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COMPOSITION AND FUNCTIONAL PROPERTIES
OF INSOLUBILIZED WHEY PROTEIN CONCENTRATES
IN MODEL GEL AND FRANKFURTER SYSTEMS

presented by

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of the requirements for

Masters degree in Food Science

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COMPOSITION AND FUNCTIONAL PROPERTIES
OF INSOLUBILIZED WHEY PROTEIN CONCENTRATES
IN MODEL GEL AND FRANKFURTER SYSTEMS

By

Bryan C. Beuschel

A THESIS

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ABSTRACT

COMPOSITION AND FUNCTIONAL PROPERTIES OF INSOLUBILIZED WHEY PROTEIN CONCENTRATES IN MODEL GEL AND FRANKFURTER SYSTEMS

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Bryan C. Beuschel

Liquid whey protein concentrate (WPC) was heat-processed to prepare treatments with protein solubilities ranging from 27% to 98% in 0.1M NaCl, pH 7.0. Effects of WPC on chicken breast salt-soluble protein (SSP) gels was determined by preparing SSP:WPC gels in 0.6M NaCl, 0.05M Na phosphate buffers, pH 6.0, 7.0 or 8.0 at 65 or 90°C. Elasticity of gels containing 4% SSP and 12% WPC increased with decreased WPC solubility at 65 and 90°C. Decreased WPC solubility decreased expressible moisture (EM) and increased gel strength for SSP:WPC gels at 65°C, but increased EM and decreased gel strength at 90°C. In general, SSP:WPC gels were more firm, less elastic and expressed less moisture when pH increased from 6.0 to 8.0. Results from this study suggest that WPC's with varying protein solubilities may be useful for improving yield and altering texture of processed meats under specific conditions.

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INTRODUCTION

Comminuted meat products are complex systems in which salt-soluble proteins form heat-induced gels that bind fat and water while providing texture to the product. New meat products are being introduced which contain less salt and fat. These changes can adversely affect the textural and water-holding characteristics of the meat product.

Nonmeat proteins of plant and animal origin have been used in comminuted meat products as binders, extenders and fillers to improve their textural and water-holding characteristics (Parks and Carpenter, 1987). One of these protein-containing products, whey protein concentrate (WPC), has received much attention. Several studies using whey products in processed meat systems have been conducted (Lauck, 1975; Lee et al., 1980; Thompson et al., 1982), but effects on yield and texture have been conflicting. Part of the reason that WPC has not produced consistent results when used in processed meats may lie in the inherent sample-to-sample variability in composition and functional properties of commercial whey products. Much of this WPC variability can be traced to whey processing methods and conditions. Of particular importance is the exposure of WPC to heat which

insolubilizes the proteins and changes functional properties. Little work has been done relating the solubility of WPC to its performance in heat set meat protein gels and processed meat systems.

The objectives of this study were:

- (1) To produce WPC's with a range of protein solubilities;
- (2) To characterize the WPC's;
- (3) To evaluate the effect of heating temperature, pH, and WPC solubility on the gel properties of these proteins alone and in combination with chicken breast salt-soluble proteins;
- (4) To evaluate the effect of WPC substitution on the yield and textural parameters of chicken frankfurters.

REVIEW OF THE LITERATURE

2.1 Introduction

Milk proteins have been used as ingredients in food products for many years. The extensive use of these products is directly attributable to their high nutritional value and unique functional properties in food systems (Morr, 1979). Non-fat dry milk and dried whey powder have been the principle sources of milk protein as food ingredients (Morr, 1979). Other dairy proteins include Na, Ca, and K caseinate, which are the salts of casein, and coprecipitates, which are a combination of whey proteins and casein (Tobelman, 1979). Increased emphasis has been placed on the study and use of whey protein concentrate (WPC) in food systems.

Whey is a by-product of cheesemaking. According to the American Dairy Products Institute, approximately 778.4 million kilograms of whey and modified whey products were produced in the United States in 1988 (Przybyla, 1989). About 356.3 million kilograms of these whey products were used for human consumption, 21.9 million kilograms of which were in the form of WPC.

Cheese whey is a dilute protein solution (Table 1). Consequently, there is a need to remove lactose

in order to increase the protein content of whey products. Whey protein concentrates are products in which sufficient lactose, minerals, and water have been removed to establish a protein content of 25% or more (Swartz, 1983). Depending on the method of processing, protein concentration may be as high as 90% (Morr, 1982).

Table 1. Typical composition of liquid and dry cheese whey (from USDA Handbook No. 8-1, 1976)

Component	Liquid (%)	Dry (%)
Protein	0.9	12.9
Lactose	5.1	74.5
Ash	0.5	8.4
Fat	0.4	1.1
Water	93.1	3.2

2.2 Whey Proteins

Whey proteins are collectively categorized as those nitrogenous compounds which remain in solution upon precipitation of casein at pH 4.6 (de Wit, 1981). About 20% of this fraction is not protein, but proteose-peptones and nonprotein nitrogen (de Wit, 1981). Beta-lactoglobulin (β -lg), α -lactalbumin (α -la), bovine serum albumin (BSA), and the immunoglobulins are the most important proteins in whey.

Beta-lactoglobulin is the most abundant protein, constituting greater than 60% of the whey proteins (Kim et

al., 1987). As shown in Table 2, it has an isoelectric point (PI) of 4.2 and a molecular weight (MW) of 18,400 (de Wit, 1981). It exists as a dimer with a MW of 36,700 at room temperature and pH values from 5.5 to 7.0 (Swaisgood, 1982). Each monomer contains two disulfide bridges and a free thiol group (de Wit and Klarenbeek, 1984). Dissociation of the monomers occurs above 40°C (de Wit and Klarenbeek, 1984). Beta-lactoglobulin is low in α -helix and high in β -sheet structure (Swaisgood, 1982).

Table 2. Molecular weights (MW) and isoelectric points (IP) of the major whey proteins (from de Wit, 1981)

Protein	MW	IP
β -lactoglobulin	18,400	5.2
α -lactalbumin	14,200	5.1
Bovine Serum Albumin	66,000	4.8
Immunoglobulin	160,000	5.5

The second most important whey protein is α -lactalbumin comprising about 12% of the total whey protein fraction (Morr, 1985). It is the most heat-resistant and smallest of the whey proteins (de Wit, 1981). It has a molecular weight of 14,200 and a PI of 5.1 (Table 2). The eight half-cystine molecules interact to form four intramolecular disulfide bridges which allow for a reversible conformational change under certain conditions upon heat denaturation (de Wit and Klarenbeek, 1984). It also possesses several carboxyl

groups which bind calcium tightly and are responsible for conformational change at pH 4.0.

Bovine serum albumin (BSA) makes up about 6% of the total whey protein (de Wit and Klarenbeek, 1984). Thirty-four of its half-cystine residues react to form intramolecular disulfide bridges, and one exists as a free thiol. It has a MW of 66,000 and a PI of 4.8. The BSA molecule will denature at a pH of 4.0.

The largest of the whey proteins are the immunoglobulins IgG, IgA, and IgM which represent about 9% of the total whey proteins. Immunoglobulin G has a MW of 160,000, a PI of 5.5, and makes up about 80% of the total immunoglobulin fraction (de Wit and Klarenbeek, 1984). The immunoglobulins are tetramers consisting of two light and two heavy chains bound together by disulfide bridges. They are also very heat sensitive.

2.3 Whey Protein Concentrate Production

Some of the methods for whey protein recovery include electrodialysis, precipitation by various complexing agents, gel filtration, and ultrafiltration. Electrodialysis yields a product low in salts but still relatively high in protein (Hann et al., 1983). An electrodialysis unit consists of alternating stacks of anion and cation exchange membranes with an anode and cathode at either end (Figure 1). When a current is applied, ions move toward the anode or cathode but will not pass through the charged membranes. This

results in two solutions, one of low ionic strength (D) and one of high ionic strength (C) (Hann et al., 1983).

Under the proper pH, concentration, and ionic strength conditions, complexing agents such as polyphosphate can be added to precipitate whey proteins, but the proteins may be highly denatured (Sternberg et al., 1976). Thus, these products have limited solubility and functionality in low pH foods (Morr, 1976). Gel filtration is a molecular sieving process which separates the larger protein molecules from the smaller salt and lactose molecules (Marshall, 1982). The drawbacks of this process are the low concentration of protein in the effluent and the high capital cost.

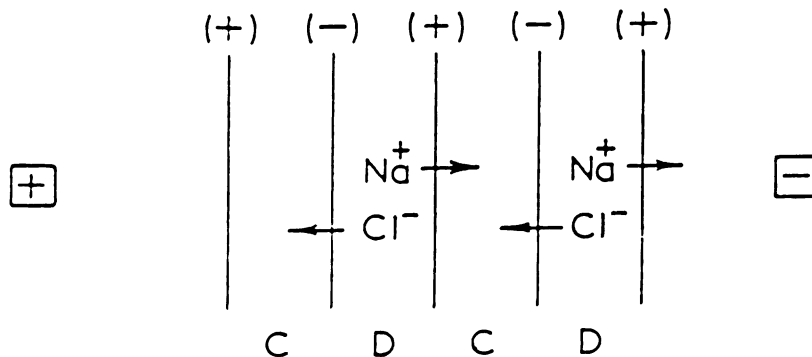


Figure 1. Diagram of an electrodialysis unit (from Hann et al., 1983).

Ultrafiltration (UF) is the most commonly used method of concentration. The whey is pumped across a membrane made of a synthetic polymer (usually polysulfone) which retains the protein while allowing water, salts and lactose to pass through (Marshall, 1982). High velocity tangential flow

reduces concentration polarization on the membrane surface which facilitates a more efficient passage of water and small molecules (Kosikowski, 1986). Multiple stages of UF can be used to make the process continuous. The retentate may also be recirculated to control the concentration of the final product. Membranes with a MW cutoff of 10,000-20,000 are most common in the food industry (Kosikowski, 1986). Figure 2 shows the configuration of a spiral-wound membrane.

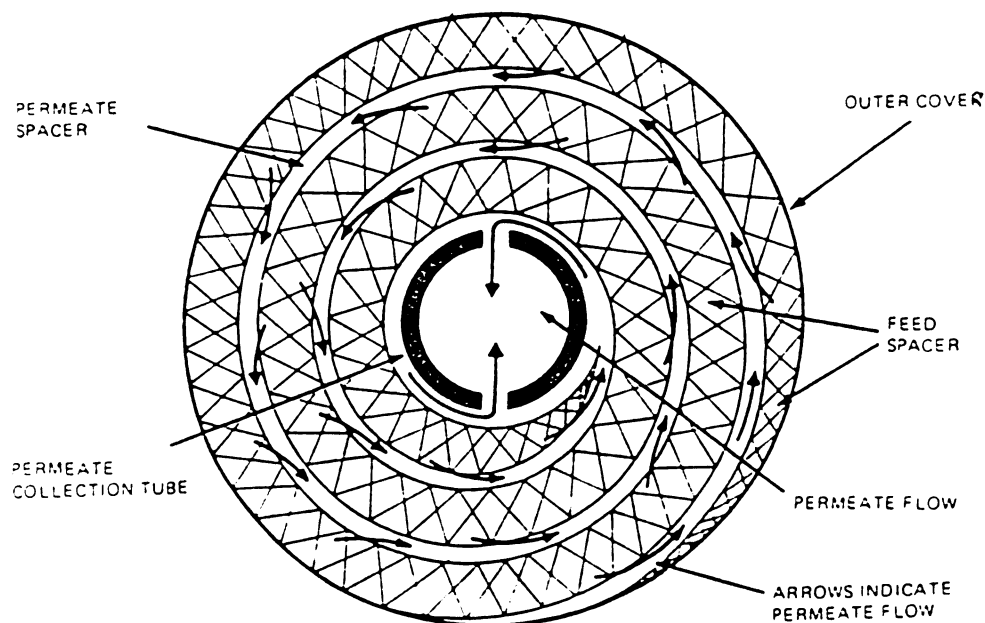


Figure 2. Configuration of a spiral-wound membrane (from Hanisch, 1986).

This configuration is popular because a large amount of surface area can be packed in a small space. Diafiltration, an adaptation of ultrafiltration, is the controlled addition of water to the retentate to remove additional salts and lactose (Kosikowski, 1986). The mild pH and temperature

conditions of ultrafiltration and diafiltration maintain the functionality of the final product.

2.4 Gelation

Gelation, a functional characteristic of some food ingredients such as proteins and certain polysaccharides, is very important to the acceptance of solid and semisolid foods. Gels contribute texture, and bind water, fat and other components in certain products (Hermansson, 1983; Smith, 1988). In many meat and dairy products, protein-protein interactions are responsible for these characteristics.

Gelation is thermally induced in some food systems, including processed meats. Denaturation is the first step in Ferry's (1948) protein gelation model. Kauzmann (1959) defined denaturation as "a process in which the spatial arrangement of the polypeptide chains within the molecule changed from that typical of the native protein to a more disordered arrangement." Ferry (1948) suggested the following mechanism: $xP_n \rightarrow xP_d \rightarrow (P_d)$ where x represents the number of protein molecules and n and d are the native and denatured forms, respectively. Protein gelation is the ordered interaction of native or partially denatured protein molecules to form an ordered three-dimensional network (Hermansson, 1978).

2.5 Muscle Proteins

Muscle proteins are classified as myofibrillar, sarcoplasmic or stromal. The myofibrillar fraction constitutes 50-55% of the total protein in muscle (Morrissey et al., 1987) and is soluble in moderate to high ionic strength solutions (Forrest et al., 1975). The sarcoplasmic proteins represent 30-34% of the total protein and are soluble in low-salt solutions. Stromal proteins make up the remainder of the muscle proteins and are insoluble in aqueous solutions. The myofibrillar proteins are the most functional of these classes in meat systems (Smith, 1988).

Myosin accounts for about 45% of the total myofibrillar protein (Morrissey et al., 1987). It has a MW of about 450,000 (Ziegler and Acton, 1984), and a PI of about 5.4 (Forrest et al., 1975). Ziegler and Acton (1984) described myosin as a hybrid molecule with two globular heads attached to a rod-like "tail." Two light chains with MW of about 20,000 are associated with each globular head (Hultin, 1985). The two light chains are further divided into two classes - DTNB [(S,S-dithiobis)-2-(nitrobenzoic acid)] light chains and alkali light chains. The DTNB light chains are so named because they are dissociated from the globular head upon treatment with DTNB (Morrissey et al., 1987). Alkaline solutions above pH 11 will cause the alkali light chains to dissociate.

When myosin is treated with the enzyme trypsin, it is cleaved near the head (Figure 3). The rod-like portion with

a MW of 150,000 is termed light meromyosin (LMM), while the head (MW=350,000) is called heavy meromyosin (HMM) (Morrissey et al., 1987). Heavy meromyosin may be further separated into HMM subfragment 1 (S-1) containing the globular head and subfragment 2 (S-2). Actin constitutes 20-25% of the myofibrillar protein (Smith, 1988) and has a MW of 43,000-48,000 (Hultin, 1985). G-actin is the globular monomeric form of actin and has a PI of approximately 4.7. It polymerizes in the presence of magnesium to form fibrous actin (F-actin). Actomyosin results when actin and myosin interact during muscle contraction (Bechtel, 1986). This interaction is the primary cause of toughening in meat that has gone into rigor.

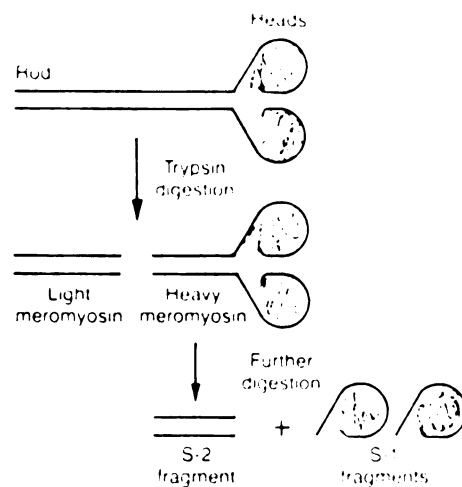


Figure 3. Proteolytic fragments of myosin (Bechtel, 1986).

