examined, Y'_{∞} increased as did the parameter A´. Any differences between the values for any one variable at a given concentration were related to variations in alpha as determined by Equation 8. Therefore, in this study, the effective molecular weight of the resultant polymer (A´) formed through thermal gelation of the proteins was correlated to the maximum apparent viscosity (Y'_{∞}). This relationship was also established by Lever (1988) in his work with thermocoagulation of beef

Table 12. Comparison of Y'_{∞} and A' values for 5% ovalbumin gels adjusted to pH 6.

Variable	Protein (%)	Y'_{∞}	A'
Ovalbumin, pH 6	3	14.60	19.74
	5	43.31	48.13
	7	105.00	119.00
Ovalbumin, pH 7	3	21.10	38.10
	5	67.50	78.10
Ovalbumin, pH 6, 3% NaCl	3	17.20	25.00
	5	63.70	77.00
Ovalbumin, pH 6, NEM[1]	5	11.20	12.50
	7	26.90	30.20

[1]20 mM N-ethylmaleimide

myofibrillar protein. Ovalbumin gelation (pH 6) at heating temperatures of 85-95°C had increased gel strength (Y'_{∞}) and A' values with increasing concentration. This increase was observed in all variables studied and agreed with other studies describing egg albumen protein gelation strength (Shimada and Matsushita, 1980a; Woodward and Cotterill, 1986). By increasing the pH to 7.0, increased gel strength (Y'_{∞}) and A' values were obtained. This corresponded with the results reported by Egelandsdal (1980) who found an ovalbumin gel strength maxima at pH 7.

Salt concentrations of 1-5% for processed meats such as sausage have been used. Levels up to 0.6 M have disrupted myofibrillar proteins which then can act as emulsifiers for fat particles necessary in processed meat products (Merkel, 1987). Because egg albumen proteins have often been used as binders in meat or fish systems, a 3% (w/v) level of NaCl was chosen to test the effect of salt on ovalbumin thermal gelation.

Addition of NaCl resulted in higher maximum apparent viscosities (Y'_{∞}) and A' values compared to those generated in the absence of salt (Table 12). This was more evident at the 5% than at the 3% ovalbumin concentration where Y'_{∞} values were 43.31 vs 63.7 for no NaCl and NaCl samples, respectively. Hegg et al. (1979) found ovalbumin gel-like precipitates with NaCl concentrations up to 0.34 M, however, no evaluation of gel strength was performed. A positive response to the textural characteristics of egg albumen gels

was found by Holt et al. (1984) with NaCl addition up to 0.1 M at pH 6.4. They suggested that NaCl addition neutralized repulsive electrical charges on polypeptide chains. Collision between chains then resulted in cohesion and flocculation, with a possible positive effect on gel structure. More recently, Van Kleef (1986) showed that gelation of ovalbumin containing 3% NaCl at pH 5-12 provided higher storage shear modulus, G'(rigidity), than the gel prepared without NaCl. Also supporting the results of this present study, Woodward and Cotterill (1986) reported increased gel strength of egg albumen at pH 6 and 7 with addition of 0.32 M NaCl.

However, the results seemed to contradict those of Egelandsdal (1980) who observed a decrease in 8% ovalbumin rigidity (g/cm) as the amount of NaCl was increased (0, 0.003, 0.03, 0.3 M) at pH 6.0. If Egelandsdal had increased NaCl to 0.52 M, the results may have been more comparable. Also, his buffer system and heating rate and time differed from the current work being discussed.

N-ethylmaleimide (NEM) has been used in protein research because of its ability to block sulfhydryl bonding (Buttkus, 1974). Utsumi and Kinsella (1985) added 20 mM NEM to gels made from 12-15% (w/v) 7S and 11S soy proteins and soy isolate to determine if these proteins required thiol/disulfide interchange reactions for hard gel formation.

In this study, 20 mM NEM was added to 5% ovalbumin

gels (pH 6) to test the dependency of the thermocoagulation reaction on thiol/disulfide interchange. The dependency of the ovalbumin thermal gelation on this reaction was confirmed (Table 12). NEM samples with protein concentrations of 5 and 7% had 74% lower maximum apparent viscosity (Y'_{∞}) and A values than controls. The influence of NEM on 5% protein samples was even more than at lower concentrations (3%). While NEM substantially repressed gelation, some residual gelling did occur. This most likely was due to other reactions involving hydrophobic, hydrogen, and/or electrostatic bonding. While Matsudomi et al.(1987) could detect no effect of NEM on the binding of lysozyme to heat denatured ovalbumin, low protein concentrations (0.1%) and turbidity measurements rather than gelation were examined. Free sulfhydryl (SH) and total disulfide (SS)-sulfhydryl determinations on ovalbumin gelation are presented later in the results and discussion section.

Five percent ovalbumin maximum apparent viscosity (Y'_{∞}) and A' values were compared to those of the other three proteins with the following ranking resulting: conalbumin > lysozyme $\simeq$globulin > ovalbumin (Table 13). Differences were not quite as large at 3% protein but the same general trend was found.

Five percent binary mixtures of ovalbumin with the other egg albumen proteins showed that ovalbumin decreased the gel strength of the other proteins to varying degrees depending upon the protein studied (Table 13). Gel strength

Table 13. Comparison of Y'_{∞} and A' values for isolated egg albumen proteins and selected binary mixtures adjusted to pH 6.

Variable	Protein (%)	Y'_{∞}	A'
Ovalbumin	3	14.60	19.74
	5	43.31	48.13
Conalbumin	3	47.90	57.10
	5	119.00	133.00
Lysozyme	3	30.40	38.50
	5	109.00	199.00
Globulins	3	31.00	32.90
	5	93.20	95.10
C/O[1]	3	15.40	19.50
	5	76.20	88.60
L/O[2]	3	42.10	160.00
	5	72.40	97.90
G/O[3]	3	25.00	27.80
	5	83.20	88.30

[1]Conalbumin + Ovalbumin, 1:1
[2]Lysozyme + Ovalbumin, 1:1
[3]Globulins + Ovalbumin, 1:1

of conalbumin and lysozyme were reduced by 36 and 34%, respectively. The effect on globulin gelation was the smallest with addition of ovalbumin decreasing maximum apparent viscosity only 11%. The same general relationships were found with A′ parameter values. Comparison of these results can be made to the work of Johnson and Zabik (1981) who examined the same proteins′ gelation (2.6%) ability in a custard model system possessing a composition similar to milk. While the order of gel strength was the same for lysozyme, globulins and ovalbumin, conalbumin produced the weakest gel. Possibly, the additional salts involved, such as calcium chloride or variations in heating conditions resulted in the difference. Also, a different pH (8 vs 6), was used in Johnson and Zabik′s study which was higher than the reported isoelectric point of conalbumin (6.1-6.6). In this study, the protein was adjusted to a pH below its isoelectric point.

Verification of the Mathematical Model

To verify application of the mathematical model (Eq. 5) to ovalbumin gelation, predicted and experimental normalized apparent viscosity (Y′) were plotted versus log TTH for three temperatures of ovalbumin at pH 6 (Figure 8). Protein levels were maintained at (5% w/v) and Y′ data were collected at 85, 90 and 95°C to test agreement with the predicted values calculated as previously described using parameters (a) = 0.461, alpha = 0.75, and A′ = 48.1, (Tables

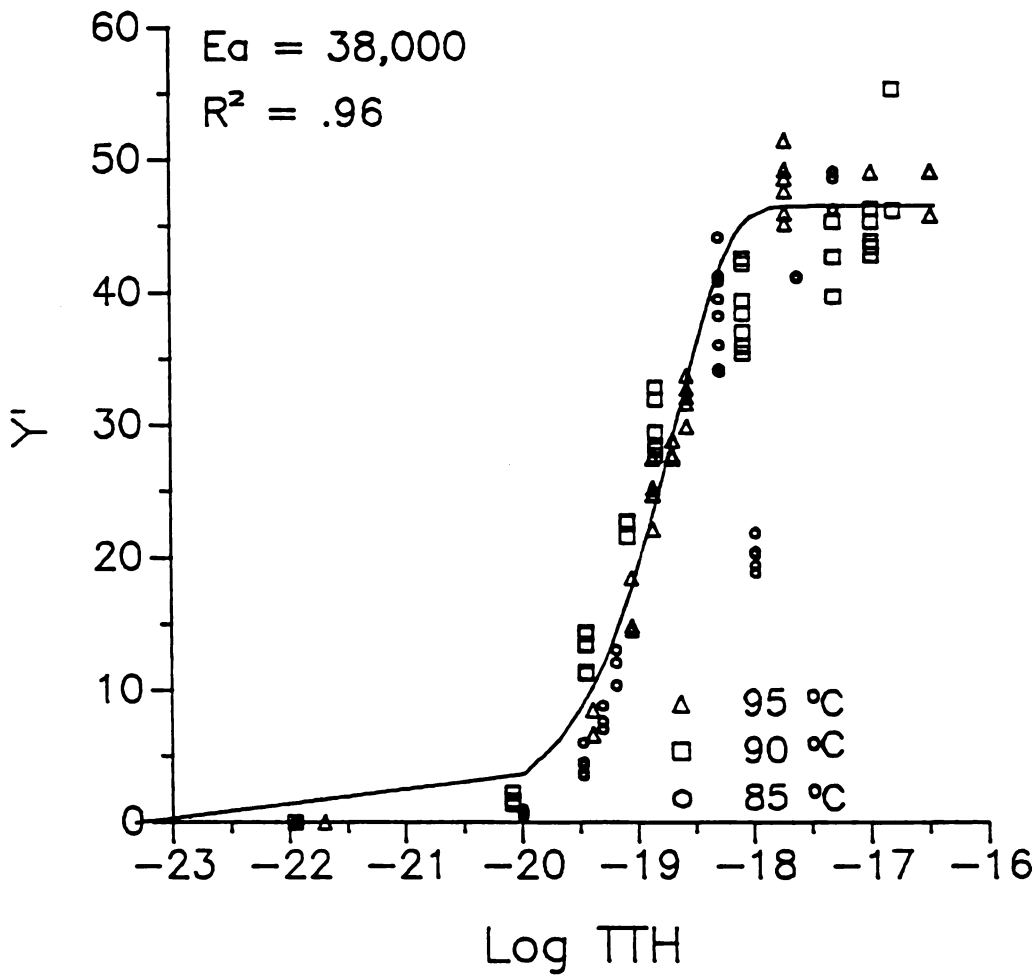


Figure 8. Comparison of predicted and experimental Y' versus TTH values for three process temperatures and 5% ovalbumin concentration. Solid line represents model prediction values.

10 and 12). The solid line in Figure 8 represents the curve produced fixing these parameters into the mathematical model (Eq. 5). Good correlation (R^2 = 0.96) resulted from plotting the gelation experimental data of three different temperatures used. This supports the hypothesis that the mathematical model (Eq. 5) has the ability to predict ovalbumin gelation as a function of the time-temperature history of the process.