Several minor myofibrillar proteins also exist. Tropomyosin is a two-stranded coiled-coil with an α -helix configuration and a MW of 65,000-70,000 daltons (Hultin, 1985). Tropomyosin molecules lie end-to-end along the

grooves of the actin α -helix. Troponin consists of three subunits, one of which is sensitive to calcium (Troponin C). Actinin is a class of minor myofibrillar proteins that are involved in the physical structure of actin (Maruyama, 1971).

2.6 Meat Protein Gelation

Proteins exhibit changes in enthalpy when their structure and conformation change upon heating (Wang, 1990). Differential scanning calorimetry (DSC) makes it possible to measure these changes and determine transition and denaturation temperatures. Wright et al. (1977) found that isolated myosin yielded one, two, or three transition temperatures (T_m), depending on the salt concentration of the solution. These transitions occurred at 43, 49.5, and 60.5°C when observed at pH 6.1 and an ionic strength of 1.0. However, at pH 7.0 and .046M salt concentration, only one T_m (55°C) was found. Similarly, Samejima et al. (1983) in studies with myosin and subfragments prepared from rabbit skeletal muscle, observed large shifts in the values of T_m as well as a reduction in the number of transitions when pH and ionic strength were changed. A DSC thermogram of prerigor native chicken breast muscle (pH 5.6) revealed peaks at 57, 62, 67, 72 and 79°C (Xiong et al., 1987). They attributed the peak at 57°C to myosin and the peak at 79°C to actin. Conformational changes in sarcoplasmic protein structure resulted in peaks at 62, 67 and 72°C.

Under certain environmental conditions, some denatured protein might refold into the native conformation. In this case, rescanning of the same sample by DSC should produce an identical thermogram. Results by Wright et al. (1977) and Quinn et al. (1980) indicated the denaturation of myofibrillar proteins is not reversible. Asghar et al. (1985) reported a gelation mechanism in which polypeptide chains cross-link to form five to six crystalline regions per molecule. Other molecules locate themselves between these strands, accounting for the gel's flexibility.

Hultin (1985) described the gelation of meat proteins and indicated myosin was the most important component. F-actomyosin acts as a cross-linker with free myosin on heating (Yasui et al., 1982). Optimal gelation occurred when the weight ratio of myosin-to-actomyosin was 4:1. The myosin head irreversibly aggregates through the oxidation of -SH groups which, in turn, contributes to the formation of the three-dimensional protein network (Hultin, 1985; Samejima et al., 1981). During heating the tail portion of myosin undergoes a partially irreversible helix-to-tail transition and then participates in the three-dimensional network.

Factors which affect gel strength include heating rate, pH and ionic strength. Foegeding et al. (1986) studied the influence of heating rate on gel rigidity of myosin, fibrinogen, and albumin singly or in combination. They found that rapid heating (ie. 70°C for 20 min) produced a

less stable gel than heating by a linear 12°C/hr increase to 70°C. They suggested that because myosin and fibrinogen are multi-domain proteins, the variation in gel matrix could be due to the association of proteins in different denatured states. Montejano et al. (1984) reported similar results with fish surimi.

Salt concentration is another factor influencing gel strength. Ishioroshi et al. (1979) heated myosin solutions ranging from 0.1M to 0.6M KCl to 65°C at pH 6.0. The resulting gels showed the highest shear modulus between 0.1M and 0.2M KCl. A finer three-dimensional network was formed at 0.2M rather than at 0.6M KCl concentration. Work done by Hermansson et al. (1986) confirmed this observation. Maximum gel strength at concentration of 0.6M KCl has been reported at pH 6.0 (Ishioroshi et al., 1979; Yasui et al., 1980). However, the pH for optimal gel strength has been shown to be affected by salt concentration (Ishioroshi et al., 1983). Maximum gel strength for myosin in 0.2M KCl occurred at pH 5.5 and at pH 6.0 for a 0.6M KCl myosin solution.

2.7 Whey Protein Concentrate Gelation

A functional property which makes WPC valuable in food systems is the ability to form firm heat-set gels which imbibe large quantities of water (Hermansson and Akesson, 1975a, 1975b). Schmidt and Morris (1984) described whey protein gelation as the physical manifestation of heat-

induced protein denaturation reactions occurring at high protein concentration. They suggested that whey protein gelation follows the classical two step model illustrated by Ferry (1948). They cautioned, however, that more work is needed to understand the initial protein unfolding.

Beta-lactoglobulin is the most important protein in the formation of WPC gels with BSA, α -lactalbumin and the immunoglobulins playing lesser roles (Kim et al., 1987). de Wit and Klarenbeek (1984) used DSC to determine the temperature at which individual whey proteins unfold in a 0.07M phosphate buffer with a pH of 6.0. They observed transition temperatures of 68, 83, 89 and 70°C for α -la, β -lg, IgG and BSA, respectively. In spite of its low T_m , α -la is the protein least effected by heat treatment because it is able to refold into its original configuration upon cooling (de Wit and Klarenbeek, 1984).

Paulsson et al. (1986) used dynamic rheological testing to study the gelation of individual whey proteins in a 1% NaCl solution. They found that at pH 6.6, the minimum protein concentrations necessary to form gels from BSA and β -lactoglobulin were 1% and 2%, respectively. Alpha-lactalbumin failed to form a gel at protein concentrations up to 20% (w:v). Gels made from BSA were purely elastic, while β -lg gels were more viscoelastic.

There are several factors which can affect the rate of formation, appearance, and physical properties of WPC gels. The process history of the product can greatly affect its

functional properties. Mangino et al. (1987) pasteurized milk, whey, and retentate alone or in combination with each other and made WPC. They found that pasteurization of the whey had no effect on gel strength, but pasteurized milk had decreased gel strength at pH 6.5. Pasteurization of retentate decreased gel strength at pH 8.0. Dunkerley and Zadow (1981) reported that gels formed from WPC made from cheddar whey pretreated at 72°C for 15 sec had gel firmness comparable to egg white at a pH range of 3 to 8. When whey pretreatment was 80°C for 15 sec, the resulting WPC produced gels which exhibited poor firmness at pH 3 and 5, but increased firmness at pH 8.

Components of WPC may affect gelation. Schmidt et al. (1978a) compared time necessary to gel different WPC's at 100°C. Tubes were pulled at 30 sec intervals, cooled in an ice bath and visually compared with reference whey protein gels with subjective gel firmness ratings of 0 to 5. Gelling time was that time necessary to form a gel with a rating of 4. They reported that differences in gelling time could not be explained by compositional differences. However, the WPC giving the fastest gel formation did contain higher protein:lactose and protein:fat ratios than other WPC's. They suggested that specific protein composition and denaturation may play a role. In addition, they observed that dialyzed WPC (100°C/15 min) resulted in stronger, more cohesive, less springy, and more translucent gels than did non-dialyzed WPC.

Calcium, a salt present in relatively high concentrations in whey, has been shown to affect the rheological properties of WPC gels (Mulvihill and Kinsella, 1987). Minerals are important because they affect the ionic strength which influences the attractive and repulsive forces between proteins in solution (Hermansson and Akesson, 1975b). Schmidt et al. (1978a) found that hardness and resistance to penetration of dialyzed WPC gels were maximized with addition of CaCl_2 from 5 mM to 20 mM and decreased with addition of 25 mM CaCl_2 . Addition of 0.2 M to 0.5 M NaCl to WPC gels increased resistance to penetration. Hardness values of WPC gels increased with NaCl concentrations up to 0.3 M, but decreased at 0.4 M NaCl. Addition of CaCl_2 at or above 5 mM or NaCl at or above 0.1 M decreased both springiness and cohesiveness of WPC gels. Johns and Ennis (1981) reported that replacement of calcium ions by sodium in acid casein whey improved the isoelectric protein solubility when WPC was produced by ultrafiltration. Gels made from this WPC exhibited increased hardness, cohesiveness and springiness with increased replacement of calcium by sodium.

Disulfide bonds play an integral role in the structure of heat induced whey protein gels (Schmidt and Morris, 1984). Protein molecules may be cross-linked by oxidation of SH groups to disulfide bonds and/or SH-induced disulfide interchange reactions (Shimada and Cheftel, 1989). Schmidt et al. (1978b) reported that addition of cysteine up to 25 mM

increased whey protein gel strength, but 100mM dramatically decreased gel strength. These gels will dissolve if a sulfhydryl reagent is added (Hillier et al., 1980). Using regression and response surface analysis, Schmidt et al. (1979) found that predicted maximum values for hardness, springiness, and cohesiveness of gels formed from dialyzed WPC occurred at cysteine concentrations of 9.7mM, 10.3mM, and 13.9mM, respectively.

While disulfide bonds are important in whey protein gel formation, Kohnhorst and Mangino (1985) determined that sulfhydryl content did not vary enough in the WPC gels to be an accurate predictor of gel strength. They developed a model in which calcium concentration was inversely related and hydrophobicity was directly related to gel strength. Shimada and Cheftel (1988) observed that apparent number of S-S bonds, as calculated from SH groups and half-cysteine content, did not change significantly as the protein concentration was increased. They concluded that increased gel strength due to increased protein concentration was not dependent on an increase in the total number of S-S bonds.

The pH of a whey protein solution is very important in determining the textural characteristics of the resultant heat induced gel (Schmidt and Morris, 1984). Schmidt et al. (1978b) reported that gel strength of WPC decreased as pH increased from 7.0 to 9.0. This finding has been confirmed by other researchers (Zirbel and Kinsella, 1988; Shimada and Cheftel, 1988). Hillier et al. (1980) noted that alkaline

pH's slowed gelling times. They postulated that the proteins became more negatively charged facilitating electrostatic repulsion and less protein-protein interaction. Zirbel and Kinsella (1988) also found that Whey Protein Isolate (WPI) gel springiness and cohesiveness increased as pH increased from 6.0 to 8.0. They hypothesized that neutral pH values enhance SH/S-S interchange reactions resulting in more elastic gels. At pH 2.5, whey protein gels were inelastic. They attributed gel formation at this pH to hydrogen bonding due to increased protonation of carboxyl groups. Dunkerley and Zadow (1988) described the texture of WPC gels at pH 3.0 and below as a smooth gel, at 3.7 to neutrality as smeary or pasty, and from 7.3 to 7.8 as moist and rubbery. At neutral pH's, hydrophobic interactions contribute strongly to whey protein gel formation (Shimada and Cheftel, 1988).

2.8 Multicomponent Gel Systems

A mixed gel system contains two or more different polymers capable of forming a gel. Individual components may have an adverse or favorable effect on gelation and texture depending on the specific food system (Oakenfull, 1987). Thus, selection of different gelling ingredients can be used to impart specific textural qualities in a food product.

Morris (1985) described the components within a gel system as either "active" or "inactive." "Active"

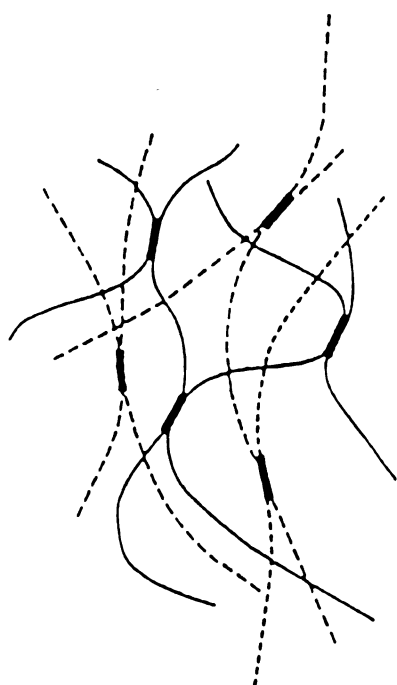
components form part of the structure while "inactive" components reside within the network. He further divided mixed gel networks into three categories: interpenetrating, phase separated, and coupled.

Interpenetrating gels are gels in which the individual components form separate networks that associate only by entanglement (Fig. 4a). A phase separated gel consists of a matrix of one component gel enclosed within the matrix of another component gel (Fig. 4b). An example is two polymers which form heat-set gels at different temperatures. A third gel is termed the coupled network (Fig. 4c). In this system, different polymers associate to form one gel network.

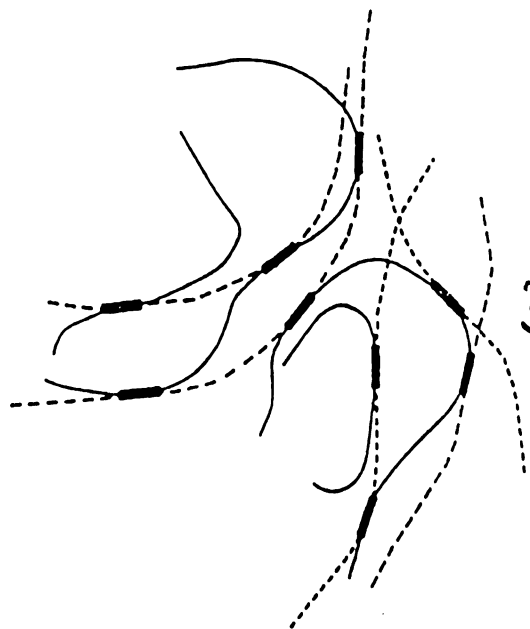
Filled gels contain particulate inclusions within the spaces formed by the three-dimensional structure of the network (Oakenfull, 1987). The filler may include air in bread or fat droplets in comminuted meat products. Morris (1985) reported that "inactive" fillers could be used to decrease the water loss and shrinking of gels, but have been neglected by food scientists.

Much work has been done in the area of multicomponent gels. Haga and Ohashi (1984), using scanning electron microscopy, observed the microstructure of heat-induced myosin B:soy protein cold insoluble fraction (CIF) gels. They found that there was some association between myosin B and soy protein CIF prior to heating and concluded it was due to disulfide bonds between the two proteins. When

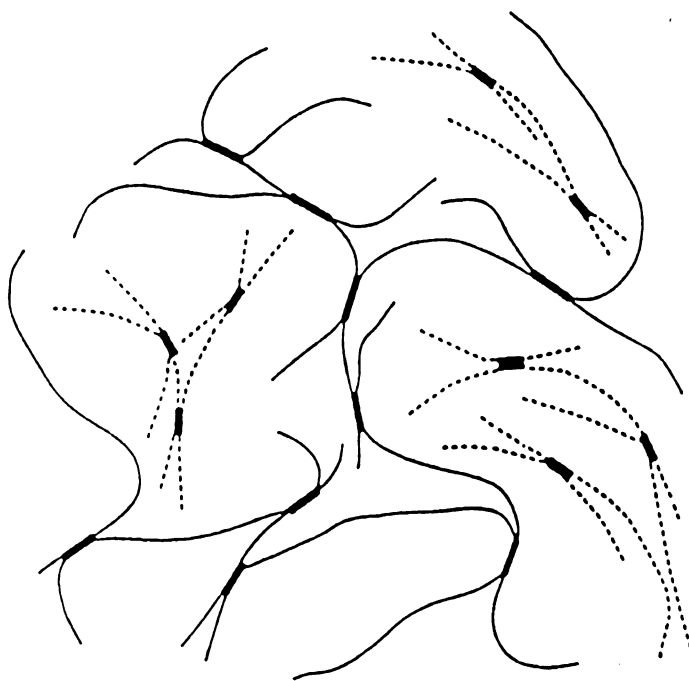
Figure 4. Mixed gel networks. (a) Interpenetrating network. Different polymers associate only by entanglement. (b) Phase separated network. Polymers form pure gels, one within the network of the other. (c) Coupled networks. At least on junction zone involves both types of polymers. (from Oakenfull, 1987).