Temperature was then held constant (90°C) and the predictive ability of the mathematical model (Eq. 5) was tested using the model parameters calculated for 3, 5 and 7% ovalbumin thermal gelation. Calculated parameters for (a), alpha, and A´ (Tables 9 and 11) were fixed into the mathematical model (Eq. 5) and the curve represented by the solid line in Figure 9 was generated. Correlation of experimental Y´ values to the prediction curve generated was calculated by using the nonlinear regression technique as previously described. Good correlation coefficients were obtained, 0.98, 0.97 and 0.95 for 3, 5, and 7% ovalbumin gelation, respectively. It was concluded that the mathematical model (Eq. 5) predicted the thermal gelation of ovalbumin as a function of protein concentration and the temperature-time history of the process.

One further test of the model was conducted using another method, water holding capacity (WHC), to evaluate ovalbumin gelation. Thermal gelation of protein-water systems occurs as a two-step process according to Ferry put

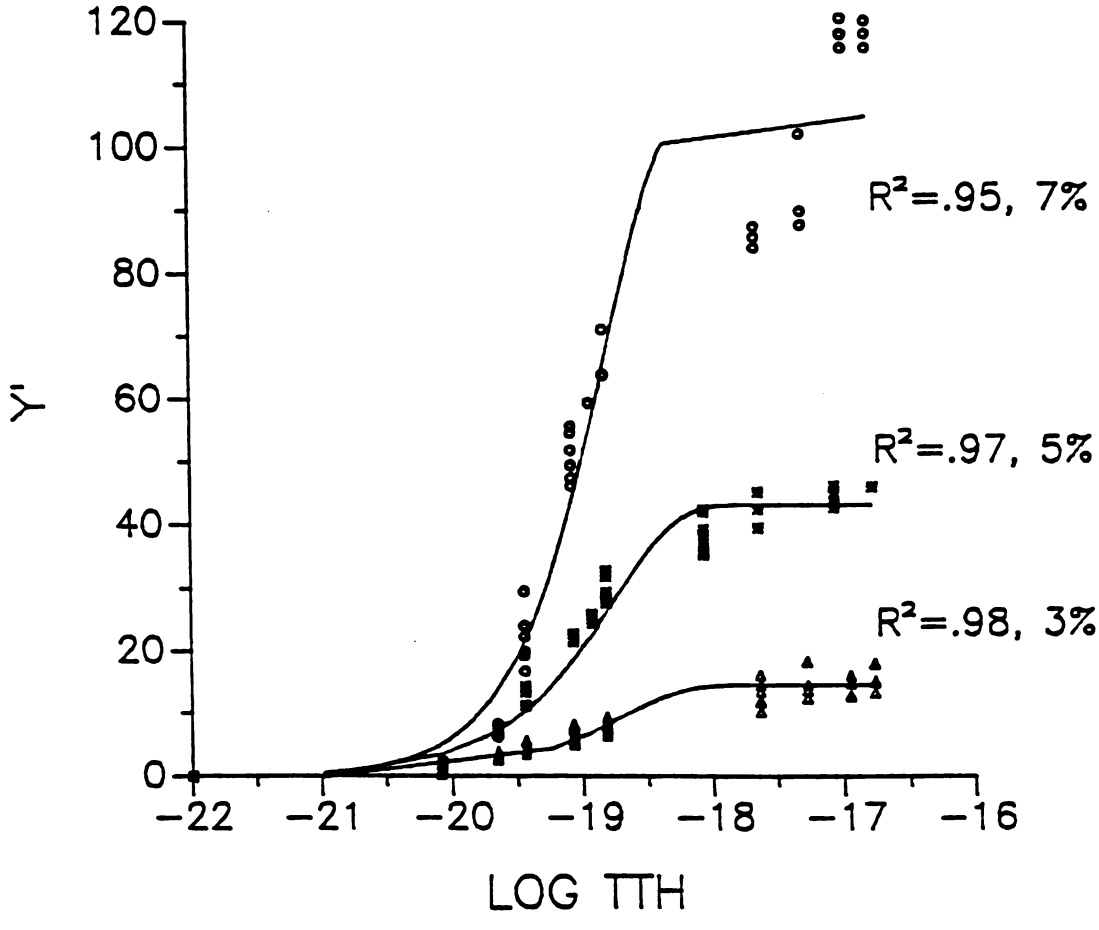


Figure 9. Comparison of predicted and experimental Y' versus TTH values for 3 ovalbumin concentrations at 90°C. Ea= 38,000 cal/mole.
Solid line represents prediction values.

(1948) and Shimada and Matsushita (1980a). The first stage involves denaturation of native protein. This is followed by a second stage of protein-protein interactions where water is entrapped into a 3-dimensional network as the process proceeds. Water holding capacity as used in this study measured the ability of the protein gel to hold water within the matrix of an undisrupted gel. Therefore, determining ovalbumin WHC at varying times during the gelation process, the degree of gelation in this study was evaluated.

WHC values obtained at 5% ovalbumin concentration, pH 6 and processed at 90°C were substituted into the mathematical model (eq. 5) in place of experimental Y′ values. Predetermined parameters (Tables 9 and 11) were used to predict the gelation process as described previously. TTH values were calculated based on temperature come up times for the apparent viscosity experiments. These values were assumed to be a good estimation of the actual TTH for the process since the same water bath was used to process samples for experiments. When the WHC values were plotted versus TTH values previously calculated, good correlation (R^2=0.93) resulted (Figure 10). This further helped to support the hypothesis that the mathematical model (Eq. 5) is valid in predicting ovalbumin thermal gelation. This also supports the assumption that back extrusion apparent viscosity data can be used as an indication of gel strength. In addition, these results agreed with the findings of put

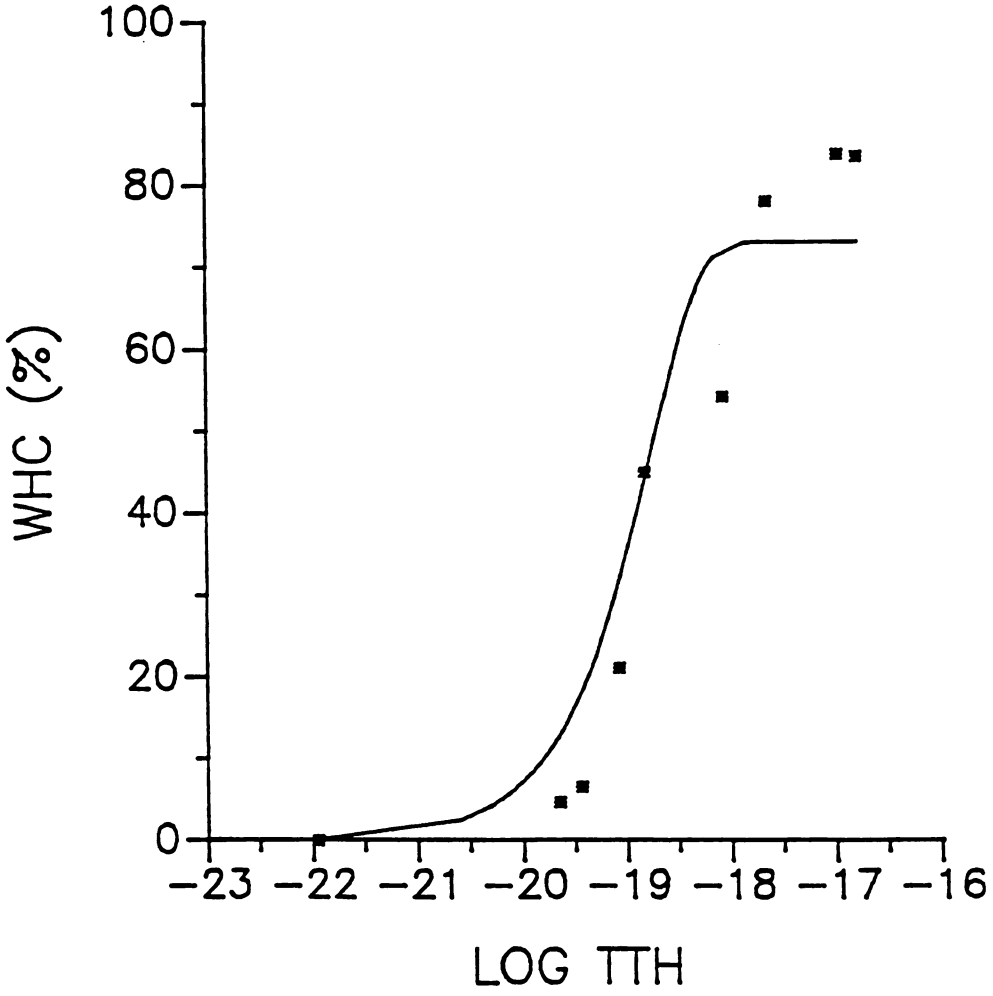


Figure 10. Average water holding capacity versus log TTH for ovalbumin thermal gelation, pH 6, 90°C. R^2 = 0.93.

Furukawa and Ohta (1982) that water-binding of proteins (soy) was correlated to gel strength. WHC data will be discussed additionally in the following section.

Evaluation of Functional Characteristics of Ovalbumin Thermal Gelation.

Water Holding Capacity of Ovalbumin Gels

Water-binding of protein gels has been studied as a means to evaluate the ability of proteins to entrap water during formation of a three dimensional network. Water binding of proteins is often reported as expressed serum or moisture which is the amount of water removed from a gel after centrifugation or pressing. It has also been presented as water holding capacity which is the amount of water retained in a gel after centrifugation. Therefore, water holding capacity and expressed serum are inversely related. A description of ovalbumin gel samples used for water holding capacity determination has been presented in Table 7.

pH and time. Water holding capacity (WHC) of 5% ovalbumin gels adjusted to pH 6 and 7 and heat-set at 85, 90, 95°C were are compared in Figure 11. Significant differences were found for the effect of time on WHC for these samples ($p<0.001$). After samples were heated at 85°C for 90 min, a significant increase in WHC occurred up to 300 sec of put