(a)



(c)



(b)

heated individually, soy protein CIF formed a more dense structure than myosin B. Upon mixing, the denatured soy protein CIF formed a continuous network around the myosin B which served to reinforce the structure. In a similar study, Haga et al. (1986) found gel formability increased and the gel network became coarser when myosin content was held constant and soy protein CIF was increased. When soy protein CIF was held constant while increasing myosin B, breaking energy values increased and the gel network became more fibrous. Moritaka et al. (1980) reported that agarose and gelatin form separate networks when the mixture was gelled. At high concentrations, each interferes with the other's network formation. In studying the effects of meat protein on pectate and alginate gels, Hughes et al. (1980) observed that myoglobin and bovine serum albumin inhibited alginate gel formation with increasing pH, but caused gel formation in the presence of pectate below pH 6.0. Aguilera and Kessler (1989) showed a synergistic effect on gel firmness when mixing whey protein concentrate (67% protein) and skim milk powder (36.7% protein) in equal proportions to give a total solids nonfat content of 10%.

Addition of α -la to a 2% BSA solution (1% NaCl, pH 6.6) increased gelling temperature and decreased gel strength (Paulsson et al., 1986). At pH 4.0, addition of increasing concentrations of β -lg to a 2% BSA solution also increased the gelation temperature over that of BSA alone. Mixed gels

exhibited the characteristics of BSA gels up to a β -lg concentration of 5%.

Foegeding and Lanier (1987) stated that interaction between muscle and nonmeat proteins when processed to higher temperatures, did not add strength to comminuted meat products even though model systems indicated a gel strengthening effect. They suggested that nonmeat proteins act like a sponge to hold water and lipids in the protein matrix which increase gel strength.

2.9 Texture Analysis

Gelation is important in forming the texture and mouth feel of food products. Much work has been done to develop methods of objectively testing texture and to correlate them to sensory analysis (Voisey et al., 1975; Bourne, 1978; Szczesniak, 1968). Two instrumental methods currently being used are stress and strain to failure using uniaxial compression and texture profile analysis (TPA).

Hamann (1983) reviewed structural failure as it relates to some foods, including protein gels. Uniaxial compression involves application of a force to a core of food with known diameter and length. The core is placed between two parallel plates and the force at failure and distance traveled by the plunger are measured. Stress at failure is then calculated by dividing force by the cross-sectional area and strain is the distance compressed divided by length of the core.

With materials such as protein gels where deformations at failure may be large, apparent or true stress and strain at failure must be calculated. Equations are available to correct for large deformations (Hamann, 1983). The Poisson ratio relates change in diameter to change in length (Hamann, 1983).

Bourne (1978) reviewed TPA for measurement of textural properties of food. He modified the method for use with an Instron Testing Machine. A piece of food with known dimensions was compressed twice in reciprocation simulating mastication. Figure 5 shows a typical TPA curve.

Seven textural parameters were generated from the curve. Fracturability was defined as the force at the first significant peak on the curve. Hardness was the peak force during the first cycle. Cohesiveness was the ratio of the positive force during the second cycle to the positive force during the first cycle. Adhesiveness was the negative force of the first cycle which represented the force necessary to pull the plunger away from the food. Springiness was the height the food recovered between the end of the first "bite" and the beginning of the second "bite." Gumminess was the product of hardness and cohesiveness. Chewiness was the product of gumminess and springiness.

Some of these TPA parameters have been defined in terms of sensory evaluation. Hardness is the force required to bite through the specimen (Hamann, 1988). Szczesniak (1963) defined cohesiveness as the strength of the internal bonds

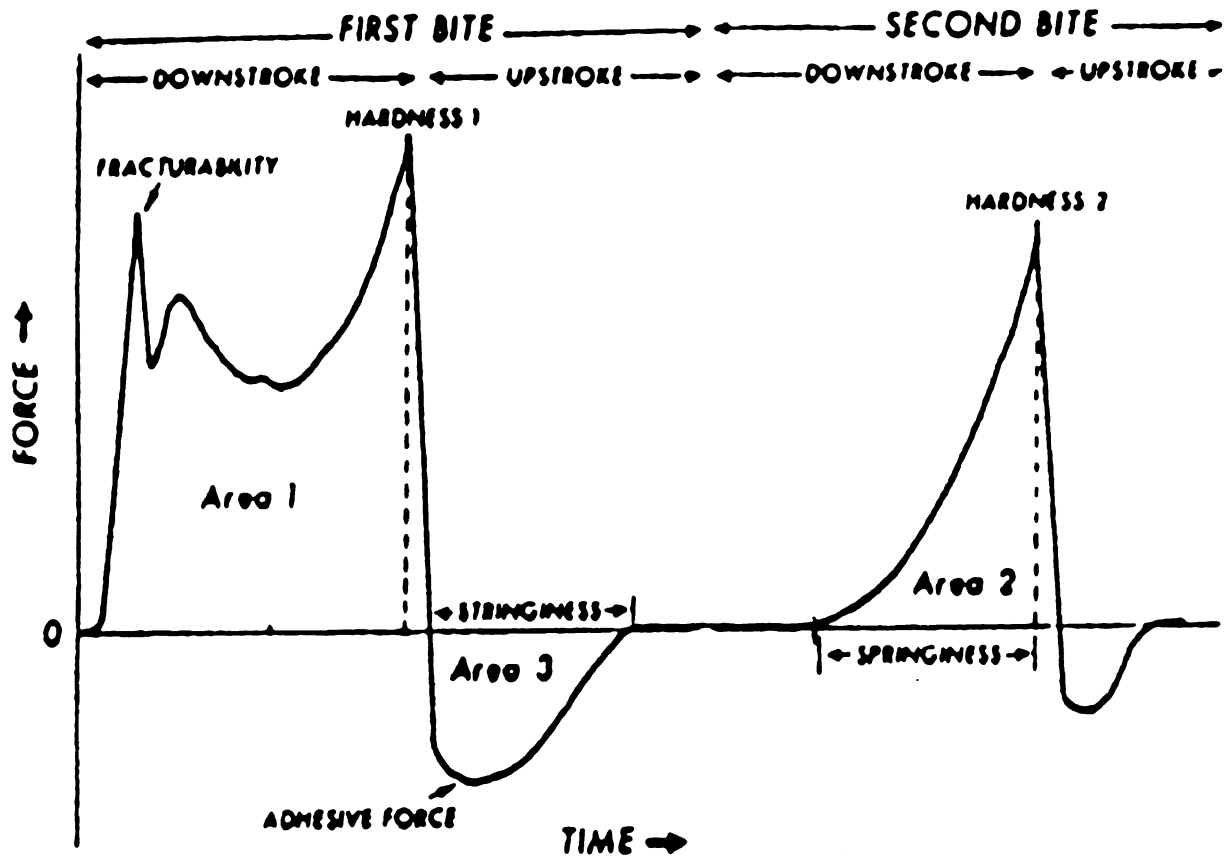


Figure 5. General texture profile analysis curve from the Instron Universal Testing Machine (from Bourne, 1978).

making up the product, chewiness as the energy required to masticate solid food sufficiently for swallowing and Gumminess as the energy necessary to ready semisolid food for swallowing.

Instrumental testing parameters have been correlated to sensory notes, processing and formulating parameters and to each other. Sensory "hardness" is highly correlated with breaking strength while sensory "springiness" is correlated

to deformation at failure (Toda et al, 1978). Voisey et al. (1975) evaluated commercial wieners by sensory and instrumental analysis and found a strong correlation ($R = 0.89$) between axial force required for failure and sensory "chewiness." High correlations were obtained between shear force and sensory elasticity, firmness, and chewiness; stress relaxation and elasticity; total expressible fluid and juiciness; and expressible moisture and wateriness for frankfurters (Lee et al., 1987).

Brady et al. (1985) studied the correlation among five Instron TPA parameters and eight sensory texture profile parameters on beef and beef-soy loaves. They found some relationships, but concluded more research was needed. Keeton et al. (1984) examined the effect of some nonmeat proteins as well as the presence or absence of sodium tripolyphosphate on the sensory and TPA parameters of frankfurters. The correlations between TPA and sensory evaluations were either small or not significant. They concluded that the sensory testing and TPA were measuring different textural attributes.

Processing conditions and product formulation are important in determining textural parameters. Siripurapu et al. (1987), working with sausage, found that several textural parameters including hardness at first and second bite increased with temperature, holding time and fat:protein ratio. Cohesiveness, springiness, and chewiness were minimal at cooking temperatures of 70 to 75°C (Singh et

al., 1985). Saliba et al. (1987) used a torsion test and two cycle uniaxial compression test to determine the effects of heating rate and sugar concentration on the textural parameters of frankfurter batters and found both had major effects on shear stress but minor effects on true shear strain.

2.10 Nonmeat Ingredients in Meat Systems

Nonmeat binders may be used in meat systems because raw material price and availability may change from day to day (Rongey and Bratzler, 1966). Current regulation permits the addition of 3.5% of final weight of WPC, modified whey, sodium caseinate and non-fat dry milk to comminuted meat products (USDA, 1990). Soy protein isolate (SPI) is limited to 2.0% of final weight.

The effects of milk proteins on fat binding, water binding and texture of meat products have been studied (Lee et al., 1980; Parks and Carpenter, 1987; Lauck, 1975; Thompson et al., 1982). Comer (1979) examined potato starch, wheat flour, textured soy protein, skim milk powder, sodium caseinate, and vegetable protein and found that they had a positive effect on cook stabilities of frankfurters, but a negative effect on texture. Nitrogen solubility index and emulsifying capacity were both found to be poor predictors of the functional properties of nonmeat proteins in meat systems (Comer, 1979; Comer and Dempster, 1981).

Rongey and Bratzler (1966) added high-temperature-processed nonfat dry milk (NFDM) to bologna at 3.5, 10.0, 15.0, and 20% and found percent moisture, percent protein, and percent shrink after 7-day storage at 38°C to be the same as the all-meat control. Addition of NFDM at 3.5% produced a higher yield than the control. Lauck (1975) substituted partially delactosed whey for meat in frankfurters and found that it equaled the control in stabilizing fat. Lee et al. (1980) studied the addition of WPC, dry whey, and NFDM to a processed meat loaf. Binding strength as measured by Instron shear did not differ significantly among the treatments. Higher sensory ratings for flavor were given to the product containing dry whey. The authors attributed this to the lactose, which is known to enhance flavor.

Parks and Carpenter (1987) evaluated soy protein isolate (SPI), soy protein concentrate (SPC), soy flour (SF), milk protein hydrolysate (MPH), autolyzed yeast (AY) and spray dried NFDM in frankfurters. They found the SPI and NFDM were the most effective emulsion stabilizers of the six binders. In addition, all of the nonmeat proteins except SPI decreased compression values.

Smick and Geist (1988) evaluated cooked yield and sensory characteristics of further processed turkey products, with 3.0% added WPC. Three trials were conducted with injected whole turkey breast muscle and one with chunked/formed turkey breast. Cooked yield was

significantly increased for two trials with added WPC over a control, and one trial showed significantly decreased cooked yield with WPC addition. The variation could not be explained. Samples with added WPC were rated significantly below the control for color, appearance and off-flavor, but similar to the control for overall acceptability. The researchers suggested that decreasing the percent WPC might improve sensory attributes.

MATERIALS AND METHODS

3.1 Whey Protein Concentrate Production

Liquid whey protein concentrate (WPC) from ultrafiltration of Parmesan cheese whey was obtained as a gift from Foremost Whey Products (Clayton, WI). The cheese whey was defatted with a cream separator and ultrafiltration was performed at 18.3°C. The WPC was cooled to 4°C for transport to Michigan. Total solids was determined according to AOAC (1980) 16.032 with modifications. Two grams of WPC was accurately weighed into an aluminum pan and dried 16 hr at 100°C in a convection oven (Blue M Electric Co, Model OU 490, Blue Island, IL). Total solids was calculated by dividing dried sample weight by the original weight x 100.

3.2 Preparation of Whey Protein Concentrate Treatments

Liquid WPC was divided into three batches to give three replicates from which four treatments were prepared. One treatment was a control and did not receive any additional heat processing. A lab model Spiratherm heat exchanger (Cherry-Burrell, Cedar Rapids, Iowa) was used to prepare two treatments by heating for 30 sec at 78.2°C or 92.2°C. The fourth treatment was prepared by heating WPC in three liter

Erlenmeyer flasks in an autoclave at 126.7°C for 30 min. Heat treatments were chosen based upon a preliminary study done at the Michigan State University Dairy Plant in which WPC was produced by ultrafiltration. Preliminary heat treatments of 71.6°C/30 sec, 83.9°C/30 sec, 93.3°C/30 sec and 126.7°C/30 min produced WPC's with solubilities of 98.5%, 96.7%, 59.3% and 37.5%, respectively. After processing, all treatments were poured into stainless steel pans to a depth of approximately 4 cm and frozen at -20°C. Frozen WPC was transferred to freeze drier pans and freeze dried with a model 42 Virtis freeze drier (Gardner, NY). Vacuum was maintained below 100 mm of Hg. Condenser temperature was -62.2°C and shelf temperature was 26.7°C. After drying, portions of the WPC receiving no heat treatment and the WPC heated at 78.2°C/30 sec were blended to give a fifth treatment with a solubility of 80%. Dried WPC was passed once through a Kitchen Aid food grinder (Model FG-A) with a 4 mm plate to reduce particle size and stored in low density polyethylene freezer bags (1.75 ml thickness) at -11°C for further use.

3.3 Analytical Methods

3.3.1 Protein

Protein content was determined according to AOAC (1984) 24.038-24039. A conversion factor of 6.38 was used for WPC samples and 6.25 for frankfurter samples. Protein content

was determined in triplicate for WPC and in duplicate for frankfurters.

3.3.2 Nonprotein Nitrogen

Nonprotein nitrogen (NPN) of WPC's was described as Kjeldahl nitrogen soluble in 12.5% trichloroacetic acid (TCA) (Kim et al., 1987) and was expressed as protein equivalents using a conversion factor of 6.38. Three grams of WPC were weighed accurately into a 100 ml beaker and 40 ml of 0.1M NaCl solution was added. The mixture was stirred for 30 min, transferred to a 50 ml volumetric flask and made to the mark with 0.1M NaCl. Contents were poured into a 100 ml beaker and 20 ml of a 43.75% TCA solution was added. This mixture was centrifuged at 4000 x g for 10 min at 4°C (Sorvall model RC2-B, Norwak, Conn.). Ten milliliter aliquots were analyzed for Kjeldahl nitrogen as described above. Percent nonprotein nitrogen, expressed as protein equivalents, was calculated as follows:

$$\text{NPN} = \frac{N \times 6.38 \times 70 \text{ ml}}{\text{sample wt}} \times 100$$

where:

N = mg Kjeldahl nitrogen/ml

6.38 = conversion factor for milk protein

70 = number of milliliter in original solution

sample wt = gms WPC originally weighed out

Each replicate was analyzed in duplicate.

3.3.3 Whey Protein Solubility

Solubility was determined as described by Morr et al. (1985). One-half gram of WPC was accurately weighed into a 100 ml beaker and enough 0.1M NaCl solution was added with stirring to form a paste. Volume was brought to about 40 ml with addition of 0.1M NaCl. The beaker was kept in a weigh boat containing ice while stirring to keep the solution cool. The solution was stirred at a rate which just failed to form a vortex. The pH was adjusted to 7.0 with either 0.1M HCl or NaOH and checked every 10 to 15 min. After one hour the solution was transferred to a 50 ml volumetric flask and made to the mark with additional 0.1M NaCl solution. After inverting several times to mix, the solution was centrifuged at 20,000 x g for 30 min at 2°C. The supernatant was poured through #1 Whatman filter paper and 10 ml aliquots were analyzed for protein as described earlier. Percentage solubility was calculated as follows:

$$\text{Protein Sol. (\%)} = \frac{\text{Supernatant protein conc. (mg/ml)} \times 50}{\text{Sample wt (mg)} \times \frac{\text{Sample protein content (\%)}}{100}} \times 100$$

Solubility of WPC was determined in duplicate.