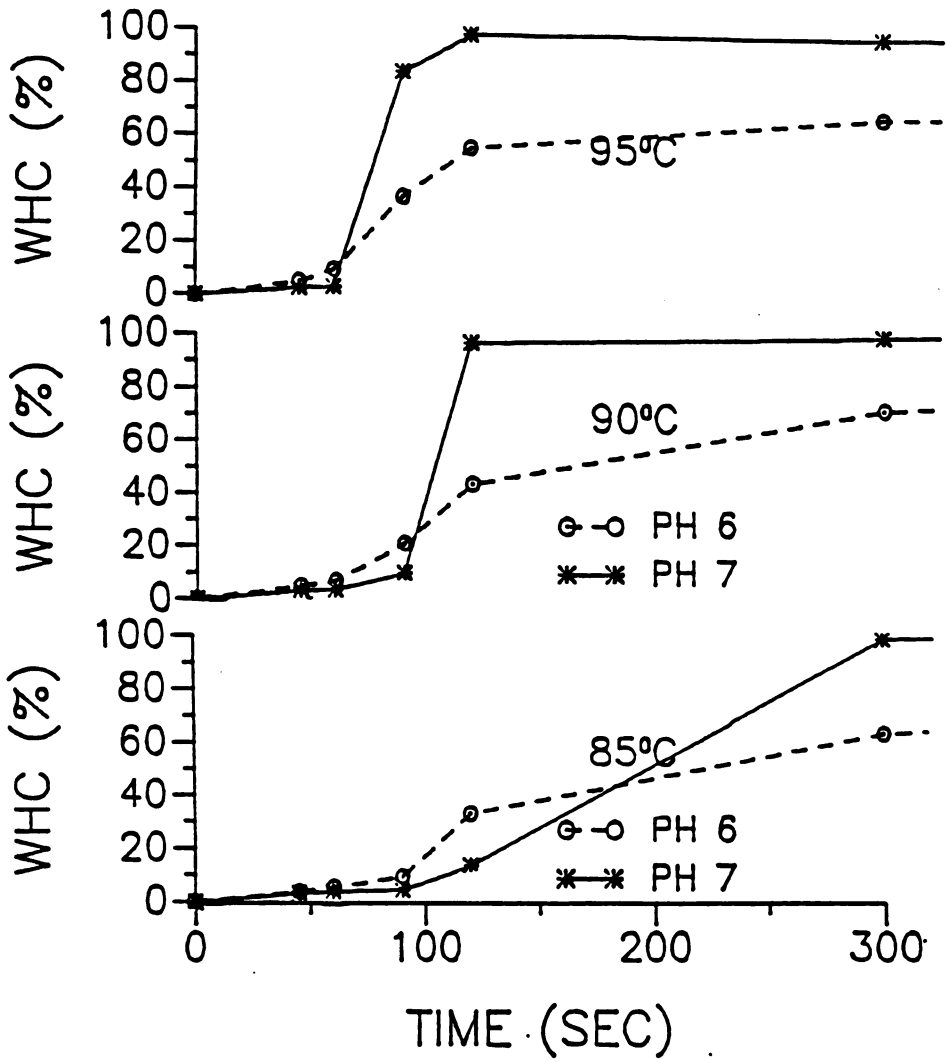


Figure 11. Average water holding capacity of 5% ovalbumin adjusted to pH 6 or 7 and heat-set at 85, 90, or 95°C. n=2

heating. Heating from 300 to 3600 sec showed no significant differences as WHC leveled off. No significant differences in WHC occurred initially after heating at 90 and 95°C for 45 or 60 sec. However, significant increases in WHC occurred for samples heated at 90 and 95°C, up to 300 sec. WHC remained constant after 600 sec of heating at 90°C and 300 sec at 95°C.

Water holding capacity of gels at pH 7 were greater than those of pH 6. Differences were shown for the effect of pH at the three process temperatures ($p < 0.01$, 85°C and < 0.001, 90 and 95°C). Both the maximum WHC and the apparent rate in which maximum WHC was reached were shown to be different (Figure 11). Samples at pH 7 appeared to reach their maximum water holding capacity at faster rate than the pH 6 gels. Also, significant pH-time interactions occurred for WHC at 85, 90 and 95°C. These results correlated with large differences found between the gel strengths of these samples. Maximum apparent viscosity was 43.3 and 67.5 for samples adjusted to pH 6 and 7, respectively. Results of statistical analysis of data are presented in the appendix (Table C).

Woodward and Cotterill (1986) examined gel hardness and expressed serum (ES) of egg albumen gels. Significant pH-temperature-time interactions occurred for ES. ES was lower at pH 7 than pH 6 when determined at 85 and 90°C for gels cooked for 10, 30 or 50 minutes. Gossett and Baker (1983) found decreasing ES as pH increased greater than pH 7 in egg

albumen. In general, the results of these two studies were in agreement with the data reported here, however, some variation in the effect of pH on egg albumen and ovalbumin could be expected due to the differences in the protein.

Temperature and time. The data in Figure 12 demonstrate the effect of temperature on WHC of 5% ovalbumin gels adjusted to pH 6 prior to heating. In general little differences in WHC for gels occurred after heating for 300 sec. Therefore WHC data obtained from cooking times from 45 to 300 sec at 85, 90 and 95°C were statistically analyzed (Appendix, Table B). Significant increases in WHC were found ($p<0.001$) as temperature was increased up to 300 seconds. These results agreed with the gel strength data which showed a rate dependence on temperature. Interactions between temperature and time for water holding capacity data were also found ($p<0.001$).

Water-binding of protein gels can be influenced by some of the same factors which control texture. Water-binding of soy protein gels were reported by Furukawa and Ohta (1982) to correlate to gel strength. Woodward and Cotterill (1986) found decreased ES with increasing heating temperature for egg albumen heated for ten minutes. Minimal decreases in ES occurred when samples (pH 6 and 7) were heated for 30 or 50 minutes. Gels increased in hardness with increasing temperature and time up to ten minutes and then remained constant when heated 30-50 minutes at 80, 85

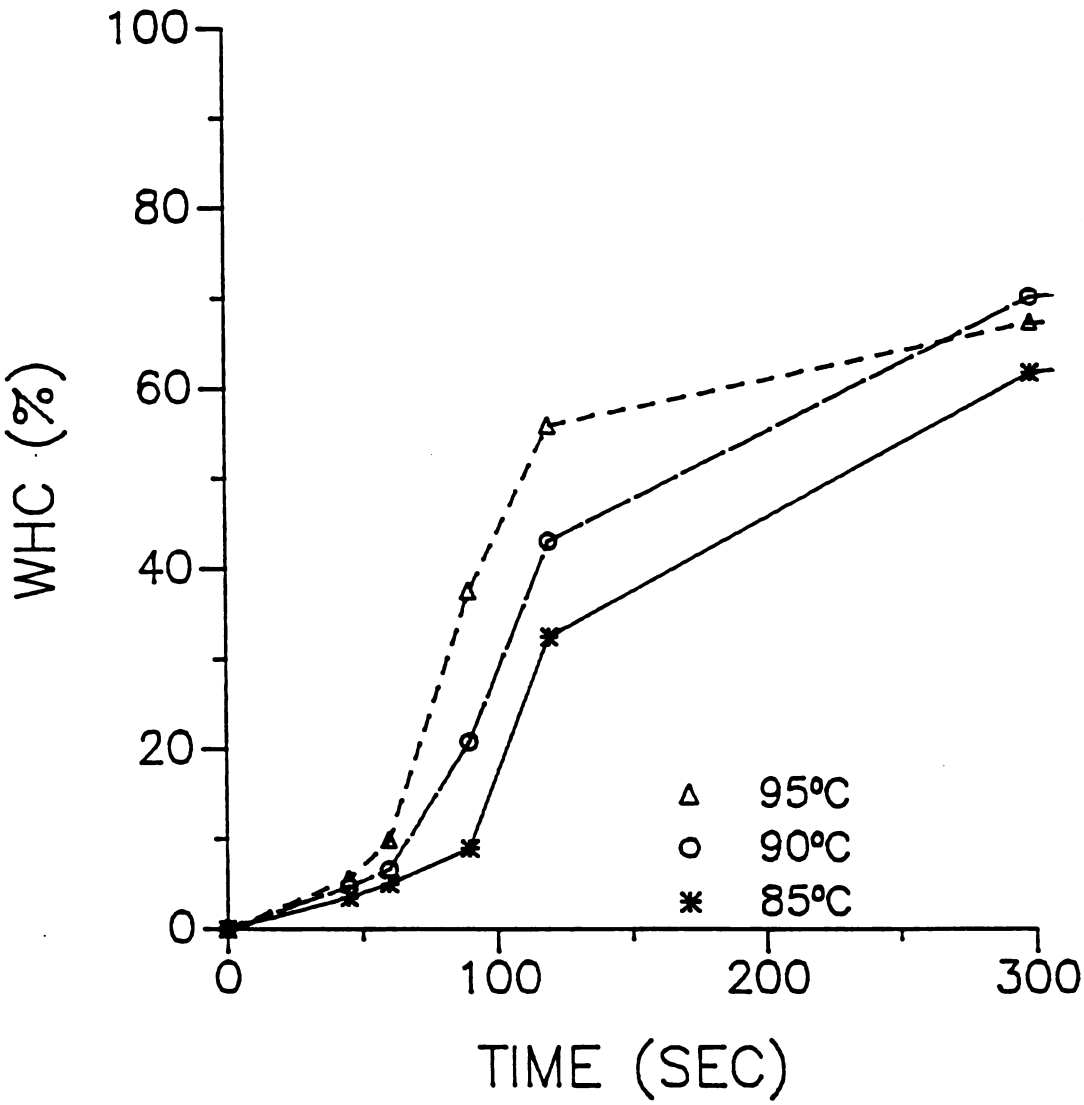


Figure 12. Water holding capacity of 5% ovalbumin, pH 6, heat–set at 85, 90 and 95°C. Duplicate determinations, n= 2.

and 90°C. In general, these results agreed with those found in this study with ovalbumin. This implies that both water binding and gel strength were indicators of the protein denaturation/ gelation reaction. This reaction occurred with increased time and temperature up to a maximum and then stopped. No further change in water holding capacity or gel strength occurred until extessive evaporation or gel break down occurred after heating gels for greater than 3600 sec.

Protein concentration and time. Water holding capacity of 3% ovalbumin heat-set gels was significantly lower than that of the 5 and 7% gels at pH 6 when evaluated at gelation times up to 300 sec (Appendix Table D). The WHC's of higher concentration gels were not significantly different from each other ($p < 0.001$) (Figure 13). Water holding capacity significantly increased after heating 60 seconds. Significant interactions occurred for protein concentration and time for gelation at pH 6 ($p < 0.001$). This correlated with gel strength data which showed increased maximum apparent viscosity with increased protein concentration. Y'_{∞} were 14.6, 43.3 and 105 for 3, 5 and 7% ovalbumin gels adjusted to pH 6.

Gelation of ovalbumin, as measured by WHC, occurred very quickly at pH 7, with slightly higher water holding capacities for the 5% compared to the 7% samples (Figure 14). Three percent samples had no significant differences in water holding capacity compared to the 5 and 7% samples

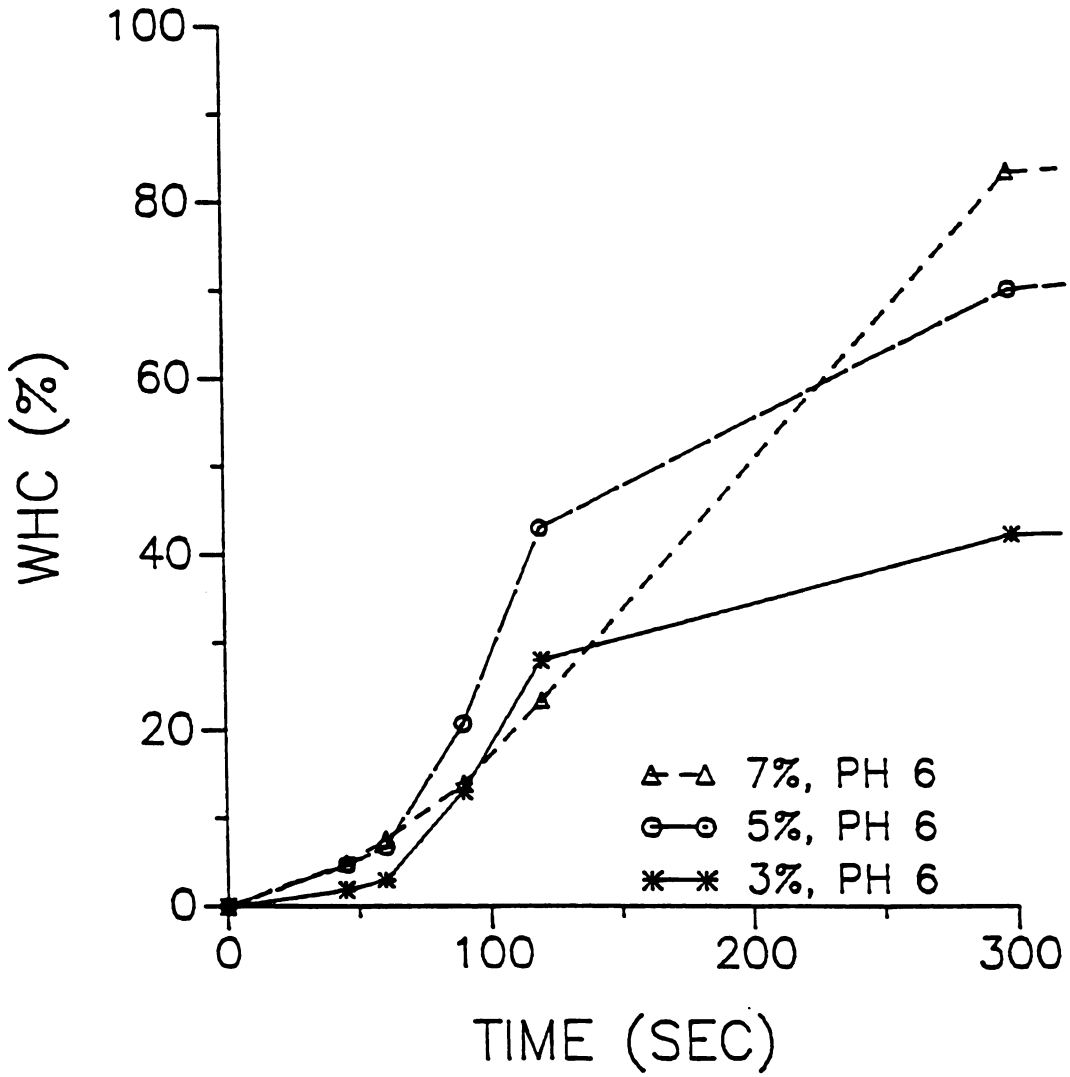


Figure 13. Water holding capacity of ovalbumin gels adjusted to pH 6 prior to heating at 90°C. Duplicate determinations, n=2.

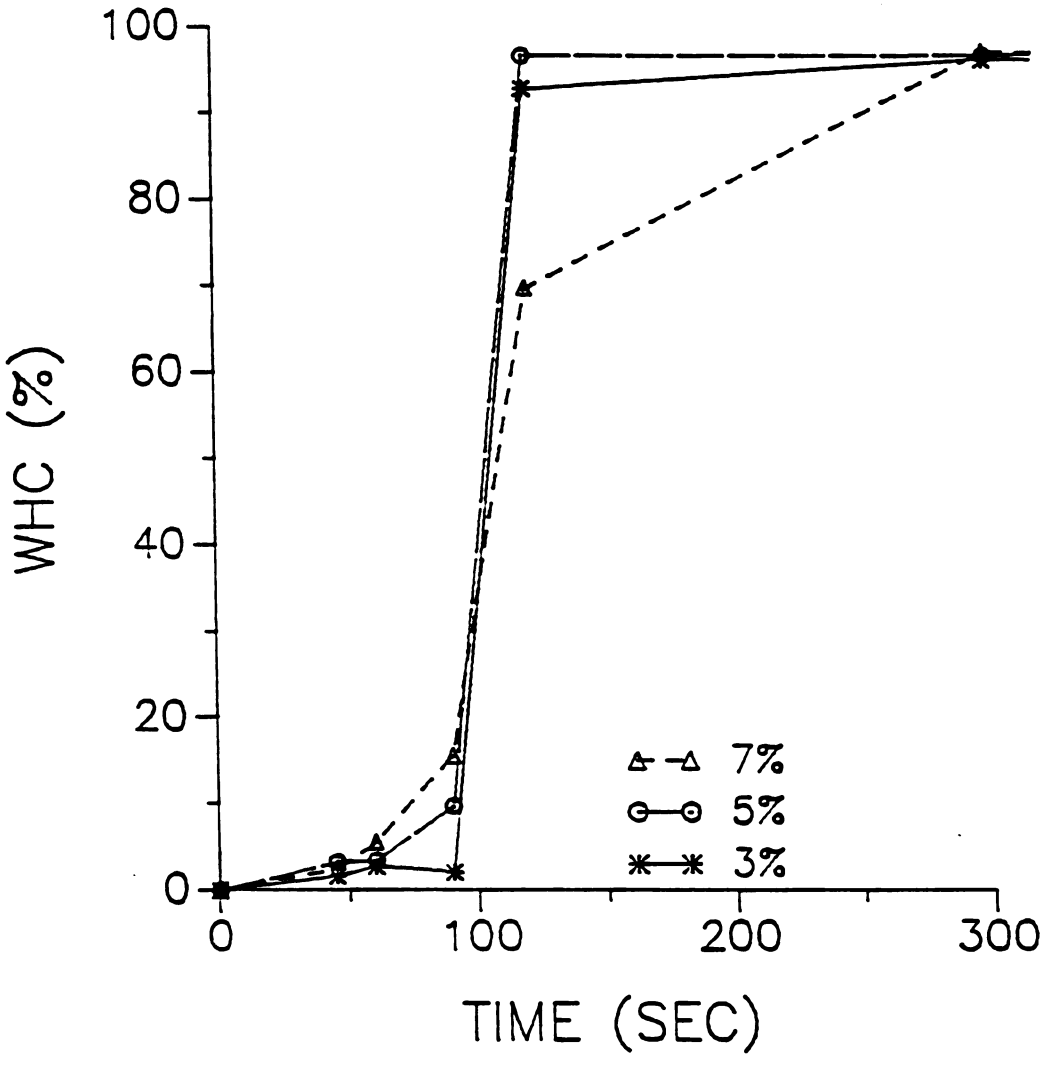


Figure 14. Water holding capacity of ovalbumin gels adjusted to pH 7 prior to heating at 90°C. Duplicate determinations, n = 2.

(Appendix Table D). Increases in heating time greater than 60 minutes showed significantly higher water holding capacity. Also, interactions between protein concentration and time occurred.

In general, the effect of protein concentration on water holding capacity for samples at pH 6 agreed with gel strength data while pH 7 WHC data differed from the gel strength results. Maximum apparent viscosity (gel strength) obtained from gels with pH 7 increased as protein concentration increased. Y'_{∞} values were 21.1 and 67.5 for 3 and 5% protein, respectively. The gel strength optimum for maximum ovalbumin thermal gelation has been reported to be at pH 7 (Egelandsdal, 1980; Hayakawa and Nakai, 1985). This increased gel strength indicates that at this pH the ovalbumin thermal gelation reaction is favored compared to gelation at pH 6. If this is extrapolated to WHC, even at low concentrations (3%) the reaction may occur more readily and reach maximum WHC very rapidly (Figure 14). Hermansson and Lucisano (1982) demonstrated that a change in blood plasma gels structure affected water binding and texture characteristics (penetration force) differently. This may also apply to ovalbumin texture and water binding at pH 7.

Interactions between pH and egg albumen concentration were not observed by Woodward and Cotterill (1986). ES was lower for pH 7 than pH 6 gels which agreed with the results of this study using ovalbumin. Increasing protein content caused a significant increase in gel hardness and decrease

in ES. While in general this was true for WHC ovalbumin gels at pH 6 (Figure 13), no clear trend was found for pH 7 ovalbumin gels (Figure 14).

Free Sulfhydryl and Total Sulfhydryl-Disulfide Content of Ovalbumin Gels

Protein cross-linking by disulfide bond formation has been shown to be important for heat coagulation or gelation (Cunningham and Cotterill, 1962; Seideman et al., 1963; Beveridge et al., 1980). Therefore, it was expected that enhancement or inhibition of this reaction by changes in total SS-SH or free SH would affect ovalbumin gel texture characteristics. A description of the ovalbumin gels analyzed for total SS-SH and free SH content is provided in Table 6, page 76.

NEM and salt addition. Addition of NEM decreased the total SH-SS means of 5% ovalbumin gels adjusted to pH 6 prior to heat-setting at 90°C compared to the control (13.46 and 7.52 moles/10^5 g protein, respectively) (Table 14). A significant decrease in the free SH means also occurred (5.44 for control and 0.10 for NEM). This indicated that addition of NEM inhibited free SH and total SH-SS causing a reduction in sample maximum apparent viscosity (Y'_∞) of 48.1 vs 11.2 (Table 12). Since free SH of ovalbumin gels were almost totally inhibited by NEM addition, total SH-SS values

Table 14. Comparison of chemical addition (ca) and time (t) effect on total sulfhydry-disulfide and free sulfhydryl content of 5% ovalbumin, pH 6.[1,2]

Time (sec)	Control		NEM		NaCl			
	Total	Free	Total	Free	Total	Free	Total	Free
	(moles SH-SS or SH /g protein X 10^5)[3]						$\bar{X}_t$	$\bar{X}_t$
0	14.18	5.88	10.48	0.16	10.71	2.89	11.79[x]	2.98[z]
300	13.35	5.19	6.03	0.08	9.91	2.90	9.76[xy]	2.73[z]
3600	12.84	5.25	6.06	0.09	9.35	2.88	9.42[y]	2.74[z]
$\bar{X}_{ca}$	13.46[a]		7.52[b]		9.99[c]			
$\bar{X}_{ca}$		5.44[d]		0.01[e]		2.89[f]		

[1]Time means within columns ($p<0.05$) and added chemical means within rows ($p<0.001$) with different letters are significantly different.
[2]Two replications for each mean.
[3]SH+SS=total sulfhydryl-disulfide, SH=free sulfhydryls.

represented the S-S content of the gels. Before heating occurred, the NEM samples showed an inhibition in total SH-SS and free SH, 14.18 and 10.48 moles/10^5, control vs NEM, respectively. As time of heating increased, both values decreased 42% and 54%, for total and free, respectively. Possibly, some of the NEM was not completely solubilized until samples were heat-set. More likely, as the ovalbumin monomer was heated, the unfolding of the protein exposed additional SH groups which were then blocked by NEM.

Hayakawa and Nakai (1985) studied the contribution of free SH groups to thermal gelation of ovalbumin. As heating temperature increased above 85°C, free SH decreased and gel strength increased. A significant decrease was also observed for total SH-SS between time 0 and 3600 sec (Table 15, appendix Table G, $p < 0.05$). This relationship was also shown for the control samples of this present study when time 0, and 3600 sec, free SH values were compared (Appendix Table G, $p<0.05$), Table 16).