3.3.4 Fat

Fat content of WPC was determined as described in AOAC (1980) 16.199b - 16.200. One gram of WPC was accurately weighed into a Mojonnier extraction flask. Ten milliliters of distilled deionized H₂O was added with shaking until

homogeneous. After adding 1.25 ml of NH_4OH , the flask was heated in a 70°C water bath for 15 min with occasional shaking and then cooled. Ten milliliters of 200 proof ethanol, 25 ml of ethyl ether, and 25 ml of petroleum ether were added with 1 min of vigorous shaking after each addition. Distilled water was added if necessary to bring the aqueous/ether interface into the neck of the flask. The top layer was poured into a previously weighed dish and the stopper and lip of the flask were washed with 1:1 (v:v) ethyl ether:petroleum ether. The above extraction was repeated using 4 ml of ethanol instead of 10 ml. The ether was evaporated over a steam bath without spattering and the pans dried in a convection oven for 1 hr. After cooling in a desiccator for 45 min, the pans were weighed and percentage fat was calculated. Each replicate was analyzed in duplicate.

Fat content of frankfurters was determined according to AOAC (1980) 24.005 (a). Determinations were performed in duplicate.

3.3.5 Moisture

Moisture determination of WPC was performed as described in AOAC (1980) 16.192 with modifications. Flat-bottom aluminum dishes were dried in a convection oven for 1 hr, cooled for 45 min in a desiccator, and weighed in pairs. One dish held the sample, and the other was used as a cover. One to 1.5 gm of WPC sample was accurately weighed into a

dish and the other dish was loosely put on top as a cover. Samples were dried in a vacuum oven at 95°C and less than 100 mm of Hg until a constant weight was reached. Each replicate was analyzed in duplicate.

Moisture of frankfurters was determined in duplicate according to AOAC (1980) 24.003 (a).

3.3.6 Ash

Ash content of WPC was determined as described by AOAC (1980) 16.192. Porcelain crucibles were ignited overnight in a muffle furnace at 600°C, cooled in a desiccator and weighed. One gram of WPC was accurately weighed into each dish and charred over a Bunsen burner until no smoke formed. Samples were ignited in a muffle furnace at 600°C until ash was light gray or white. Crucibles were cooled in a desiccator and weighed. Replicates were analyzed in duplicate.

3.3.7 Calcium

Calcium content of WPC was determined using the ashed samples. Ten milliliters of 3N HCl was added to the crucible and gently boiled for 10 min using a watch glass as a cover. After cooling, crucible contents were volumetrically transferred through #1 Whatman filter paper into a 50 ml volumetric flask. The flask was made to the mark with 0.1M HCl which contained 0.1% potassium chloride and 0.4% strontium chloride to prevent interferences. A Perkin-Elmer (Model 303, Norwalk, Conn.) atomic absorption

spectrometer was used with an air-acetylene flame, calcium lamp wavelength of 422.7 nm and slit width of 0.7 mm. The linear range was determined with 2 ppm and 5 ppm calcium standards (Sigma Chemical Co, St. Louis, MO). Samples were diluted with 0.1M HCl if necessary to bring them into the linear range of the instrument. Each replicate was analyzed in duplicate.

3.3.8 Lactose

Lactose content of WPC was determined enzymatically using an analysis kit (Boehringer-Mannheim, catalog #176303, West Germany). Each replicate was analyzed once.

3.4 Electrophoresis of Whey Protein Concentrate

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the total protein and soluble protein fractions of the five WPC treatments using a modification of the method described by Laemmli, (1970). Total protein fractions were prepared by adding enough WPC to a 25 ml volumetric flask to give 5mg/ml of protein. The flask was made to the mark with 0.625M Tris buffer (pH 7.2) containing 2% SDS and 10% glycerol. After mixing, 10 ml were put into a capped test tube along with 3 drops of β -mercaptoethanol and heated in boiling water for 10 min. A few grains of bromophenol blue were added as a tracking dye.

Soluble protein fractions of each WPC treatment were prepared in 0.1M NaCl and pH 7.0 as described earlier (Morr

et al., 1985), except that sufficient WPC was used to make a solution of 5 mg of protein per milliliter before centrifuging. Nine milliliters of sample solution, 1 ml of 10% SDS solution, and 3 drops of β -mercaptoethanol were combined in a capped test tube and heated in boiling water for 10 min. Five ml of this solution was dialyzed in Spectrapor dialysis tubing (Spectrum Medical Supplies, Los Angeles, CA) for 18 hr with one buffer change against 0.2M ethylenediaminetetraacetic acid, 25mM Tris, pH 7.2; containing 0.2% SDS, glycerol (20% by weight) and bromophenol blue.

Proteins were electrophorized on slab polyacrylamide gels using a SE 600 series vertical slab unit (Hoefer Scientific Instruments, San Francisco, CA). A stock solution containing 30% (w:v) acrylamide and 0.8% (w:v) N'-N methylenebisacrylamide in distilled water was made for preparation of the stacking and resolving gels. The stacking gel was 4% acrylamide in 0.1% SDS, 0.125M tris buffer, pH 6.8 and the resolving gel was 14.5% acrylamide in 0.1% SDS, 0.375M tris buffer, pH 8.8. The electrode buffer contained 0.025M Tris, 0.192M glycine, 0.2% SDS, pH 8.3. The resolving gel was 1.5 cm x 12 cm. Forty or sixty micrograms of protein were applied to each lane of the gels. Molecular weight standards (SDS-6H and SDS-7, Sigma Chemical Co., St. Louis, MO) were run simultaneously as references. Current was held constant at 30 mA until the tracking dye migrated into the resolving gel where it was increased to 50

mA, until the tracking dye reached the bottom of the gel (5 hr). A model IP-17 power supply (Heathkit, Benton Harbor, MI) was used. Gels were stained overnight in 9:45:45 (v:v:v) acetic acid:water:methanol solution containing 0.4% Commassie Brilliant Blue. Destaining was accomplished with several changes of 7.5:25:67.5 (v:v:v) acetic acid:methanol:water and gels were stored in 7.5% (v:v) acetic acid solution. A Shimadzu Dual-Wave Length Thin-Layer Chromoto Scanner (Model CS-930, Kyoto, Japan) was used to quantitate protein bands by scanning at 580 nm. Areas under the peaks were compared and the percentage of each whey protein were determined for each lane. After multiplying percent \dot{A} -la by 1.056 to correct for staining differences (Foegeding, 1990), the β -lg: \dot{A} -la ratio was calculated by dividing the percent β -lg by the percent \dot{A} -la. Molecular weights (MW) were determined by comparing the relative mobility of the molecular weight standards to that of the sample. Individual proteins were identified by comparing MW's to those of published data (de Wit and Klarenbeek, 1984; Eigel et al., 1984).

3.5 Whey Protein Concentrate Gel Preparation

Twenty percent (w:w) gels from all WPC treatments were prepared in either 0.1M NaCl, 0.1M Na phosphate buffer, pH 7.0, or 0.6M NaCl, 0.1M Na phosphate buffer, pH 7.0. Whey protein concentrate was weighed into a 100 ml beaker and all but 2-3 ml of buffer was added. Samples were blended with a

Polytron homogenizer (model PT10/35, Kinimatica, Switzerland) with a model PTA 10 TS generator for 30 sec at a setting of 4. After pH adjustment with 3M HCl or 4M NaOH, solutions were stored in the cooler (4°C) overnight. The next day, pH was again adjusted and enough buffer added to give the final concentration. Six grams of sample were put into 10 mm ID x 13 cm pieces of glass tubing that were stoppered at one end. Capped tubes were then heated for 30 min at 60, 70, 80 or 90°C in a Blue M model MW-1120A-1 water bath (Blue Island, IL). A blank (water) with a thermocouple was used to monitor the temperature and timing began when the desired temperature was reached. Tubes were immersed immediately in ice water for 5 min and stored at 4°C overnight for further analysis. All three replicates of the WPC's were used to prepare gels with the 0.6M NaCl, 0.1M Na phosphate buffer, but only one replicate of the WPC's was used with the 0.1M NaCl, 0.1M Na phosphate buffer.

3.6 Salt-Soluble Protein Extraction

The procedure of Wang (1990) was followed with some modifications. Chicken breast meat was purchased at a local grocery store and visible skin, fat and connective tissue removed before grinding twice through a Kitchen Aid food grinder (Model FG-A) with a 4 mm plate. Meat was blended with 4 volumes of low salt buffer (0.1M NaCl, 0.05M Na phosphate, pH 7.0) for 90 sec and stirred with a motorized propeller stirrer for 1 hr in a cooler (4°C). This solution

was centrifuged 10 min at 8800 x g and the supernatant was discarded. The pellet was resuspended in 4 volumes of low salt buffer, stirred for 1 hr, and centrifuged as above. One-third volume of 2.4M NaCl, 0.05M Na-phosphate, pH 7.0 was added to the pellet to adjust the salt concentration to 0.6M and about 2 volumes of 0.6M NaCl, 0.05M Na-phosphate buffer were added before stirring for 1 hr. The suspension was allowed to stand in the cooler overnight and centrifuged at 21,500 x g for 30 min and the pellet containing insoluble protein was discarded. Five volumes of distilled H₂O were added to the supernatant and centrifuged at 21,500 x g for 30 min to precipitate the salt soluble proteins. Pellets were combined and centrifuged twice more to concentrate them. Protein concentration was determined by Kjeldahl as described earlier. One-third volume of 2.4M NaCl, 0.05M Na-phosphate buffer, pH 6.0, 7.0, or 8.0, was added to solubilize the protein in a final salt concentration of 0.6M. Protein solutions were adjusted to the desired pH with 0.1M HCl or NaOH, if necessary. High salt buffer (0.6M NaCl, 0.05M Na-phosphate) of appropriate pH was added to give a final protein concentration of 8% (w:w).

3.7 Whey Protein Concentrate:Salt-Soluble Protein Gels

Solutions of 16, 24 and 32% (w:w) protein from the WPC control treatment were prepared in 0.6M NaCl, 0.05M Na phosphate buffer, pH 7.0. These WPC control solutions were then combined 1:1 (w:w) with 8% SSP to give protein

solutions with 4% SSP and either 8%, 12% or 16% whey protein. The protein solutions were blended for three 3 sec periods using the Polytron homogenizer with the model PTA10 TS generator at a setting of 4. In addition to the combination protein solutions, SSP protein solutions of 4 and 8% (w:w) and WPC solutions of 8, 12, 16 and 20% (w:w) in the same buffer were prepared. All solutions were allowed to stand overnight at 4°C. About 4 to 5 ml of protein solution was pipetted into 10 mm ID by 13 cm stoppered glass tubing, centrifuged at about 100 x g for 6 min to remove air bubbles, heated at 65 or 90°C for 15 min and cooled 5 min in an ice bath. Gels were stored at 4°C until analyzed.

Multicomponent gels containing the other four WPC's were also prepared as described above except the ratio of SSP:WPC for all combinations was 4%:12%. In addition, 16% solutions of each WPC treatment were prepared and gelled. All three replicates of the WPC's were used in the multicomponent gels.

3.8 Model Gel Evaluation

3.8.1 Expressible Moisture

Gel expressible moisture was measured using centrifugation as described by Jauregui et al. (1981) with some modifications. Two pieces of 9 cm and one piece of 12 cm Whatman #2 filter paper were pressed into a 50 ml polycarbonate centrifuge tube to form a sample pocket and a weight taken of the paper and tube. One to two grams of gel

was accurately weighed into the tube and centrifuged at 755 x g for 10 min, instead of for 5 min as described by Jauregui et al., (1981). The pellet was removed and the tube and paper reweighed. The difference between the initial and final weights of the paper plus tube was termed expressible moisture (EM). Percent expressible moisture was determined by dividing EM by the weight of the sample and multiplying by 100. Expressible moisture was determined for all gels except for WPC gels prepared with 0.1M NaCl, Na phosphate buffer, pH 7.0.

3.8.2 Apparent Stress and Strain at Failure

Apparent stress and strain at failure was determined for all gels as described by Hamann (1983). An Instron Universal Testing Machine (Model 4202, Canton, MA) equipped with a 50 N compression load cell was used. Cores (10 mm x 10 mm) were cut with a surgical scalpel. Samples were kept on ice until immediately before the test was performed. Lubricated parallel plates (silicon oil) were used and samples were compressed to 80% of original length. The crosshead speed was 50 mm/min and the chart speed was 72 cm/min. Force at failure in newtons and core length at failure were read from the chart. The following equations describe calculations of stress and strain (Hamann, 1983):

$$\bar{\epsilon}_h = l_o/l$$

where: $\bar{\epsilon}_h$ = strain at failure

l_o = length of core at failure (mm)

l = initial length of core (mm)

Apparent strain at failure:

$$\dot{\gamma}_z = -\ln(1 - \dot{\gamma}_h)$$

Apparent stress at failure (k Pa):

$$\dot{\sigma}_{app} = \frac{F}{cR^2 1000(1 + v\dot{\gamma}_h)^2}$$

where:

F = force at failure from chart (N)

R = radius of core (meters)

v = Poisson's ratio (0.48; from Hamann, 1983)

c = π

3.9 Model System Frankfurter Preparation

Frankfurters were prepared with thawed mechanically deboned chicken meat (Ottawa Gardens, Athens, MI), beef fat which had been ground through a 9 mm plate (ADA Beef Co., Ada, MI), water, salt and WPC. The formulation was designed to contain 12% protein, 30% fat, 56% moisture, and 2% salt. The WPC was added at 3.5% or 7.0% of the batch weight (Table 3 and Table 4). Since the WPC contained 62% protein, it was substituted for 21% of the meat protein in the formulation containing 3.5% WPC and 4.2% protein to the formulation containing 7.0% WPC. The protein content of the frankfurter formulations was held constant at 12%. Thus, the amount of meat was adjusted such that it contributed 9.9% protein to the 3.5% substituted WPC formulation and 7.8% protein to the

Table 3. Model system frankfurter formulation with 3.5% added whey protein concentrate (WPC)^a

Ingredient	Control	WPC Added ^b
Mechanically Deboned Chicken Meat	1295.5g	1097.7g
Fat	384.7g	402.8g
Water	98.0g	214.1g
Salt	36.3g	36.3g
WPC	0.0g	63.5g

^a Formulation is 12% protein, 30% fat, 56% moisture and 2% salt.

^b Whey protein concentrate was substituted for chicken meat on a protein basis.

Table 4. Model system frankfurter formulation with 7.0% added whey protein concentrate (WPC)^a

Ingredient	Control	WPC Added ^b
Mechanically Deboned Chicken Meat	1295.5g	820.1g
Fat	293.9g	344.7g
Water	188.7g	517.1g
Salt	36.3g	36.3g
WPC	0.0g	127.0g

^a Formulation is 12% protein, 25% fat, 61% moisture and 2% salt.

^b Whey protein concentrate was substituted for chicken meat on a protein basis.

7.0% substituted WPC formulation. Meat, salt, water, and WPC were added to a Hobart bowl chopper (model 84181D, Troy, OH) and chopped for 4 min. Beef fat was added and chopped four minutes or to a temperature of 40°F, whichever came first. Batter was stuffed with a hand stuffer into 50 ml pre-weighed polycarbonate centrifuge tubes, reweighed and capped. Eight tubes from each replicate were heated to an internal temperature of either 72°C or 90°C in a waterbath set at 74°C or 92°C and cooled immediately in ice water. Model system frankfurters were removed from the tubes and, after all visible fat caps were removed, they were weighed. All three replicates of the WPC's were used to prepare the 3.5% WPC substituted model system frankfurters, but only one replicate of the WPC's was used to prepare the 7.0% WPC substituted model system frankfurters.

3.10 Model System Frankfurter Evaluation

3.10.1 Cook Yield

Cook yield was determined by dividing the weight of the drained cooked model system frankfurter (with visible fat caps removed) by the weight of the uncooked batter and multiplying by 100. Eight model system frankfurters were evaluated per replicate.