It was suggested by Hayakawa and Nakai (1985) that decreased free SH (< 1 mole SH/mole ovalbumin) corresponded to a rise in SS resulting in increased gel strength. The authors concluded that conversion of SH to SS groups (oxidation) and the interaction of SH-SS groups were important to gelation. Results found in this current ovalbumin study supported the theory that interaction of the SH-SS were responsible for increased gel strength since SS groups were not shown to increase with heating. It was

Table 15. Evaluation of temperature (T) and time (t) on total sulfhydryl-disulfide content of 5% ovalbumin gels, pH 6.[1,2]

Time (sec)	Temperature (°C) 85	90	95	$\bar{X}_t$
	(moles SH-SS /g protein X 105)[3]			
0	15.03	14.18	14.57	14.58[x]
300	15.37	13.37	11.63	13.56[xy]
3600	12.64	12.84	12.21	12.02[y]
$\bar{X}_T$	14.65[a]	13.46[a]	12.21[a]	

[1]Time means within columns and temperature means within rows with different letters are significantly different. $p<0.05$
[2]Two replications for each mean.
[3]SH = free sulfhydryls, SS = disulfides

Table 16. Evaluation of temperature (T) and time (t) on free sulfhydryl content of 5% ovalbumin gels, pH 6.[1,2]

Time (sec)	Temperature (°C) 85	90	95	$\bar{X}_t$
	(moles SH/g X 10^5)[3]			
0	6.26	5.88	5.99	6.04[x]
300	5.65	5.20	4.08	4.98[xy]
3600	5.49	5.25	4.22	5.17[y]
$\bar{X}_T$	5.80[a]	5.44[a]	4.76[a]	

[1]Time means with in columns and temperature means rows different letters are significantly different. $p<0.05$
[2]Two replications for each mean. [3]SH=free sulfhydryls.

suggested by Buttkus (1974) that very few SH groups have to be oxidized before polymerization can proceed without any further decrease in measurable SH groups. For every SH group incorporated into a SS bond another SH is produced by the mechanism described by Cecil and McPhee (1959).

A reduction in SH content of egg albumen was also shown to occur by Beveridge and Arntfield (1979) as time and temperature increased up to 90°C. They proposed that oxidation of SH to SS or the formation of volatile (Pofahl and Vakaleris, 1968) and non-volatile (Horn et al, 1941) compounds was responsible.

A small decrease in free SH (0.2 mol SH/mol ovalbumin) occurred when Kitabatake et al. (1987) evaluated heat gelation of ovalbumin. This small decrease was thought to be accompanied by a concurrent small rise in SS. Therefore, disulfide bridging formed by the interchange reaction rather than oxidative polymerization of free SH was thought to be responsible for increased protein aggregation.

Blocking of the SH groups with NEM resulted in decreased but not totally inhibited gel strength for 5% protein gels (Y'_{∞}= 11.2). NEM reacted with that portion of the SH groups which were available for reaction (Buttkus, 1974). Therefore, it is possible that some free SH located in the protein's interior were still able to react in intermolecular SH-SS exchange, leading to polymerization (Buttkus, 1974). However, the dramatic decrease in gel strength (Y'_{∞}) caused by the addition of NEM proved the

importance of free SH to ovalbumin gelation. Hydrophobic bonding has also been proposed to have a major role in the heat-setting of ovalbumin (Shimada and Matsushita, 1980a; Ma and Holm, 1982; Kato et al., 1983; Hayakawa and Nakai, 1985). Egelandsdal (1980) suggested that electrostatic forces govern ovalbumin gelation at pH 5.5 to 8.0. These types of bonding may be responsible for ovalbumin gelation in the presence of NEM.

Addition of 3% NaCl also significantly decreased the total SH-SS and free SH content of ovalbumin gels heat-set at 90°C when compared to the control (Appendix Table E $p < 0.001$, Table 14). When compared to the control, gel strength of the NaCl sample increased (Y'_{∞}, 48.1 vs 77.0) even though total SH-SS and free SH contents decreased. Thus, some other bonding may be responsible for the increased gel strength resulting from NaCl addition.

Temperature, concentration and pH effect.

Analysis of the data presented in Tables 15-18 showed that temperature (85, 90, 95°C), protein concentration (3, 5, 7%) and pH (6, 7) did not have an effect on the free sulfhydryl and total disulfide content of 5% heat-set ovalbumin gels. Analyses of variance for these samples are presented in Appendix Tables F, G and H. Lack of differences in free SS and total SH-SS groups with change in concentration could be expected since results are reported per mole of protein.

Table 17. Influence of ovalbumin concentration (Con) and time (t) of ovalbumin thermal gelation at 90°C, pH 6 on total sulfhydryl-disulfides and free sulfhydryl content.[1,2]

Time (sec)	Concentration							
	3%		5%		7%			
	Total	Free	Total	Free	Total	Free	Total	Free
	(moles SH or SH+SS)/g protein X 10^5)[3]						$\bar{X}_t$	$\bar{X}_t$
0	13.81	4.99	14.18	5.86	15.99	4.65	14.66[x]	5.169[z]
300	10.81	4.16	13.35	5.19	10.49	3.31	11.55[y]	4.221[z]
3600	10.48	4.17	12.84	5.26	12.12	3.24	11.82[y]	4.223[z]
$\bar{X}_{Con}$	11.70[a]		13.46[a]		12.87[a]			
$\bar{X}_{Con}$		4.44[b]		5.44[b]		3.73[b]		

[1]Time means within columns and concentration means within rows with different letters are significantly different. $p<0.01$

[2]Two replications for each mean.

[3]SS+SH=total sulfhydryl-disulfides, SH=free sulfhydryls

Table 18. Influence of pH and time (t) of 5% ovalbumin thermal gelation at 90 C on total sulfhydryl-disulfide and free sulfhydryl content.[1,2]

Time (sec)	pH 6		pH 7			
	Total	Free	Total	Free	Total	Free
	(moles SH-SS or SH /g protein X 10^5)[3]				$\bar{X}_t$	$\bar{X}_t$
0	14.18	5.88	14.85	4.32	14.52[x]	20.40[y]
300	13.35	5.19	12.22	3.39	12.79[x]	17.16[y]
3600	12.84	5.25	14.32	3.90	13.58[x]	18.30[y]
$\bar{X}_{pH}$	13.46[a]		13.80[a]			
$\bar{X}_{pH}$		5.44[b]		3.87[b]		

[1]Time means within columns and pH means within rows with different letters are significantly different.
[2]Two replications for each mean.
[3]SH+SS=total sulfhydryl-disulfide, SH=free sulfhydryls.

The SH-SS interchange reaction is enhanced at alkaline pH's (8 and 9) but decreases rapidly as pH 6 is approached in egg albumen. At this pH the S^- concentrations were restricted thus slowing this reaction (Beveridge, 1976). While lower gel strength at pH 6 compared to pH 7 could be explained by this, no measurable differences in the free SH or total SH-SS content in the pH 6 or 7 ovalbumin samples were found. Therefore, the charge on the cysteine sulfhydryl groups rather than the amount of SS or SS groups was restrictive.

While no significant differences occurred with increased heating temperature in this study, Beveridge and Arntfield (1979) showed a decrease in free SH as temperature was increased from 70 to 90°C (40 minutes). This decrease was thought to be caused by conversion of free SH the volatile and nonvolatile compounds. The higher pH (8) and the differences between egg albumen and ovalbumin may account for the differences. In this study with ovalbumin, a significant decrease in free SH was found between the 0 time and 60 minute times for the three temperatures studied. This may have been caused by losses of free SH in the form of volatile and nonvolatile compounds (Beveridge and Arntfield, 1979).

Scanning Electron Microscopic Evaluation of Egg Protein Gels

Two techniques were used to evaluate the microstructure of egg white protein gels using scanning electron microscopy (SEM). The first procedure utilized fixation and postfixation (OTO) followed ethanol dehydration and critical point drying (CPD) of the protein gels. Low temperature scanning electron microscopy (LTSEM) was also used to evaluate gel microstructure. The later incorporated the use of a commercial unit equipped to examine gels at high vacuum and low temperatures.

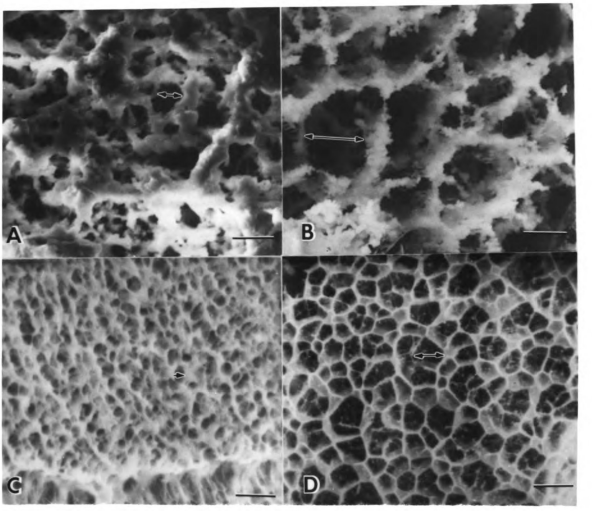
Etching

Etching or surface water removal is necessary in LTSEM to reveal surface morphology and reduce charging in a sample. In order to determine the optimum etching time as related to the appearance of gel microstructure, egg white gel micrographs were taken after one, three and six minutes of etching. Samples were not gold coated so that consecutive changes in the sample could be recorded. While charging and poorer resolution resulted, the micrographs provided insight into the effect of etching on the apparent gel structure using LTSEM. One minute etching of the pH 6 egg white gels (Figure 15a) revealed a gel structure which had varying thicknesses of a protein network and interspace sizes. Protein filaments appeared rounded and fairly smooth, and no ice crystals were observed with this low etch

time and magnification (1,000X). Samples etched six minutes resulted in pores approximately twice the size of the one minute etch (Figure 15b). After six minutes of etching, the protein appeared to consist of interconnected filaments of uneven tiny aggregates.

The etching experiment (1, 3, 6 min) was repeated for gels adjusted to pH 8 prior to heat coagulation. At this pH, egg albumen demonstrated a different, more homogeneous network than that found at pH 6. This web-like, three dimensional structure, also had interspaces which were twice as large after the six minute etching (Figure 15c) compared to the one minute procedure (Figure 15d). The longer etching process probably removed more of the water or ice surrounding the protein filaments, thus producing larger spaces between filaments and clusters. The egg albumen sample (pH 6) etched six minutes had an aggregated structure with grape-like clusters which was very similar to the structure shown by Woodward and Cotterill (1985) using the OTO, CPD method. However, the filamentous protein network of the LTSEM samples was not apparent in the samples produced by the OTO, CPD method. This filamentous structure could be attributed to the freezing technique which may have resulted in formation of very small ice crystals between the proteins thus setting up an apparent network structure (Colombo and Spath, 1981; Hermansson and Buchheim, 1981; Van Kleef, 1986). It is also possible that the 40-50% shrinkage resulting from the CPD method or the ethanol dehydration

Figure 15. Microstructures of 5% egg albumen gels formed at pH 6, 85°C and etched for different times using LTSEM. A. pH 6, 1 min, B. pH 6, 6 min, C. pH 8 1 min, D. pH 8, 6 min. Bar = 10 μ m. Interspaces = arrow.


A
B
C
D

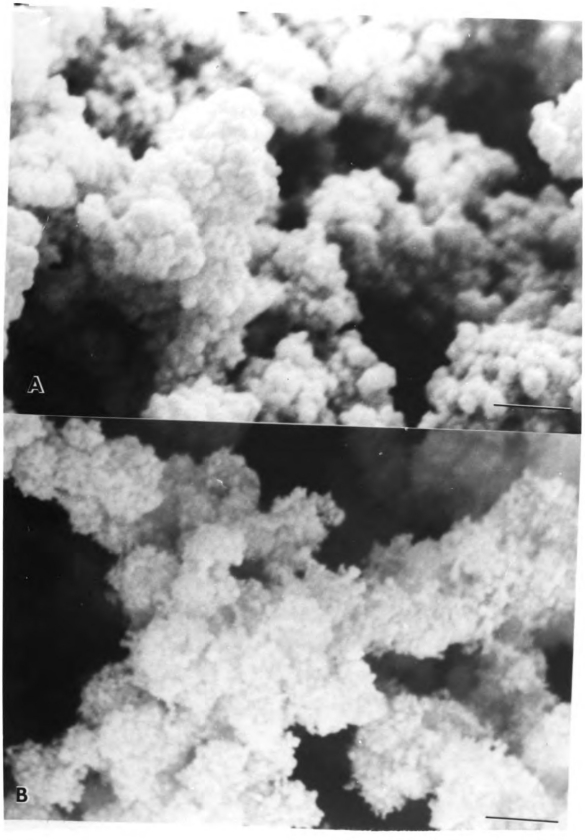
step (Colombo and Spath, 1981; Woodward and Cotterill, 1985; Crang, 1988) could have caused development of an aggregated structure which prevented visualization of the protein network.

Some similarities were observed between egg white gel matrixes produced by both methods of sample preparation. An intermediate etching time of 3 minutes was chosen for all subsequent samples using the LTSEM technique. This was long enough to produce adequate surface structure without excessive drying of the sample or charge build up.

Effect of pH

Five percent ovalbumin gels adjusted to pH 6 and 7 prior to heat setting produced non-homogeneous, grape-like, aggregated protein clusters (Figure 16a and b). This was consistent with the findings of Heertje and van Kleef (1986) who evaluated ovalbumin microstructures at pH 5 which is close to the isoelectric point. They suggested that gelation of ovalbumin at pH 5 proceeded through formation of relatively large aggregates which formed networks. The net charge of a protein in solution can have a distinctive effect on microstructure and texture. Close to the isoelectric point a low net charge exists which results in protein aggregation. It was suggested by Montejano et al. (1984) that the spherical particles in micrographs of low pH gels may be caused by random aggregation which resulted in the non-homogenous structure. Hegg et al. (1979) reported

Figure 16. Microstructures of 5% ovalbumin gels prepared using the OTO/CPD procedure. A = pH 6 , B = pH 7. Bar = 1 μm.


A
B

that thermal aggregation of ovalbumin (4.4%) in a salt free environment only took place around the isoelectric point indicating that the number of net charges on the ovalbumin molecule determines its aggregation. The round spherical aggregates found in pH 6 and 7 ovalbumin gels in the current study supported this type of protein attraction.

In order to examine the conformational changes occurring in ovalbumin, NMR measurements at pH 5 were performed and showed only partial unfolding of the ovalbumin molecule during heating (Van Kleef, 1986). This helped to explain the aggregated spherical appearance seen in OTO/CPD prepared ovalbumin gels at pH 6 and 7. These same samples were examined using the LTSEM technique, and ultrastructures were observed which were different from those seen by the OTO, CPD method (Figure 17a and b). LTSEM involves quick freezing of sample in a liquid nitrogen slush and holding at high vacuum. This limits the size of ice crystals but can't eliminate them totally. Examination of the micrographs showed interspaces which were most likely the result of the ice crystal formation. The pH 6 gel still had an aggregated appearance which exhibited interconnected network formation. Therefore, examination by LTSEM of the pH 6 gels showed an aggregated gel with less shrinkage than that produced by OTO, CPD but with larger interspaces than would be expected without ice crystal formation.

A dramatic difference in microstructure was observed using LTSEM for pH 7 gels compared to that shown by the OTO,

Figure 17. Microstructures of 5% ovalbumin gels prepared using the LTSEM procedure. A = pH 6, B = pH 7, C = pH 6 + 3% NaCl, D = pH 6 + 20 mM NEM. Bar = 10 µm. Interspaces = double headed arrows. Thin protein strands = single headed arrows.

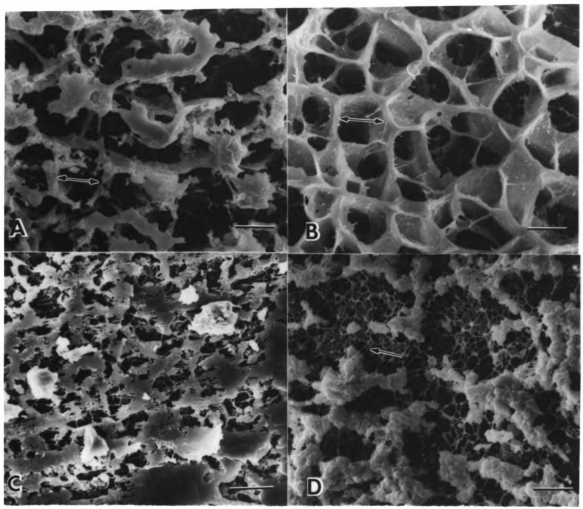
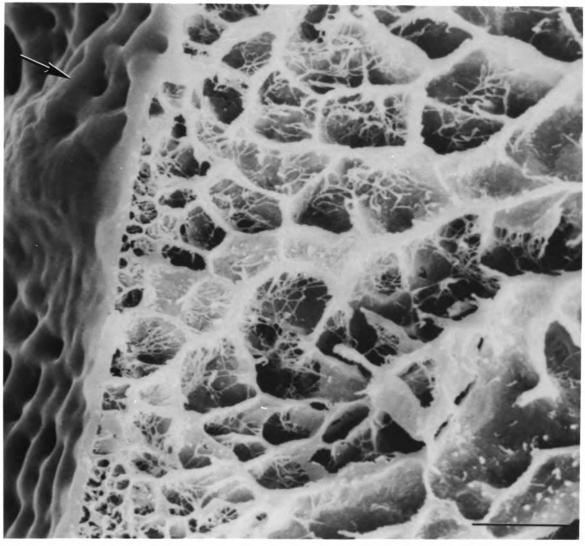

A
B
C
D

Figure 18. Cut surface of 5% ovalbumin gel prepared using the LTSEM procedure. Bar = 10 μm. Arrow = uncut surface.



CPD method (pH 6 and 7) and for the pH 6 gel using the LTSEM (Figure 17b and 18). The large interspaces can be explained by ice crystal formation. In an attempt to eliminate this artifact, Davis and Gordon (1984) added 30% sucrose to a 10% collagen gel which had demonstrated a web-like appearance when viewed using a LTSEM technique. A less open structure resulted which suggested a cryoprotectant effect. However, due to the high attraction of sucrose for water, gels prepared using 30% sucrose may not be totally comparable. High concentrations of sucrose would compete with the protein for the available water which may effect the gel structure. This may explain the more compact appearance of the gel. Addition of sucrose or other chemical additives may control ice crystal size but may introduce artifacts. Therefore, to accurately evaluate gel microstructure, the influence of chemical agents must be properly interpreted.

Optimum gel formation resulting in fine structure and optimum water-binding properties was reported by Ferry (1948) to occur through balancing of attractive and repulsive forces. The smooth ovalbumin network of the sample (LTSEM) could be explained by an increase in repulsive charge at the higher pH of 7. With the repulsive forces balancing the attractive forces, optimum gel forming ability reported for ovalbumin gelation at pH 7 could occur (Egelandsdal, 1980).

Van Kleef (1986) showed even higher gel hardness at higher pHs and high protein concentration. Complete

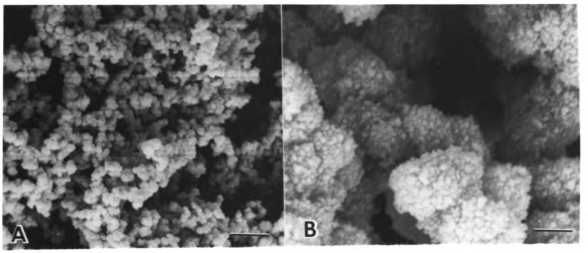
unfolding of the ovalbumin molecule occurred due to heating at pH 10 (Van Kleef, 1986). This resulted in a cellular microstructure in which sheet-like protein fibers were interconnected. The cellular structure was thought to be induced by ice crystal formation during freeze-drying. In another study, Heertje and Van Kleef (1986) examined these same samples and compared freeze-drying and CPD for sample preparation techniques. They found less difference in low pH microstructures which showed an aggregated gel structure which agreed with results of the current ovalbumin study. Microstructures of high pH gels prepared using the CPD method were amorphous, homogeneous, noncellular structures. This structure or protein network had a high breaking stress (hard gels).

It would be expected that the OTO, CPD procedure for ovalbumin gel would have shown this same amorphous structure (Heertje and Van Kleef, 1986) rather than the aggregated ultrastructure (pH 7). It is possible that ovalbumin gelation ultrastructure at pH 7 may be at a transitional point between an inflexible, aggregated protein network and increased chain flexibility which was only detected using the LTSEM procedure.

Effect of NaCl and NEM

While gel strength increased when 3% NaCl was added to 5% ovalbumin (pH 6), ultrastructure differences were small compared to the no salt added samples. These samples were

Figure 19. Microstructure of 5% ovalbumin = 3% NaCl prepared using the OTO/CPD procedure. A = 1,000X, bar = 10 μm, B = 10,000X, bar = 1 μm.


A
B

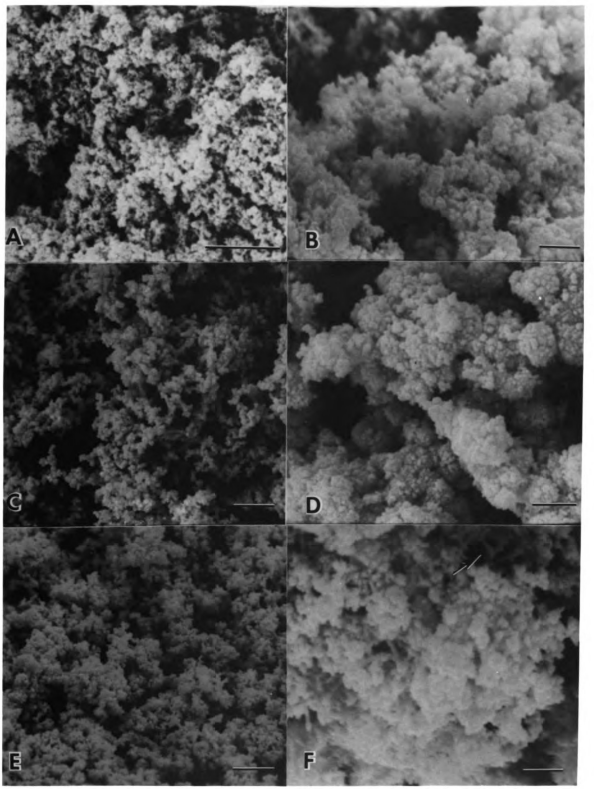
examined using the OTO, CPD procedure (Figure 19) which revealed very similar ultrastructures. Addition of salt is expected to shield repulsive charges, thus promoting protein aggregation (Hegg et al., 1979; Hatta et al., 1986). Increased aggregation caused by 3% NaCl in the gel was enough to increase maximum apparent viscosity which were 63.7 compared to 43.3 for the control. Ultrastructure of NaCl added samples (Figure 17c) prepared using the LTSEM method showed an apparent increase in the amount aggregated protein network which resulted in the increased gel strength.