3.10.2 Severe Reheat Yield

A severe reheat yield study was performed according to Smith and Brekke (1984). Two twenty-gram cores were placed in 100 ml of 95°C distilled H₂O for 10 min, cooled on paper

towel for 5 min and reweighed. Final weight divided by initial weight multiplied by 100 was termed severe reheat yield. Each replicate was analyzed in duplicate.

3.10.3 Texture Profile Analysis

Texture profile analysis was performed as described by Bourne (1978). Model system frankfurters were cut into 15 mm lengths and a 15 mm diameter core was cut longitudinally from the center of each length. A two-cycle compression was used to compress cores to 75% of their original height using an Instron Universal Testing Machine (Model 4202, Canton, MA) and a 500 N load cell. Crosshead speed was 50mm/min and chart speed was 40mm/min. Results were recorded with a chart recorder. Three cores were analyzed per replicate.

Hardness was calculated as the force at the height of the first peak on the chart recording (Bourne, 1978). Cohesiveness was defined as the ratio of the positive force during the second compression to that of the first (Bourne, 1978). These positive forces are represented by peak areas on the chart recording. The peaks on the chart recording for each determination were cut out and weighed. Cohesiveness was calculated by dividing the weight of the peak area made by the second compression cycle by the weight of the peak area made by the first compression cycle.

Springiness was described as the length of the sample core after the first compression cycle as a percentage of

the original length was calculated from the chart recording (Bourne, 1978).

3.11 Statistical Analysis and Design

A completely randomized block design was used for all experiments. A statistical software program (MSTATc, 1989) was used to compute mean square error, analysis of variance and to separate means.

3.11.1 Whey Protein Concentrate Gels

Gels from WPC in 0.1M NaCl, 0.1M Na phosphate, pH 7.0 were replicated once in preliminary experiments. Means and standard deviations of the duplicate analyses were reported.

Three replicates were analyzed of WPC gels in 0.6M NaCl, 0.1M Na phosphate, pH 7.0. A two-way analysis of variance (replicate x treatment) was performed on the stress and strain at failure data at each temperature. A 3 x 2 x 5 factorial (replicate x temperature x treatment) was used to analyze variance of the expressible moisture data, and interaction was significant ($P > 0.05$). Means were separated with Tukey's test using the mean square error term.

3.11.2 Salt-Soluble Protein:Whey Protein Concentrate Gels

Variance for the stress and strain at failure data at 65° and 90°C was computed using a two-way analysis of variance (replicate x treatments). A 3 x 2 x 5 factorial (replicate x temperature x treatment) was used to compute

variance for the expressible moisture data and significant interaction ($P > 0.05$) was found. All means were separated with Tukey's test using the mean square error term.

3.11.3 Model System Frankfurters

A 3 x 2 x 4 factorial (replicate x temperature x treatments) was used to analyze variance of the 3.5% WPC substituted model system frankfurters. Means were separated with Tukey's test using the mean square error as the error term.

Only one replicate of the experiment substituting 7.0% WPC in a model system frankfurter formulation was performed. Means and standard deviations for the analyses were calculated.

RESULTS AND DISCUSSION

4.1 Whey Protein Concentrate Composition

The unheated liquid whey protein concentrate (pH 6.3) contained 15.3% total solids. The proximate composition and other characteristics of the five WPC treatments are listed in Table 5. The average composition for all treatments was 62.4% protein, 4.7% nonprotein nitrogen, 5.4% moisture, 5.7% fat, 27.8% lactose, 3.8% ash, and 0.47% calcium. The WPC control was 98% soluble at pH 7.0 which suggests most of the protein was not denatured. Through heat treatment, WPC's with solubilities of 47.2%, 41.0%, and 27.5% were obtained. The 80% soluble WPC was obtained by mixing 98% and 47% soluble WPC's. The composition of the WPC's varied little with the exception of moisture. The 98% soluble WPC contained nearly twice as much H₂O (6.7%) as the 41% soluble treatment (3.7%). This probably resulted because the products needed to be dried in batches over a period of three weeks and a subjective method (ie. visual) was used to determine whether the product was dry.

An electrophoregram of the WPC treatments (60 ug protein/lane) is shown in Figure 6. Proteins with a MW of 70.6, 63.2, 57.5, 34.0, 17.2, and 13.7K were identified.

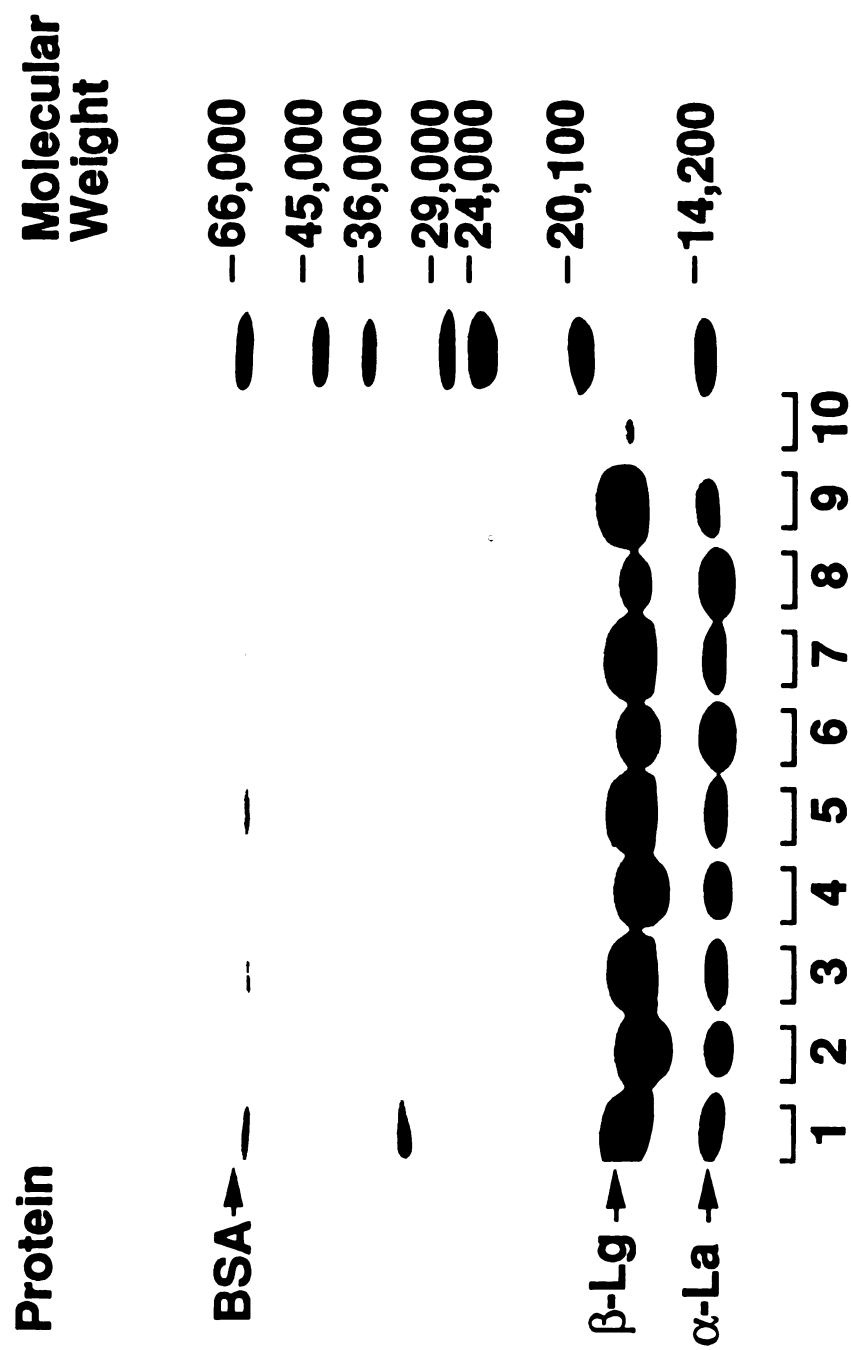
Table 5. Mean values (%) for proximate composition and solubility of whey protein concentrates subjected to different heat treatments

Characteristic	Treatment ^a					Average
	98	80	47	41	27	
Protein	61.0	61.7	62.8	63.7	62.9	62.4
NPN ^b	4.4	4.5	4.8	4.9	5.0	4.7
Solubility	98.1	80.0	47.2	41.0	27.5	----
Water	6.7	6.0	4.8	3.7	5.6	5.4
Fat	5.7	5.7	5.7	5.8	5.6	5.7
Lactose	27.5	28.1	28.3	28.2	26.7	27.8
Ash	3.8	3.7	3.8	3.9	3.6	3.8
Calcium	0.49	0.51	0.45	0.42	0.47	0.47

^a Treatments are labeled according to percent protein solubility in 0.1M NaCl, pH 7.0.

^b Nonprotein nitrogen

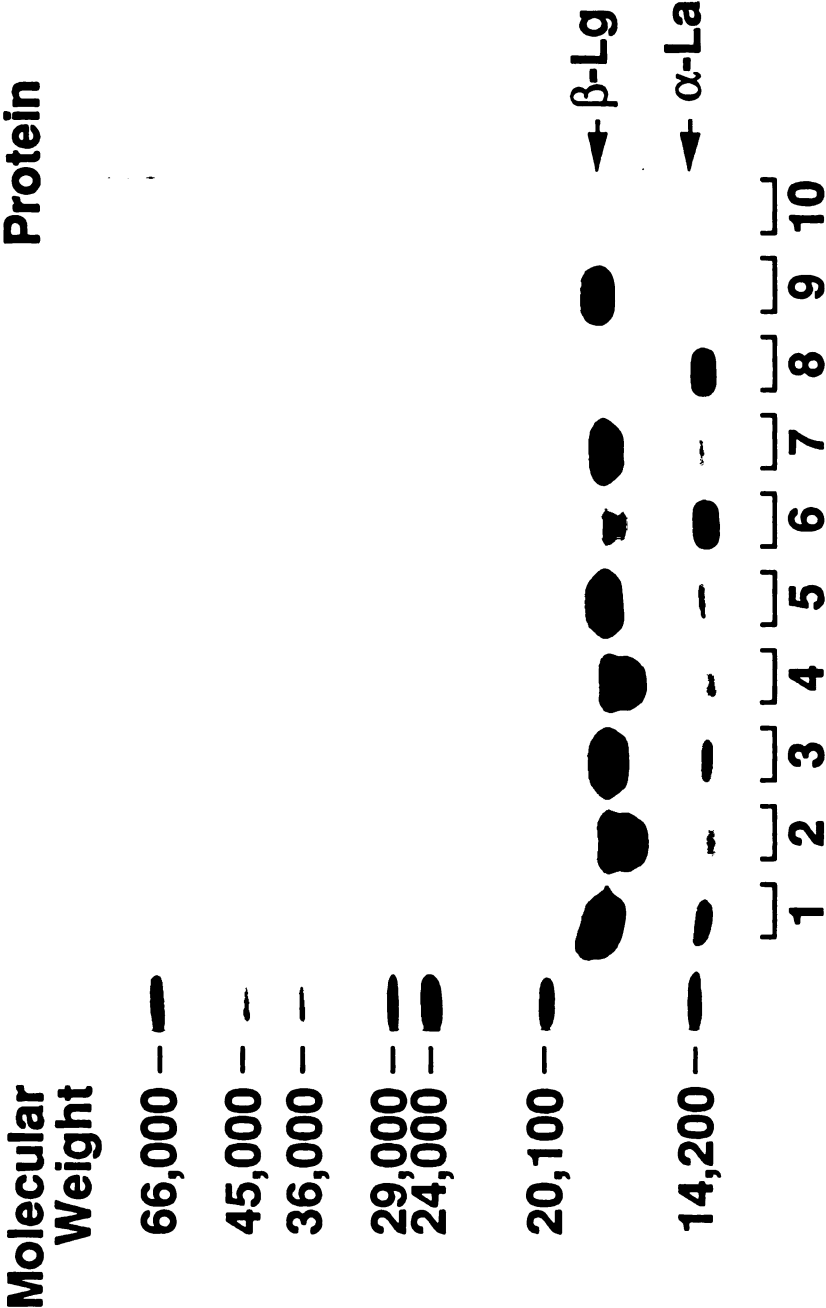
Figure 6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whey protein concentrate (WPC) showing the relative change in concentration of β -lactoglobulin (β -lg), α -lactalbumin (α -la) and bovine serum albumin (BSA) when applied at 60 ug protein/lane (1: 98% soluble WPC, 2: soluble fraction of 98% soluble WPC, 3: 80% soluble WPC, 4: soluble fraction of 80% soluble WPC, 5: 47% soluble WPC, 6: soluble fraction of 47% soluble WPC, 7: 41% soluble WPC, 8: soluble fraction of 41% soluble WPC, 9: 27% soluble WPC, 10: soluble fraction of 27% soluble WPC)



The 63.2K protein was most likely bovine serum albumin (BSA), which has a MW of 66,000 (de Wit and Klarenbeek, 1984). The proteins with MW of 70.6 and 57.5K may be monomeric forms of immunoglobulins. Eigel et al. (1984) reported that all classes of immunoglobulins contain two identical heavy polypeptides of 50-70K and two identical light polypeptides of 20K covalently linked by disulfide bonds. Proteins with MW of 17.1 and 13.7K are most likely β -lactoglobulin (β -lg) and α -lactalbumin (α -la), which have reported MW's of 18,400 and 14,200, respectively (de Wit and Klarenbeek, 1984). The band at 34.K was probably β -lg in its dimeric form which was not fully dissociated by the β -mercaptoethanol.

Electrophoresis was carried out using 40 ug protein/lane to further resolve the β -lg band (Figure 7). Two bands with MW of 17.1 and 16.4K show up only in the lanes containing the soluble protein fraction (2, 4, 6, 8). Both bands disappear with increasing heat treatment. This would suggest that they are not casein remnants, as caseins are heat stable (Schmidt and Morris, 1984). Beta-lactoglobulins exist as several genetic variants, of which A and B (MW of 18,363 and 18,277, respectively) are the most commonly cited in the literature (Eigel et al., 1984). However, SDS-PAGE, which separates on the basis of molecular size, would not be expected to separate these genetic variants. Isoelectric focusing on PAGE may be more useful for determining the identity of these bands.

Figure 7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whey protein concentrate (WPC) showing the relative change in concentration of β -lactoglobulin (β -lg), α -lactalbumin (α -la) and bovine serum albumin (BSA) when applied at 40 ug protein/lane (1: 98% soluble WPC, 2: soluble fraction of 98% soluble WPC, 3: 80% soluble WPC, 4: soluble fraction of 80% soluble WPC, 5: 47% soluble WPC, 6: soluble fraction of 47% soluble WPC, 7: 41% soluble WPC, 8: soluble fraction of 41% soluble WPC, 9: 27% soluble WPC, 10: soluble fraction of 27% soluble WPC)



Bands 2, 4, 6, 8 and 10 in Figure 7 show the soluble protein fraction of WPC treatments 98, 80, 47, 41 and 27, respectively. Nearly all of the immunoglobulin and BSA fraction disappeared from the 47% soluble fraction (72.2°C for 30 sec) and the β -lg: α -la ratio decreased dramatically indicating a loss of β -lg, but little α -la insolubilization. After severe heat treatment (27.2°C for 30 min) only a light band of about 17.1K remained.

This is in agreement with other researchers (Li-Chan, 1983; Mutilangi and Kilara, 1985) who reported that the resistance to heat denaturation increases as follows: immunoglobulins, BSA, β -lactoglobulin, and α -lactalbumin. de Wit and Klarenbeek (1984), using differential scanning calorimetry, determined the denaturation temperatures (T_d) of α -la, β -lg, immunoglobulin G and BSA in 0.7M phosphate, pH 6.0 buffer to be 68, 83, 89 and 70°C, respectively. They suggested the heat stability of α -la, despite its low T_d , was due to a high degree of refolding (<90% reversibility). Denaturation temperature is also dependent on environmental conditions such as pH, ionic strength and presence of other solutes (de Wit, 1981; Varunsatian et al., 1983; de Wit and Klarenbeek, 1984).