Because of the soft and fragile gels resulting from NEM addition, samples were not prepared using the OTO, CPD technique. However, LTSEM examination of the samples was possible and ultrastructures demonstrated aggregated protein with no network formation and very thin thread-like strands (Figure 17d). This explained the weak gel strength (Y' = 11.1) and the lack of free sulfhydryls found in these samples. Thus, ultrastructure examination of these samples demonstrated the importance of free sulfhydryls in ovalbumin thermal gelation.

Effect of Isolated Egg Albumen Proteins on Ovalbumin Ultrastructure

Ultrastructures of 5% globulin, conalbumin and lysozyme gels (pH 6) (Figure 20) were very similar when prepared using the OTO, CPD method. Low magnification (1,000X)

Figure 20. Microstructures of three egg albumen proteins (5%, pH 6) prepared using the OTO/CPD method. A = globulins, bar = 10 μm, b = globulins, bar = 1 μm, C = conalbumin, bar = 10μm, D = conalbumin, bar = 1 μm, E = lysozyme, bar = 10 μm, F = lysozyme, bar = 1 μm. Arrow = protein bridge.


A
B
C
D
E
F

showed an interconnected, aggregated protein network. Grape-like clusters were shown for all samples viewed at 10,000X magnification which agreed with results of Johnson and Zabik (1980) for the same proteins. The lysozyme sample (Figure 20e) did appear to be more compact and there was evidence of smaller protein bridges. Gel strength of 5% lysozyme was the second highest of the four proteins examined which seems to be supported by the denser structure and additional bridging. Johnson and Zabik (1981) observed a finer, more compact ultrastructure for lysozyme gels when compared to conalbumin, globulins and ovalbumin gels at pH 8. At pH 6, globulins, ovalbumin and conalbumin are closer to their isoelectric points than lysozyme which has a very high isoelectric point. However, it is unclear how this may have affected differences in gel strength or ultrastructure.

LTSEM examination of globulins, conalbumin and lysozyme showed similar aggregating protein networks to those demonstrated by ovalbumin (Figure 21). In comparison to ultrastructures shown by the CPD, OTO method, the lysozyme sample appeared more dense with thin interconnecting bridges which were more apparent (Figure 21a and b). Globulin's microstructure had the smoothest interconnected chains and interspaces of varying size (Figure 21 c and d). Next to ovalbumin, globulin gels demonstrated the lowest gel strengths at pH 6. While the ultrastructure of conalbumin appeared the most disordered with large spaces between protein chains at 1,000X, closer examination at 5,000X

Figure 21. Microstructures of three egg albumen proteins (5%, pH 6) prepared using the LTSEM method. A = lysozyme, bar = 10 m, B = lysozyme, bar = 1 μm, C = globulins, bar = 10 μm, D = globulins, bar = 1 μm, E = conalbumin, bar = 10 μm, F = conalbumin, bar = 1 μm.

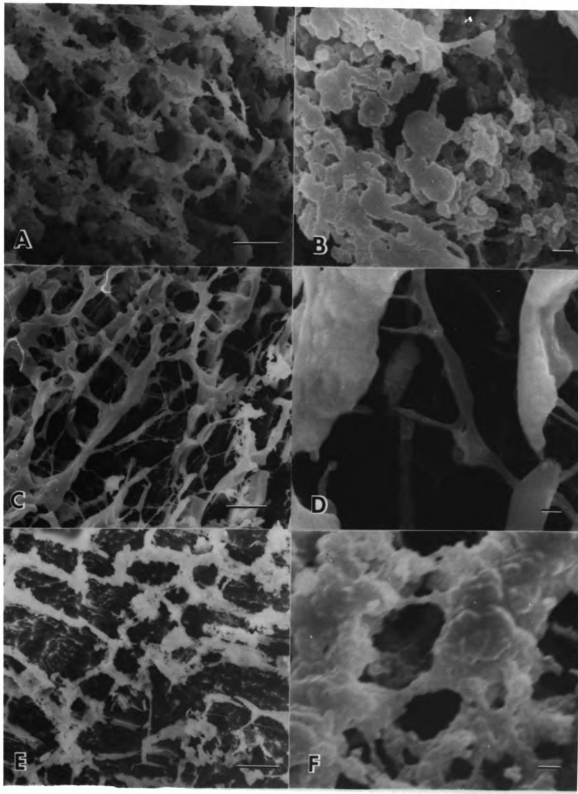
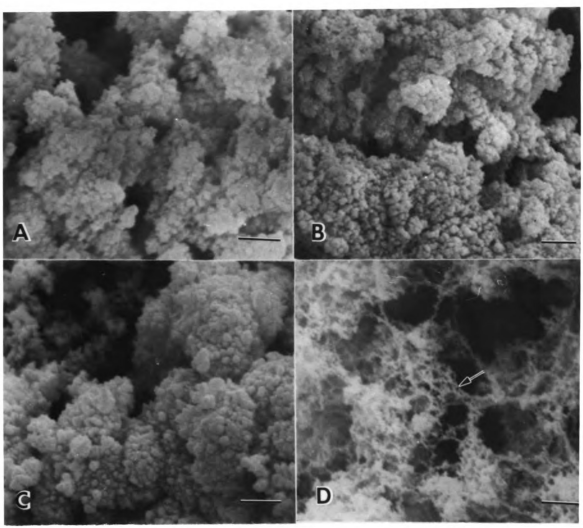

A
B
C
D
E
F

Figure 22. Microstructures of binary mixtures of egg albumen (5%, pH 6) prepared using the OTO/CPD method. A = ovalbumin (O), B = O + globulins, C = O + conalbumin, D = O + lysozyme. Bar = 10 μm.

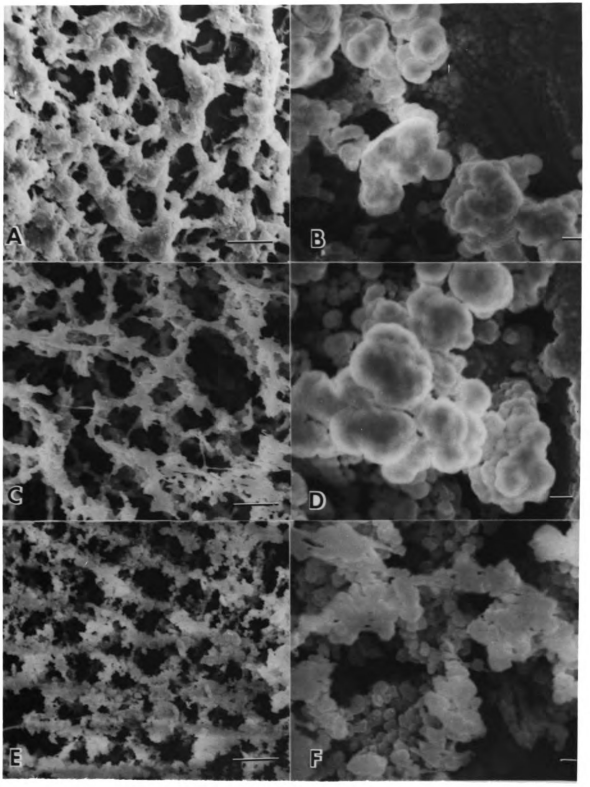

A
B
C
D

demonstrated an aggregated protein network with interconnecting chains (Figure 21 e and f).

Combinations of lysozyme, globulin and conalbumin with ovalbumin were then examined to study protein interaction as shown by ultrastructure evaluation using the OTO, CPD method. No differences were apparent between ultrastructures of ovalbumin alone, and G/O and C/O combinations (Figure 22). While the ultrastructure of lysozyme alone exhibited a network of small chains, the L/O combination microstructure dramatically illustrated fine strands of interconnecting protein. This was not seen with any other samples but could be explained by the ability of lysozyme to bind with other proteins. Matsudomi et al. (1987) found that lysozyme and ovalbumin were combined and heated they interacted due to electrostatic bonding at a molar ratio of 1.5. Cysteine residues were not found to be involved with the reaction. They suggested that interactions among such heterogeneous proteins as ovalbumin and lysozyme in egg white may facilitate heat coagulation of proteins.

LTSEM evaluation of the same combinations of egg albumen proteins (Figure 23) showed little difference between samples. The G/O samples appeared a little more disorganized and showed less thin protein network formation. Conalbumin/ovalbumin samples demonstrated fine networks of aggregated protein. Ultrastructures of the L/O sample were similar to that of G/O and C/O, showing smaller interspaces and a slightly denser appearance.

Figure 23. Microstructures of binary mixtures of egg albumen proteins (5%, pH 6) prepared using the LTSEM method. A = ovalbumin (O) = lysozyme (L), 1,000X, bar = 10 μm, B = O + L, 10,000X, bar = 1 μm, C = O + globulins (G), 1,000X, bar = 1 μm, D = O +G, 10,000X, bar 10 μm, E = O + conalbumin (C), 1,000X, bar = 1 μm, F = O + C, 10,000X, bar = 10 μm.


A
B
C
D
E
F

SUMMARY AND CONCLUSIONS

A modified mathematical model (Morgan et al., 1989; Lever, 1988) was used successfully to predict ovalbumin gelation at pH 6. Gel strength at three protein concentrations and three cook temperatures was characterized by apparent viscosity. Variable temperature-time histories (TTH) accounted for temperature increases from initial to final sample temperature during time of experimental heating. Samples' TTH values at 85, 90 and 95°C were calculated using a denaturation/gelation activation energy (E_a) equal to 38,000 cal/mole. When the combined data for the three temperatures studied were plotted against normalized apparent viscosity (Y') values, good correlation resulted indicating that the chosen E_a was correct.

When pH was increased to 7, or 3% NaCl or 20 mM N-ethylmaleimide (NEM) was added to ovalbumin, the E_a remained constant. The conalbumin gelation reaction demonstrated an E_a equal to that of ovalbumin while lysozyme, globulins or binary mixtures of these proteins possessed E_a equal to 50,000 cal/mole. When conalbumin was combined with ovalbumin (1:1), an E_a equal to 50,000 cal/mole was found.