The densitometer was used to follow the relative change in concentration of the whey proteins with changes in heat treatment. Table 6 summarizes this data for β -lg, α -la and BSA as determined from the gel loaded with 60 ug protein/lane. Beta-lactoglobulin as a percent of the total

Table 6. Percent β -lactoglobulin (β -lg), λ -lactalbumin (λ -la), Bovine Serum Albumin (BSA), and the ratio of β -lg: λ -la in whey protein concentrate (WPC) as determined by densitometric scanning of sodium dodecyl sulfate polyacrylamide gels

Treatment ^a	β -lg ^b (%)	λ -la ^b (%)	BSA ^b (%)	β -lg: λ -la ^b
98 _t	64.8	17.9	4.1	3.43
98 _s	67.6	20.8	6.8	3.08
80 _t	68.4	18.9	3.7	3.43
80 _s	68.8	23.6	6.0	2.76
47 _t	67.1	20.5	3.6	3.10
47 _s	44.5	54.3	1.2	0.78
41 _t	69.8	18.4	3.7	3.59
41 _s	32.0	67.6	0.0	0.45
27 _t	74.9	19.3	4.8	3.68
27 _s	100.0	0.0	0.0	----

^a Treatments are labeled according to their percent solubility. The _t represents the total protein fraction and the _s represents that protein soluble in 0.1M NaCl, pH 7.0.

^b Percentage β -lg, λ -la, and BSA were determined assuming equal staining. The β -lg/ λ -la ratio was corrected for staining differences by multiplying λ -la by 1.056 prior to calculation of the ratio (Foegeding, 1990).

whey proteins in the total protein fraction was higher than that reported by Kim et al. (1987), but similar to values obtained by Foegeding (1990). Bovine serum albumin values were in agreement with the work of Kim et al. (1987). Beta-lactoglobulin as a percent of total protein decreased from 68% to 32% as WPC solubility decreased from 98 to 41%. Alternately, α -la increased from 21 to 68% over the same solubility range. This indicates the resistance to heat insolubilization of α -la, compared with β -lg. Bovine serum albumin was completely absent from the soluble fraction of WPC 41, indicating its heat lability.

4.2 Effect of Salt Concentration on Apparent Stress at Failure of Whey Protein Concentrate Gels

Table 7 shows apparent stress at failure for gels prepared by heating WPC solutions (20% protein in 0.1M NaCl, 0.1M Na phosphate, pH 7.0) at four temperatures (60, 70, 80 and 90°C). Gel strength for WPC 98 increased eleven-fold when the temperatures increased from 70° to 80°C. Significant increases in strength were also observed for WPC 80, WPC 47, and WPC 27 when the temperature increased from 70° to 80°C. As WPC insolubilization decreased to 41%, the temperature needed for gel formation increased. Suspensions of WPC 41 did not form gels at any temperature. Gel strength decreased for WPC 98, doubled for WPC 47 and stayed the same for WPC 80 and WPC 27 when the temperature increased from 80° to 90°C. Since the T_d of β -lg is near 80°C (de Wit and Klarenbeek, 1984), the greatest changes in

Table 7. Apparent stress at failure for 20% (w:w) protein whey protein concentrate (WPC) gels in 0.1M NaCl, 0.1M Na phosphate, pH 7.0^a

Treatment ^b	Apparent Stress at Failure (kPa)			
	Temperature			
	60°C	70°C	80°C	90°C
WPC [98]	< 4.0	8.1 (0.65)	91.6 (8.3)	73.7 (1.6)
WPC [80]	no gel	< 4.0	53.1 (1.9)	50.6 (3.1)
WPC [47]	no gel	no gel	10.0 (1.3)	19.3 (1.3)
WPC [41]	no gel	no gel	no gel	no gel
WPC [27]	5.8 (0.61)	12.8 (1.0)	20.5 (1.0)	23.8 (0.9)

^a Average of three determinations with standard deviations in parenthesis.

^b Numbers in brackets represent % protein solubility in 0.1M NaCl, pH 7.0.

NOTE: Samples designated "< 4.0" formed weak gels that were below the sensitivity of the test. Samples designated "no gels" flowed when the tube was inverted.

Table 8. Apparent stress at failure for 20% (w:w) protein whey protein concentrate (WPC) gels in 0.6M NaCl; 0.1M Na phosphate, pH 7.0

Treatment ^a	Apparent Stress at Failure (kPa)		
	Temperature		
	70°C	80°C	90°C
WPC [98]	< 4.0	91.4 ^b	94.1 ^b
WPC [80]	no gel	42.0 ^c	59.8 ^c
WPC [47]	no gel	6.5 ^e	12.6 ^d
WPC [41]	no gel	< 4.0	< 4.0
WPC [27]	17.2	21.9 ^d	25.2 ^d

^a Numbers in brackets represent % protein solubility in 0.1M NaCl, pH 7.0.

^{b-e} Means within the same column followed by the same letter do not differ significantly (P < 0.05)

NOTE: Samples designated "< 4.0" formed weak gels that were below the sensitivity of the test. Samples designated "no gel" flowed when the tube was inverted.

gel strength would be expected near this temperature for WPC's with most of the β -lg in the native form prior to heating. Zirbel and Kinsella (1988) reported that strength of 20% whey protein isolate gels (pH 7.0) increased when heating temperature increased from 70°C to 80°C, but did not increase upon heating to 90°C. However, Schmidt et al. (1978b) reported an increase in the strength of WPC gels (7.5% protein) with an increase in temperature from 80-110°C. Schmidt and Illingworth (1978) suggested that higher temperatures gave rise to more intermolecular bonding which resulted in harder gels.

Gels formed from WPC in 0.6M NaCl, 0.1M Na phosphate, pH 7.0 exhibited similar gel strength characteristics (Table 8). Only WPC 27 formed a gel at 70°C. Gel strength of WPC 27 increased from 17.2 kPa at 70°C to 21.9 kPa at 80°C. The increase at 90°C was not as great (25.2 kPa). Whey protein concentrate 47 gels were twice as firm (12.6 vs. 6.5 kPa) at 90°C than at 80°C. The WPC 80 gels also showed some increase in firmness when the temperature increased from 80°C to 90°C. Gels formed from WPC 98 did not increase in strength when the temperature increased from 80°C to 90°C (91.4 vs. 94.1 kPa). The WPC 41 treatments did not form gels with measurable strength.

At both salt concentrations, insolubilization of the WPC prior to gel preparation had a significant effect on gel strength. Gel strength was four times greater for the control (WPC 98) than for the highly insolubilized treatment

(WPC 27) at 80°C, regardless of salt concentration. Similarly, apparent stress at failure decreased by 50% when WPC solubility decreased from 98% to 80% at a heating temperature of 80°C. Denaturation of whey proteins can greatly affect the properties of subsequent gels (Kinsella, 1984). Dunkerley and Zadow (1981) reported that gels from WPC given a pretreatment of 80°C/15 sec formed less firm gels than WPC with pretreatment of 72°/15 sec.

An interesting observation can be made when comparing WPC 27 and WPC 41. At both salt concentrations, WPC 27 formed a soft gel while WPC 41 formed no measurable gel. A possible explanation for this may lie in the viscosity of the WPC solutions prior to gelling. WPC 41 formed a thick but pourable solution while WPC 27 formed a paste and needed to be stuffed into the tubes. Thus the values obtained for gel strength for WPC 27 may actually have been a function of the viscosity rather than heat-induced gelation.

Because the WPC 27 gels became more firm with increasing temperature, high viscosity of the solution may be only a partial explanation. Ferry's (1948) model depicts gelation as a two-step process where protein molecules first partially unfold before they interact to form a matrix. Since much of the protein in the WPC 27 was already unfolded, gel matrix formation may have been facilitated at a lower temperature. A third possibility is the gels might be the result of aggregation rather than gelation. The unfolded and aggregated proteins have more surface area and

hydrophilic groups exposed to hold water. In addition, these reactive groups may randomly associate which would account for the increased firmness. Unlike a gel where the network is ordered, this aggregated system is random which would account for the low gel strength compared to the more undenatured treatments.

There was no consistent relationship between salt concentration and gel strength. Whey protein concentrate 98 in 0.1 NaCl heated to 70°C did not form a gel that was measurable, whereas the same treatment in 0.6M NaCl formed a weak gel (8.1 kPa). This difference in gel strength was too small to conclude that salt concentration was the cause. At 80°C, WPC 80 in 0.1M NaCl produced a firmer gel (53.1 kPa) than the same treatment combination in 0.6M NaCl (42.0 kPa). However, at 90°C, gel firmness was greater for WPC 80 in 0.6M NaCl (59.8 kPa) than in 0.1M NaCl (50.6 kPa). The high salt concentrations gave firmer gels at 80°C and 90°C for WPC 47 than the low salt concentration under the same conditions. WPC 27 in 0.6M NaCl produced a firmer gel at 70° (17.2 kPa) than it did in 0.1M NaCl at 70° (12.8 kPa), but no differences in gel firmness were found between high and low salt at 80°C or 90°C. Only one replicate of the low salt treatment was produced, so statistical comparisons between low and high salt treatments could not be made. Schmidt et al. (1978a) used texture profile analysis (TPA) hardness and a probe penetration test to evaluate the effect of NaCl concentration on the strength of gels made from

dialyzed and non-dialyzed WPC (10%) protein. They found that penetration and hardness values were maximized at 0.2M NaCl for the dialyzed samples, but salt concentration had little effect on gels made with undialyzed WPC.

4.3 Effect of Salt Concentration and Temperature on Apparent Strain of Whey Protein Concentrate Gels

Tables 9 and 10 contain the apparent strain values of WPC gels prepared with NaCl concentrations of 0.1M and 0.6M, respectively. Shear strain is the amount of deformation at gel failure and has been associated with sensory springiness (Toda et al., 1978). Several trends are evident from the data. Heating temperature did not affect the strain of gels at either salt concentration. This is consistent with the finding of Kim (1987). Fish surimi gels were prepared using three heating schedules (40°/30 min, 90°/15 min or a combination of these treatments) and strain did not differ among treatments.

Insolubility of WPC had a large impact on strain values. Strain at failure at 80° and 90°C for WPC 47 gels were half that of WPC 98 gels, regardless of salt concentration. At NaCl concentration of 0.1 and 0.6M and temperature of 80° and 90°C, there was no difference in strain of WPC 47 and WPC 27 gels. Whey protein concentrate 47 did not form gels at 60° or 70°C. Shear strain at failure is a good measure of protein functionality or denaturation (Hamann, 1988). Kim et al. (1986) found that increased freeze-thaw cycles decreased strain at failure of

Table 9. Apparent strain at failure for 20% protein (w:w) whey protein concentrate (WPC) gels in 0.1M NaCl, 0.1M Na phosphate, pH 7.0^a

Treatment ^b	Apparent Strain at Failure			
	Temperature			
	60°C	70°C	80°C	90°C
WPC [98]	TWTM	0.82 (0.09)	0.76 (0.04)	0.80 (0.05)
WPC [80]	no gel	TWTM	0.58 (0.03)	0.64 (0.04)
WPC [47]	no gel	no gel	0.31 (0.04)	0.35 (0.01)
WPC [41]	no gel	no gel	no gel	no gel
WPC [27]	0.31 (0.04)	0.37 (0.04)	0.30 (0.01)	0.34 (0.04)

^a Average of three determinations with standard deviations in parenthesis

^b Numbers in brackets represent % protein solubility in 0.1M NaCl, pH 7.0.

NOTE: TWTM designates gels too weak to be detected by the test. "No gel" designates samples which flowed when the tube was inverted.

Table 10. Apparent strain at failure for 20% (w:w) protein whey protein concentrate (WPC) gels in 0.6M NaCl, 0.1M Na phosphate, pH 7.0

Treatment ^a	Apparent Strain at Failure		
	Temperature		
	70°C	80°C	90°C
WPC [98]	TWTM	0.95 ^b	0.79 ^b
WPC [80]	no gel	0.68 ^c	0.73 ^b
WPC [47]	no gel	0.34 ^d	0.34 ^c
WPC [41]	no gel	no gel	TWTM
WPC [27]	0.30	0.34 ^d	0.36 ^c

^a Numbers in brackets represent % protein solubility in 0.1M NaCl, pH 7.0.

^{b-d} Means within columns followed by the same letter do not differ significantly ($P < 0.05$)

NOTE: TWTM designates gels too weak to be detected by the test. "No gel" designates samples which flowed when the tube was inverted.

Alaskan pollack surimi gels. Park et al. (1987) reported a similar trend with gels made from beef semimembranosus muscle that had been stored frozen for 0 to 8 months. As storage time increased, strain at failure values decreased.

Salt concentration had no apparent effect on strain at failure of WPC gels. Schmidt et al. (1979) reported that cohesiveness of gels made from non-dialyzed WPC (10%) was maximized at 0.2M NaCl.

4.4 Effect of Protein Insolubilization on Expressible Moisture of 20% Whey Protein Concentrate Gels

Expressible moisture (EM) was calculated for WPC gels (20% protein) in 0.6M NaCl, pH 7.0 buffer (Table 11). Heating temperature did not significantly affect the EM of gels made from WPC 98, WPC 41, or WPC 27 ($P > 0.05$). Expressible moisture decreased from 36.1% to 21.4% when the heating temperature of WPC 80 gels was increased from 70°C to 80°C. Gels made from WPC 47 also expressed less moisture (34.7% vs. 28.2%) when heating temperature increased from 70°C to 80°C. Expressible moisture did not significantly change ($P > 0.05$) for either treatment when the temperature was increased from 80°C to 90°C. Since the T_d of β -lg is near 80°C (de Wit and Klarenbeek, 1984), most of the β -lg may have already denatured and formed a gel. Thus, EM did not change when the temperature increased from 80°C to 90°C because the gel was already formed. The decrease in EM for these two treatments between 70°C and 80°C coincides with an increase in gel firmness, indicating that a 3-dimensional

Table 11. Expressible moisture (EM) for 20% (w:w) protein whey protein concentrate (WPC) gels in 0.6M NaCl; 0.1M Na phosphate, pH 7.0

Treatment ^a	Expressible Moisture (%)		
	Temperature		
	70°C	80°C	90°C
WPC [98]	23.5 ^{defg}	19.6 ^g	22.2 ^{efg}
WPC [80]	36.1 ^c	21.4 ^{fg}	23.2 ^{defg}
WPC [47]	34.7 ^c	28.2 ^d	27.2 ^{de}
WPC [41]	44.2 ^b	44.7 ^b	39.5 ^{bc}
WPC [27]	24.4 ^{defg}	26.2 ^{def}	28.2 ^d

^a Numbers in brackets represent % protein solubility in 0.1M NaCl, pH 7.0.

^{b-g} Means followed by the same letter do not differ significantly (P < 0.05)

matrix formed at 80°C and entrapped the moisture. Expressible moisture remained high over the temperature range evaluated for WPC 41 because of the lack of gel formation. The lack of a gel matrix to physically entrap water resulted in high EM values.

Gel expressible moisture was greatly affected by the degree of WPC insolubilization. Treatments with the most soluble protein expressed the least amount of moisture when heated above 80°C. Gels from the most severely insolubilized product expressed less moisture than gels made from WPC 41. de Wit and de Boer (1975) reported that water uptake by WPC powder increased with increasingly severe heat treatment. They attributed this phenomenon to the changed geometry of the aggregates and enhanced water-binding by denatured proteins. This may explain why WPC 27 treatment expressed less moisture than WPC 41 treatments.

4.5 Salt-Soluble Protein:Whey Protein Concentrate Gels: Effect of Whey Protein Concentrate Concentration on Gel Properties.

4.5.1 Apparent Stress at Failure

Gels were made in which salt-soluble protein (SSP) concentration was held constant at 4% and WPC 98 was varied from 8% to 16% (protein basis) in 0.6M NaCl, 0.05M Na phosphate buffer, pH 7.0. None of the SSP gels alone failed so apparent stress was calculated at 80% compression (Table 12). Apparent stress at 80% compression tripled when SSP concentration increased from 4% to 8% and gels were heated

Table 12. Apparent stress at failure (kPa) for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination

Treatment ^a	Ratio ^b %SSP:%WPC	Apparent Stress at Failure (kPa)	
		Temperature	
		65°C	90°C
SSP	4:0	24.0 ^d	< 4.0
SSP	8:0	76.7 ^c	53.7 ^e
WPC (98)	0:8	no gel	< 4.0
WPC (98)	0:12	no gel	15.3 ^f
WPC (98)	0:16	no gel	28.1 ^f
WPC (98)	0:20	no gel	94.9 ^d
SSP:WPC (98)	4:8	24.8 ^d	46.7 ^e
SSP:WPC (98)	4:12	27.1 ^d	97.7 ^d
SSP:WPC (98)	4:16	15.2 ^d	134.3 ^c

^a Numbers in parenthesis represent % protein solubility in 0.1M NaCl, pH 7.0.

^b Ratio is based on protein content.

^{c-f} Means within the same column followed by the same letter do not differ significantly ($P < 0.05$).

NOTE: Samples designated "< 4.0" formed weak gels that were below the sensitivity of the test. Samples designated "no gel" flowed when the tube was inverted.

at 65°C. The 4% SSP gel heated at 90°C was too soft to measure while the 8% SSP gels were weaker at 90°C than at 65°C indicating that too much heat may destabilize the gel matrix of salt-soluble protein. Using viscosity index as an indicator of gel strength, Wang (1990) found that gel strength of 3% SSP gels at pH 6.5 or 7.5 decreased at holding temperatures above 70°C.

Whey protein concentrate alone did not form a gel at any concentration when heated to 65°C. Results from differential scanning calorimetry of WPC show a large peak between 70°C and 80°C which corresponds closely to the denaturation temperature of β -lg (de Wit, 1981; de Wit and Klarenbeek, 1984). Since β -lg accounts for half or more of the total protein of WPC, this may explain why no gelation took place below 70°C. Apparent stress at failure increased from 15.3 to 94.9 kPa when protein concentration increased from 12 to 20% at a holding temperature of 90°C.

There was no significant difference in apparent stress at failure when the ratio of SSP:WPC 98 was increased from 4:8 to 4:16 ($P > 0.05$) at a holding temperature of 65°C (Table 12). Gel strength of the 4% SSP was not significantly different than the combination gels at 65°C indicating the SSP was probably solely responsible for the gel network. The WPC might have been acting as an inactive filler within the SSP gel network as described by Morris (1985).

When holding temperature was increased to 90°C, apparent stress at failure doubled when the SSP:WPC changed from 4:8 to 4:12, and tripled when the ratio increased from 4:8 to 4:16. It is interesting to compare the apparent stress at failure of the 20% WPC gel (94.9 kPa) with that of the SSP:WPC 4:16 gel (134.3 kPa). At 4% protein SSP alone formed a weak gel (< 4.0 kPa) at 90°C. These results suggest a greater than additive effect of the two components on gel strength at equal total protein concentrations. Burgella et al. (1985) working with minced fish (MF):WPC combination gels found that low ratios gave a gel with less strength than an additive relationship would have predicted. When the MF:WPC ratio was high, gel strength approximated an additive relationship.

4.5.2 Apparent Strain at Failure

As mentioned previously, SSP gels did not fail and strain values were recorded at 80% compression. Apparent strain at failure for gels prepared from WPC with protein concentrations of 12 and 20% did not differ significantly at 90°C ($P < 0.05$), though the 16% WPC gel showed less elasticity (Table 13).

Apparent strain at failure decreased significantly ($P < 0.05$) for SSP:WPC gels made at 65°C as the proportion of WPC increased from 8% to 16%. The whey proteins may be affecting the ability of SSP to crosslink as the number of crosslinks is associated with the elastic modulus of a gel

Table 13. Apparent strain at failure for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination

Treatment ^a	Ratio ^b %SSP:%WPC	Apparent Strain at Failure	
		Temperature	
		65°C	90°C
SSP	4:0	1.61 ^c	TWTM
SSP	8:0	1.61 ^c	1.61 ^c
WPC (98)	0:8	no gel	TWTM
WPC (98)	0:12	no gel	0.90 ^d
WPC (98)	0:16	no gel	0.69 ^f
WPC (98)	0:20	no gel	0.88 ^d
SSP:WPC (98)	4:8	1.20 ^d	0.88 ^{de}
SSP:WPC (98)	4:12	1.04 ^{de}	0.83 ^{def}
SSP:WPC (98)	4:16	0.81 ^e	0.72 ^{ef}

^a Numbers in parenthesis represent % protein solubility in 0.1M NaCl, pH 7.0.

^b Ratio is based on protein content.

^{c-f} Means within columns followed by the same letter do not differ significantly (P < 0.05).

NOTE: TWTM designates gels too weak to be detected by the test. "No gel" designates samples which flowed when the tube was inverted.

(Hamann, 1983). When the three SSP:WPC combinations (4:8, 4:12, 4:16) were heated at 90°C, strain values did not differ significantly ($P < 0.05$). Apparent strain at failure for the combination gels was similar to that of the WPC gels at a holding temperature of 90°C, indicating WPC was the determining component of strain at this temperature. This is also evidenced by the fact that SSP:WPC gels were more elastic when heated at 65°C (before WPC gelation) than when heated at 90°C.

4.5.3 Expressible Moisture

Expressible moisture of SSP gels heated at 65°C and 90°C decreased by one-third and one-half, respectively, as protein concentration was raised from 4 to 8% (Table 14). This is most likely due to a more dense network formed by the gel with higher percentage protein. Holding temperature did not have a pronounced effect on the EM of SSP at either concentration.

Gels prepared from WPC 98 at 90°C showed a significant decrease ($P < 0.05$) in EM as protein concentration increased from 8% to 20%. Thus, water-holding capacity of the gel was increased as protein concentration increased because a more organized, dense network was produced.

Expressible moisture for the SSP:WPC gels at 65°C was not concentration dependent. All combination gels expressed significantly less moisture ($P < 0.05$) than 4% SSP at 65°C.

Table 14. Expressible moisture for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination

Treatment ^a	Ratio ^b %SSP:%WPC	Expressible Moisture (%)	
		Temperature	
		65°C	90°C
SSP	4:0	38.1 ^c	32.3 ^e
SSP	8:0	13.1 ^e	16.8 ^f
WPC (98)	0:8	no gel	63.5 ^e
WPC (98)	0:12	no gel	41.8 ^d
WPC (98)	0:16	no gel	31.8 ^e
WPC (98)	0:20	no gel	20.3 ^f
SSP:WPC (98)	4:8	16.0 ^{de}	17.8 ^f
SSP:WPC (98)	4:12	17.7 ^{de}	7.3 ^g
SSP:WPC (98)	4:16	21.2 ^d	5.7 ^g

^a Numbers in parenthesis represent % protein solubility in 0.1M NaCl, pH 7.0.

^b Ratio is based on protein content.

^{c-g} Means within columns followed by the same letter do not differ significantly (P < 0.05).

NOTE: Samples designated "no gel" flowed when the tube was inverted.

This was probably due to whey protein:water interactions within the interstitial spaces of the SSP gel network.

Expressible moisture was not different between 65°C and 90°C for the 4% SSP:8% WPC 98 combination gels, but EM decreased by more than one-half for the 4:12 and 4:16 combinations when holding temperature increased to 90°C. This may indicate that at 90°C the gel structure formed a phase separated network, as described by Oakenfull (1987). The SSP formed a matrix at about 65°C and the whey proteins formed a gel within the matrix of the SSP gel. As mentioned earlier, WPC 98 formed a very weak gel at 90°C. Thus, the WPC concentration in the 4% SSP:8% WPC combination was not sufficient to form a gel capable of physically entrapping water. Burgella et al. (1985) suggested a similar gel network for minced fish (MF):WPC and MF:egg white co-gels.

4.6 Effects of pH on Properties of Salt-Soluble Protein, Whey Protein Concentrate, and Salt-Soluble Protein:Whey Protein Concentrate Gels

4.6.1 Apparent Stress at Failure

The gels were prepared from SSP, WPC, and 4% SSP:12% WPC (w:w) in 0.6M NaCl buffer at pH 6.0, 7.0 and 8.0. There was a general trend for SSP gels to weaken as the pH increased from 6.0 to 8.0 at both holding temperatures (65°C and 90°C) and protein concentrations (4.0% and 8.0%) (Tables 15, 16, 17). The exception to the trend was 8% SSP at 65°C, which formed the firmest gel at pH 8.0. Wang (1990) used back extrusion to measure the viscosity index of chicken

Table 15. A
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Treatment^a

SSP

SSP

WPC (98)

WPC (80)

WPC (41)

SSP:WPC (9)

SSP:WPC (8)

SSP:WPC (7)

^a Numbers

in 0.1M

^b Ratio i

^{c,d} Means

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Table 15. Apparent stress at failure for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination at pH 6.0

Treatment ^a	Ratio ^b %SSP:%WPC	Apparent Stress at Failure (kPa)	
		Temperature	
		65°C	90°C
SSP	4:0	32.3 ^d	17.3 ^d
SSP	8:0	87.2 ^c	72.4 ^c
WPC (98)	0:16	no gel	28.3 ^d
WPC (80)	0:16	no gel	10.6 ^d
WPC (41)	0:16	no gel	no gel
SSP:WPC (98)	4:12	< 4.0	21.1 ^d
SSP:WPC (80)	4:12	9.0 ^d	19.1 ^d
SSP:WPC (41)	4:12	81.3 ^c	29.0 ^d

^a Numbers in parenthesis represent % protein solubility in 0.1M NaCl, pH 7.0.

^b Ratio is based on protein content.

^{c,d} Means within columns followed by the same letter do not differ significantly ($P < 0.05$).

NOTE: Samples designated "< 4.0" formed weak gels that were below the sensitivity of the test. Samples designated "no gel" flowed when the tube was inverted.

Table 16. Apparent stress at failure for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination at pH 7.0

Treatment ^a	Ratio ^b %SSP:%WPC	Apparent Stress at Failure (kPa)	
		Temperature	
		65 °C	90 °C
SSP	4:0	24.0 ^f	< 4.0
SSP	8:0	76.7 ^{cd}	53.7 ^f
WPC (98)	0:16	no gel	28.1 ^g
WPC (80)	0:16	no gel	35.4 ^g
WPC (47)	0:16	no gel	< 4.0
WPC (41)	0:16	no gel	< 4.0
WPC (27)	0:16	no gel	8.2 ^h
SSP:WPC (98)	4:12	27.1 ^{ef}	97.7 ^c
SSP:WPC (80)	4:12	52.1 ^{de}	81.4 ^d
SSP:WPC (47)	4:12	106.4 ^c	61.9 ^{ef}
SSP:WPC (41)	4:12	90.9 ^c	54.2 ^f
SSP:WPC (27)	4:12	105.5 ^c	70.0 ^{de}

^a Numbers in parenthesis represent % protein solubility in 0.1M NaCl, pH 7.0.

^b Ratio is based on protein content.

^{c-h} Means within columns followed by the same letter do not differ significantly (P < 0.05).

NOTE: Samples designated "< 4.0" formed weak gels that were below the sensitivity of the test. Samples designated "no gel" flowed when the tube was inverted.

Table 17. Apparent stress at failure for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination at pH 8.0

Treatment ^a	Ratio ^b %SSP:%WPC	Apparent Stress at Failure (kPa)	
		Temperature	
		65°C	90°C
SSP	4:0	< 4.0	< 4.0
SSP	8:0	104.7 ^c	< 4.0
WPC (98)	0:16	no gel	59.4 ^e
WPC (80)	0:16	no gel	38.3 ^f
WPC (41)	0:16	no gel	< 4.0
SSP:WPC (98)	4:12	25.1 ^d	102.4 ^c
SSP:WPC (80)	4:12	45.6 ^d	86.7 ^d
SSP:WPC (41)	4:12	84.1 ^c	38.2 ^f

^a Numbers in parenthesis represent % protein solubility in 0.1M NaCl, pH 7.0.

^b Ratio is based on protein content.

^{c-f} Means within columns followed by the same letter do not differ significantly (P < 0.05).

NOTE: Samples designated "< 4.0" formed weak gels that were below the sensitivity of the test. Samples designated "no gel" flowed when the tube was inverted.

breast SSP gels at pH 5.5, 6.5 and 7.5. Gels prepared at pH 5.5 were found to be the strongest, followed by pH 6.5 and 7.5. Ashgar et al. (1984) also determined maximum gel rigidity of chicken meat myosin at pH 5.6. No gels were formed from WPC alone at any pH when holding temperature was 65°C. This is in agreement with Burgess and Kelly (1979) and de Wit et al. (1988) who found that WPC prepared from sweet whey by ultrafiltration required temperatures of 67-79°C for gelation.

At 90°C, whey protein concentrate 41 did not form measurable gels at pH 7.0 and 8.0 and no gelation occurred at pH 6.0. Apparent stress at failure for WPC 98 and WPC 80 was not significantly different ($P < 0.05$) at pH 6.0 and 7.0. At pH 8.0 apparent stress at failure was 59.4 kPa for WPC 98 and 38.3 kPa for WPC 80 (Table 17). This is in general agreement with de Wit et al. (1988) that there is a decrease in gel strength with increasing denaturation. The general trend of increased stress at failure with increased pH is in agreement with Morr and Foegeding (1990). However, this is not in agreement with a study done by Zirbel and Kinsella (1988). They prepared 20% whey protein isolate (WPI) gels at pH 6.0, 6.4, 7.0 and 8.0 and found that hardness increased with increased pH at 70°C, but decreased with increased pH when heated to 80°C and 90°C. Whey proteins become more negatively charged when the pH increases above their isoelectric point, which inhibited protein-protein interactions through electrostatic repulsion

and may be responsible for the weaker gels (Zirbel and Kinsella, 1988).

Researchers have shown that addition of calcium to whey protein isolate (WPI) and WPC may increase gel strength (Schmidt et al., 1978a,b; Zirbel and Kinsella, 1988; Johns and Ennis, 1981). The calcium may interact with the negatively charged proteins at higher pH's and partially reduce the electrostatic repulsion. This may explain the higher apparent stress at failure values at higher pH's in our study.

When SSP:WPC gels were heated at 65°C, apparent stress at failure increased significantly ($P < 0.05$) as the solubility of WPC decreased at all three pH's. One possible explanation for this is the unfolded proteins may contain more exposed reactive groups such as carboxyls and hydrophobic groups which react with the SSP to enhance gel strength. However, a better explanation is the unfolded proteins acted as an inactive component of a filled gel. The denatured whey proteins, which bind water well (de Wit and de Boer, 1975), absorbed water which firmed the gel.

Whey protein concentrates 98 and 80 decreased the strength of SSP:WPC gels at 65°C and pH 6.0 compared to the 4% SSP control (Table 16). It is possible that since whey proteins are less negatively charged at the lower pH, they were able to come into closer contact with the myosin and interfere with the gelling process.

At a holding temperature of 90°C and pH 6.0 (Table 15), apparent stress at failure for the SSP:WPC gels did not differ significantly ($P > 0.05$) with respect to WPC insolubilization. There was also no significant difference in stress at failure between WPC 98 and SSP:WPC 90 or WPC 80 and SSP:WPC 80. Whey protein concentrate 41, which did not gel at 16% protein and pH 6.0, did form a gel when 4% SSP was added. However, it is likely that the SSP formed the gel matrix and WPC 41 acted as a filler as indicated earlier. Other techniques such as scanning electron microscopy would be useful to determine the role of each component.