Calculated model parameters for the basic model were as follows: 1)"a" , the reaction rate, 2) alpha which is a relative measure of the molecular entanglement as a result of gelation, 3) A', a measure of the effective polymer (gel) molecular weight and 4) Y'_∞ , maximum apparent viscosity of

the gel. Parameters "a", alpha and A´ were used in the mathematical model and apparent viscosity values of ovalbumin gelation were predicted. Comparison of experimental values obtained at three temperatures and one protein showed concentration showed good correlation to the predicted values. When cook temperature was held constant and protein concentration was varied, good correlation was also obtained. To further test the model, water holding capacity (WHC) data were substituted for apparent viscosity values. Good correlation was found when WHC at 90°C was plotted against TTH values predicted by the model. This supported the assumption that apparent viscosity was a valid means in which to follow ovalbumin gelation. Also, these results indicated that the mathematical model accurately predicted ovalbumin gelation within the range of variables tested.

Calculated model parameters for the protein samples were compared. Reaction rates (a) for samples (ovalbumin variables and conalbumin) with Ea = 38,000 ranged from 0.425 to 4.2 E+18 with most samples close to 1.0 E+18. Reaction rates for samples with E_a = 50,000 varied from 0.37 to 4.32 E+26 with the exception of lysozyme + ovalbumin (L/O) mixtures. Combination L/O had reaction rates of approximately one order of magnitude greater than the rest of the samples. No clear relationship between rate and protein concentration was observed for these samples.

Values of alpha (molecular entanglement) were close to

1.0 for most gels. This is within the range for alpha reported in the literature for low molecular weight polymers. The exception was the L/O combination which had high alpha values of 5 for 3% and 2 for 5% protein concentration. The ability of lysozyme to readily bind with other proteins may have resulted in the higher values. This would indicate that a higher molecular weight polymer resulted due to the heating of the L/O combination.

As samples were heated to 60 min, apparent viscosity became constant. By assuming an infinite cook time, the mathematical model was simplified and a maximum apparent viscosity value was calculated (Y'_{∞}). This value was proportional to model parameter A' (effective molecular weight of the polymer) and was assumed to be a measure of the functional characteristic, gel strength. Y'_{∞} was then compared to WHC and the total disulfide and free sulfhydryl content of ovalbumin gels.

Maximum apparent viscosity and WHC were higher for ovalbumin gels at pH 7 than pH 6 which was in agreement with other reported studies. No differences were observed either in total SS-SH or free SH in these samples. Increased heating temperature increased the rate at which gelation occurred as evaluated by either apparent viscosity or WHC. Increasing the protein concentration resulted in increased ovalbumin gel strength at pH 6 and 7. While a significant increase in WHC was observed between 3% and higher protein concentrations at pH 6 no differences were found in WHC as

protein concentration increased for samples with a pH of 7. It is possible that once a critical protein concentration is reached, maximum WHC results. This critical protein concentration may vary with pH. No differences were observed for total SS-SH or free SH with change in temperature or protein concentration. After 60 minutes, cooking decreased total SS-SH and free SH. This was thought to be the result of loss of volatile and nonvolatile compounds.

Addition of 3% NaCl enhanced ovalbumin gel strength, however, total SS-SH and free SH were significantly reduced. Therefore, increased gel strength resulted from some other type of bonding. Addition of NEM inhibited gelation by significantly reducing the total SS-SH and free SH. Residual gelation was thought to be due to internal SS-SH reactions or another type of bonding.

Gel strength of 5% protein concentrations were ranked in the following order: conalbumin> lysozyme $\simeq$ globulins > ovalbumin. Gel strength increased as protein increased. Therefore, since ovalbumin is present in the egg albumen at concentrations greater than five times the other proteins, it would still contribute substantially to binding and structure of foods. Combinations of ovalbumin and other proteins resulted in varying amounts of decreasing gel strengths for the proteins (11-36%).

Dramatic differences were observed in the ultrastructures of ovalbumin samples at pH 6 and 7 when

compared using the LTSEM procedure. Ultrastructures of the pH 6 samples were similar when prepared using the CPD/OTO method and LTSEM methods. In the LTSEM samples, aggregated clusters of spheres were found in an inhomogeneous network with large interspaces created due to ice crystals. The ultrastructure of the pH 7 gel was very similar to this when prepared using the CPD/OTO method. However, an interconnecting homogeneous network was observed for the pH 7 gel prepared using the LTSEM technique.

Using LTSEM, the microstructure of ovalbumin gels containing NEM showed groups of aggregated proteins among thread-like strands. This observation supported the gel strength and total SS-SH and free SH results. Using LTSEM, it was shown that addition of 3% NaCl slightly increased the observed amount of aggregated protein in the ultrastructure. However, no differences were observed using the OTO/CPD method.

Conalbumin, globulin, lysozyme and ovalbumin ultrastructures (pH 6) were similar using either method. Lysozyme did show a slightly more compact ultrastructure using the OTO/CPD method with some evidence indicating formation of small protein bridges at the high magnification (10,000X).

Using LTSEM, ultrastructures of binary mixtures of ovalbumin and the other proteins were also very similar. However, using the OTO/CPD method numerous networks of small fragile protein strands were observed with the L/O sample.

This was thought to be the result of the protein binding ability of lysozyme. This binding may facilitate heat coagulation of egg albumen. Binary mixtures of L/O had maximum viscosity values near those of C/O mixtures even though conalbumin had the highest Y_{∞}' when gelled alone.

More than one electron microscopic technique should be utilized to evaluate ultrastructure of high moisture, low protein gels. These gels are fragile and their ultrastructure can be easily altered by added chemicals, dehydration or freezing techniques. As demonstrated in this study, LTSEM microstructures helped to demonstrate the effects of pH and addition of a sulfhydryl blocking agent on gel structure in relation to the textural characteristic of gel strength. The OTO/CPD method illustrated enhanced protein binding by lysozyme which was not shown using the LTSEM technique. Therefore, comparison SEM allows for better evaluation of possible artifacts and for better interpretation of samples' ultrastructures.

SUGGESTIONS FOR FUTURE RESEARCH

The results of the present investigation suggest further examination of the following areas:

1) While egg albumen gelation has been studied extensively, it would be valuable to investigate both low (~ 68- 80°C) and high temperature gelation using the modified mathematical model (Eq. 5). Since egg albumen is often used as an additive to enhance binding in processed foods, model prediction parameters would have more practical value. Also, a larger pH range (pH 5-8) could be investigated. Comparison of E_a for egg albumen and the individual proteins could also be made.

2) It would be of value to use NMR as a measurement of egg albumen (Goldsmith and Toledo, 1985) gelation or isolated egg albumen proteins and examine if the data could be predicted by the mathematical model parameters.

3) Further modification or development of the model could be attempted so that values of B (Eq. 4) could accommodate concentration differences while Mm (molecular weight of the monomer, Eq. 4) would remain constant. Examining protein gelation over a larger concentration range than used in this study is recommended.

4) Globulins (G2 and G3) have not been characterized very well. Also, very little information is currently available pertaining to their thermal characteristics other than that reported by Johnson and Zabik (1981) and in this study. Therefore, temperature, time, concentration, pH, and salt effects on gelation/aggregation could be investigated.

5) Interactions of lysozyme with globulins and conalbumin as a result of heating could be evaluated. Resultant polymers could be studied by using a high-performance liquid chromatograph equipped with a variable wavelength UV monitor (Matsudomi et al., 1987). Comparative SEM as used in this study is also suggested. Increases in the model parameter alpha were observed for gelled mixtures of lysozyme/ovalbumin. It would be valuable to know if gelation of globulin and conalbumin mixtures with lysozyme also produced this effect. The extent of lysozyme binding in egg albumen could be evaluated in this manner.

6) The effect of salts (other than NaCl) commonly used by the food industry on individual protein's gelation could also be studied using the techniques described in this study.

7) Finally, egg protein interactions with other proteins capable of thermal gelation at comparable temperatures could be evaluated utilizing the techniques used in this study.

APPENDIX

APPENDIX

Table A. Nomenclature of terms used in equations.

Term	Description
η	apparent viscosity at a given temperature and time function, K-sec
η_0	apparent viscosity of the protein solution before heating
Y'	normalized apparent viscosity
Y'_∞	maximum normalized apparent viscosity
a	reaction rate constant for protein denaturation and cross-linking
A'	a measure of the effective molecular weight of the unfolded polymerized gel
α	relative measure of molecular entanglement of gel network formation during shear
C	percent protein, dry weight basis
E_a	denaturation/gelation activation energy, cal/mol
B	constant (denaturation viscosity material constant)
M_m	theoretical molecular weight of the monomer
M_c	initial monomer concentration
P_c	reactive polymer species concentration

Table B. Mean squares of analyses of variance of temperature and time on water holding capacity for 5% ovalbumin, gelation pH 6.

Source	Degrees of Freedom	Mean Squares
Temperature (T)	2	433.94 ***
Time (t)	3	1,953.27 ***
T X t	6	88.95 **
Error	12	14.22

*** p <0.001, ** p < 0.01

Table C. Mean squares of analyses of variance of protein concentration and time on water holding capacity for ovalbumin heat-set at 90°C.

Source	Degrees of Freedom	Mean Squares	
		pH 6	pH 7
Concentration	2	50.56***	185.39**
Time	3	9,960.16***	1,601.69***
T X t	6	156.17*	27.59***
Error	12	4.82	7.94

*** p < 0.001, ** p < 0.01, * p < 0.05

Table D. Mean squares of analyses of variance of pH and time effect on water holding capacity for 5% ovalbumin heat-set at 85, 90, and 95°C.

Source	Degrees of Freedom	Mean Squares		
		85 (°C)	90 (°C)	95 (°C)
pH	1	399.92*	1,040.66***	2,925.78***
time (t)	6	6,659.17***	6,355.63***	5,195.89***
pH X t	6	288.00**	461.84***	452.74***
Error	14	49.32	29.23	7.58

*** p < 0.001, ** p < 0.01, * p < 0.05

Table E. Mean squares of analyses of variance of added chemicals and time for total SS-SH and free SH of 5% ovalbumin, pH 6, and heat-set at 90°C.

Source	Degrees of Freedom	Mean Squares	
		Total	Free
Chemicals (C)	2	53.40***	42.64***
Time (t)	2	9.83*	0.12
C X t	4	2.55	0.09
Error		1.66	1.06

*** p < 0.001, * p < 0.05

Table F. Mean squares of analyses of variance of pH and time for total SS-SH and free SH for 5% ovalbumin and heat-set at 90°C.

Source	Degrees of Freedom	Mean Squares	
		Total	Free
pH	1	0.34	7.39
Time (t)	2	3.00	0.67
pH X t	2	1.79	0.05
Error	6	4.94	1.69

Table G. Mean squares of analyses of variance of temperature and time for total SS-SH and free SH for 5% ovalbumin, pH 6.

Source	Degrees of Freedom	Mean Squares	
		Total	Free
Temperature (T)	2	4.67	1.62
Time (t)	2	13.26*	8.44*
T X t	4	1.37	1.59
Error	9	1.97	1.32

* $p < 0.05$

Table H. Mean squares of analyses of variance of protein concentration and time for total SS-SH and free SS for 5% ovalbumin heat-set at 90°C, pH 6.

Source	Degrees of Freedom	Mean Squares	
		Total	Free
Concentration (C)	2	4.82	4.40
Time (t)	2	17.82**	1.79**
C X t	4	2.88	0.10
Error	9	2.05	2.26

** $p < 0.01$

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