Apparent stress at failure increased for SSP:WPC 98, SSP:WPC 80 and SSP:WPC 41 gels as pH increased from 6.0 to 7.0 (Tables 15 and 16). At pH 7.0 and 90°C (Table 16) gel strength of SSP:WPC 98 was three times that of WPC 98 alone (97.7 vs. 28.1 kPa). Similarly, gel strength of SSP:WPC 80 was double that of WPC 80 alone (81.4 vs. 35.4 kPa) under the same conditions. Since 4% SSP did not form a measurable gel, it appears that the combination of SSP and WPC 98 or WPC 80 has a greater than additive effect on gel strength at pH 7.0 and 90°C. Results from Table 17 show this is also the case at pH 8.0. Whey protein gels at neutral and alkaline pH are stronger because intermolecular S-S bonds and hydrophobic interactions are primarily responsible for gel network, whereas hydrogen bonding predominates at acid pH resulting in a weaker gel (de Wit and de Boer, 1975;

Shimada and Cheftel, 1988). Schmidt et al. (1978b) prepared gels by blending peanut protein flour (PF) and WPC at pH 7.0 and found that addition of PF to 12.5% of total protein did not affect gel strength, but increasing PF to 25% of total protein caused a 50% reduction in gel strength from the WPC control.

Apparent stress at failure decreased for SSP:WPC gels at pH 7.0 and 8.0 and a heating temperature of 90°C as WPC insolubilization increased (Tables 16 and 17). Regardless of the degree of WPC insolubilization, apparent stress at failure for the SSP:WPC combinations was greater than the sum of the individual components. The reason for the strengthening of the network of combination gels by WPC was probably different for the solubilized WPC than the insolubilized WPC. The SSP:WPC 98 most likely formed a phase separated gel network as described by Oakenfull (1987). The SSP gelled at about 65° and the WPC gelled within the interstitial spaces of the SSP network between 70 and 90°C. On the other hand, SSP:WPC 41 was primarily a filled gel with SSP the active component and WPC 41 the inactive component. This is because much of the whey protein was already insolubilized, making it unable to form a gel. The ability of the insolubilized whey proteins to hold water gave support to the SSP gel network. The SSP:WPC 80 gels may have been a combination phase separated network/filled gel with the insolubilized whey proteins

binding water (Oakenfull, 1987). Electron microscopy would be helpful to discern the exact nature of the gel structure.

4.6.2 Apparent Strain at Failure

Apparent strain at failure values for gels at pH 6.0, 7.0 and 8.0 appear in Tables 18, 19 and 20. None of the SSP gels that were self-supporting failed at or before 80% compression, indicating SSP alone forms a very elastic gel.

Gels prepared from WPC were less elastic than those made from SSP, regardless of pH or degree of WPC insolubilization. Whey protein concentrate 98 gels heated at 90°C exhibited greatest strain at failure at pH 8.0 (0.98) and lowest at pH 7.0 (0.69) while WPC 80 had a maximum strain at pH 8.0 (0.85) and a minimum at pH 6.0 (0.50). There was no consistent trend in strain with regard to change in pH at 90°C. This was consistent with other work done by Morr and Foegeding (1990) with WPC. Shimada and Cheftel (1988) reported that as pH increased, elasticity of gels prepared from WPI (9% protein) in aqueous solution increased.

Strain at failure values for gels made from WPC at 90°C and pH 6.0 (Table 18) decreased from 0.87 to 0.50 as solubility decreased from 98 to 80 ($P < 0.05$). At pH 7.0 (Table 19), strain at failure for WPC 98 gels was not significantly different from that of WPC 80 gels ($P > 0.05$), but gels made from WPC 27 were much less elastic than those made from WPC 98 or WPC 80 ($P < 0.05$). Whey protein

Table 18. Apparent strain at failure for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination at pH 6.0

Treatment ^a	Ratio ^b %SSP:%WPC	Apparent Strain at Failure	
		Temperature	
		65°C	90°C
SSP	4:0	1.61 ^c	1.61 ^c
SSP	8:0	1.61 ^c	1.61 ^c
WPC (98)	0:16	no gel	0.87 ^d
WPC (80)	0:16	no gel	0.50 ^e
WPC (41)	0:16	no gel	no gel
SSP:WPC (98)	4:12	TWTM	0.45 ^e
SSP:WPC (80)	4:12	0.71 ^d	0.46 ^e
SSP:WPC (41)	4:12	1.61 ^c	0.94 ^d

^a Numbers in parenthesis represent % protein solubility in 0.1M NaCl, pH 7.0.

^b Ratio is based on protein content.

^{c-e} Means within columns followed by the same letter do not differ significantly ($P < 0.05$).

NOTE: TWTM designates gels too weak to be detected by the test. "No gel" designates samples which flowed when the tube was inverted.

Table 19. Apparent strain at failure for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination at pH 7.0

Treatment ^a	Ratio ^b %SSP:%WPC	Apparent Strain at Failure	
		Temperature	
		65 °C	90 °C
SSP	4:0	1.61 ^c	TWTM
SSP	8:0	1.61 ^c	1.61 ^c
WPC (98)	0:16	no gel	0.69 ^g
WPC (80)	0:16	no gel	0.82 ^{fg}
WPC (47)	0:16	no gel	TWTM
WPC (41)	0:16	no gel	TWTM
WPC (27)	0:16	no gel	0.29 ^h
SSP : WPC (98)	4:12	1.04 ^e	0.83 ^{fg}
SSP : WPC (80)	4:12	1.36 ^d	0.91 ^f
SSP : WPC (47)	4:12	1.38 ^d	1.07 ^{de}
SSP : WPC (41)	4:12	1.61 ^c	1.20 ^d
SSP : WPC (27)	4:12	1.28 ^d	0.96 ^{ef}

^a Numbers in parenthesis represent % protein solubility

^b in 0.1M NaCl, pH 7.0.

^c - Ratio is based on protein content.

Means within a column followed by the same letter do not differ significantly ($P < 0.05$).

NOTE: TWTM designates gels too weak to be detected by the test. "No gel" designates samples which flowed when the tube was inverted.

Table 20. Apparent strain at failure for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination at pH 8.0

Treatment ^a	Ratio ^b %SSP:%WPC	Apparent Strain at Failure	
		Temperature	
		65°C	90°C
SSP	4:0	TWTM	TWTM
SSP	8:0	1.61 ^c	TWTM
WPC (98)	0:16	no gel	0.98 ^d
WPC (80)	0:16	no gel	0.85 ^d
WPC (41)	0:16	no gel	no gel
SSP = WPC (98)	4:12	1.05 ^e	0.91 ^d
SSP = WPC (80)	4:12	1.38 ^d	0.87 ^d
SSP = WPC (41)	4:12	1.61 ^c	1.20 ^c

^a Numbers in parenthesis represent % protein solubility

^b in 0.1M NaCl, pH 7.0.

^c, ^d, ^e Ratio is based on protein content.

Means within columns followed by the same letter do not differ significantly ($P < 0.05$).

NOTE: TWTM designates gels too weak to be detected by the test. "No gel" designates samples which flowed when the tube was inverted.

concentrate 98 and WPC 80 did not show significant differences ($P > 0.05$) in apparent strain at failure at pH 8.0 (Table 20). Since neither WPC 41 or WPC 47 formed gels, it is not possible to determine from these results if strain at failure decreased with increased insolubilization or if strain at failure values are dependent on a specific degree of insolubilization.

Gels made from SSP:WPC 41 at 65°C and pH 6.0, 7.0 and 8.0 (Tables 18, 19, 20) did not fail at 80% compression (highly elastic). Thus, the insolubilized whey proteins were acting as a filler within the SSP gel matrix and had little effect on strain values (Morris, 1985). Lanier et al. (1985) added heat-denatured surimi to a gel preparation. They found that 40% added denatured proteins decreased strain values by 12% while 50% addition decreased strain values by 28%. Apparent strain at failure values at 65°C and pH 7.0 and 8.0 were significantly lower for SSP:WPC 98 than for SSP:WPC 80 ($P < 0.05$). Salt-soluble protein:whey protein concentrate 98 did not form a measurable gel at pH 6.0 and apparent strain at failure (65°C) for SSP:WPC 80 was one-half (0.711) that of the same treatment at pH 7.0 (1.36) and pH 8.0 (1.38). The number of crosslinks is related to the elasticity of the gel (Hamann, 1983). As mentioned previously, whey proteins are less electronegative at lower pH (Zirbel and Kinsella, 1988) which may allow them to interfere with crosslinking of the SSP and decrease elasticity.

When SSP:WPC 98 and SSP:WPC 80 were heated at 90°C, the whey proteins gelled and WPC exerted more influence on strain than SSP. The combination gels containing WPC 41 were less elastic than those containing soluble WPC because SSP was largely responsible for the matrix. Gels made from SSP:WPC 41 heated at 65°C were probably less elastic than those heated at 90°C because of thermal breakdown of the SSP gel matrix at the higher temperature. Gels from SSP:WPC 98 and SSP:WPC 80 at pH 6.0 and 90°C were less elastic than at pH 7.0 or 8.0 because weak bonds, such as hydrogen bonds, are responsible for the gel network at acid pH's (Shimada and Cheftel, 1988; de Wit and de Boer, 1975).

4.6.3 Expressible Moisture

Tables 21, 22 and 23 summarize the expressible moisture (EM) for SSP, WPC and multicomponent gels at pH 6.0, 7.0 and 8.0, respectively. The data show that gel EM decreased by two-thirds when the SSP concentration increased from 4% to 8% at a holding temperature of 65°C ($P > 0.05$), regardless of pH. Additionally, EM for 4% and 8% SSP gels was not affected by pH.

Wang (1990) reported that 3% SSP gels heated to 65°C expressed less moisture when prepared at pH 6.5 or 7.5 than at pH 4.5 or 5.5. Electron micrographs revealed that gels prepared at pH 4.5 were highly aggregated with little or no network. Gels at pH 5.5 contained an irregular matrix with globular, thick protein filaments. At pH 6.5 and 7.5 gel

Table 21. Expressible moisture for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination at pH 6.0

Treatment ^a	Ratio ^b %SSP:%WPC	Expressible Moisture (%)	
		Temperature	
		65° C	90° C
SSP	4:0	37.6 ^d	38.6 ^c
SSP	8:0	13.5 ^f	13.5 ^e
WPC (98)	0:16	no gel	30.6 ^d
WPC (80)	0:16	no gel	32.2 ^d
WPC (41)	0:16	no gel	no gel
SSP : WPC (98)	4:12	55.0 ^c	32.5 ^d
SSP : WPC (80)	4:12	39.8 ^d	30.3 ^d
SSP : WPC (41)	4:12	19.6 ^e	28.9 ^d

- a Numbers in parenthesis represent % protein solubility
 b in 0.1M NaCl, pH 7.0.
 c - Ratio is based on protein content.
 Means within columns followed by the same letter do not differ significantly (P < 0.05).

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Table 22. Expressible moisture for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination at pH 7.0

Treatment ^a	Ratio ^b %SSP:%WPC	Expressible Moisture (%)	
		Temperature	
		65 °C	90 °C
SSP	4:0	38.0 ^c	29.3 ^d
SSP	8:0	13.5 ^e	16.6 ^e
WPC (98)	0:16	no gel	31.8 ^d
WPC (80)	0:16	no gel	31.9 ^d
WPC (47)	0:16	no gel	34.5 ^d
WPC (41)	0:16	no gel	52.2 ^c
WPC (27)	0:16	no gel	31.2 ^d
SSP = WPC (98)	4:12	17.7 ^d	7.3 ^f
SSP = WPC (80)	4:12	11.5 ^e	7.6 ^f
SSP = WPC (47)	4:12	11.8 ^e	9.8 ^f
SSP = WPC (41)	4:12	13.0 ^e	10.8 ^f
SSP = WPC (27)	4:12	12.4 ^e	15.6 ^e

a Numbers in parenthesis represent % protein solubility

b in 0.1M NaCl, pH 7.0.

c - Ratio is based on protein content.

Means within columns followed by the same letter do not differ significantly ($P < 0.05$).

Table 23. Expressible moisture for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) gels alone and in combination at pH 8.0

Treatment ^a	Ratio ^b %SSP:%WPC	Expressible Moisture (%)	
		Temperature	
		65°C	90°C
SSP	4:0	38.2 ^c	34.7 ^d
SSP	8:0	12.1 ^{de}	17.3 ^e
WPC (98)	0:16	no gel	33.4 ^d
WPC (80)	0:16	no gel	34.5 ^d
WPC (41)	0:16	no gel	46.7 ^c
SSP : WPC (98)	4:12	17.3 ^d	7.1 ^f
SSP : WPC (80)	4:12	15.7 ^{de}	8.9 ^f
SSP : WPC (41)	4:12	11.2 ^e	11.6 ^f

^a Numbers in parenthesis represent % protein solubility

^b in 0.1M NaCl, pH 7.0.

^{c-f} Ratio is based on protein content.

Means within columns followed by the same letter do not differ significantly (P < 0.05).

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matrices were well defined which resulted in good water holding capacity.

As mentioned earlier, WPC's alone were only able to form gels at 90°C. Expressible moisture of gels prepared from WPC 98 and WPC 80 did not differ significantly ($P > 0.05$) at pH 6.0, 7.0 or 8.0 (Tables 21, 22, 23). In contrast, Shimada and Cheftel (1988) observed an increase in water-holding capacity of gels made from WPI when pH increased from 6.5 to 7.5. Water-holding capacity for WPC 41 gels was significantly less ($P < 0.05$) than for WPC 98 or WPC 80 gels at pH 7.0 (Table 22) and 8.0 (Table 23). Whey protein concentrate 41 did not gel at pH 6.0 and exhibited slightly less EM at pH 8.0 than pH 7.0. Gels prepared from WPC 41 at pH 8.0, though very weak, were stronger than at pH 7.0, resulting in less EM at pH 8.0. Expressible moisture at pH 7.0 for WPC 27 gels did not differ significantly from that of WPC 98 or WPC 80 gels ($P < 0.05$).

At 65°C, expressible moisture for SSP:WPC gels prepared from WPC 98, WPC 80 and WPC 41 decreased significantly when pH was increased from 6.0 to 7.0, but was not significantly different at pH 7.0 and 8.0. Combination gels prepared from WPC 98 expressed more moisture than those prepared from WPC 41 at pH 6.0, but the opposite was true at pH 7.0 and 8.0. As was explained earlier, soluble native proteins are less negatively charged at pH 6.0 and may interfere with the gelation of the SSP. The insolubilized whey proteins in WPC

41 might bind water which reduced EM (de Wit and de Boer, 1975).

It is interesting to note that at pH 7.0 and 8.0 and 65°C (Tables 22 and 23), SSP:WPC 98 gels expressed half the moisture of 4% SSP gels. Whey protein concentrate 98 was probably acting as a filler by binding water within the interstitial spaces of the SSP gel matrix in the combination gel (Morris, 1985).

The effect of whey protein gelation on EM can be seen when SSP:WPC gels were processed at 90°C. As was the case at 65°C for all SSP:WPC gels, EM decreased as pH increased from 6.0 to 7.0 (Tables 21 and 22), and then did not change when pH increased to 8.0 (Table 23). This was as expected, since whey proteins form a coagulum that is not well organized at pH's less than 6.0 (Hillier et al., 1980).

Insolubilization of the WPC did not significantly effect EM for combination gels at 90°C and pH 6.0 ($P < 0.05$). At pH 7.0, EM for combination gels containing WPC 98, WPC 80, WPC 47 and WPC 41 did not differ significantly ($P > 0.05$), but SSP:WPC 27 gels expressed twice as much moisture as SSP:WPC 98 gels ($P < 0.05$). At pH 8.0, EM did not change significantly for SSP:WPC gels containing WPC 98, WPC 80 and WPC 41.

4.7

Practical Implications of Gel Model System Results

Results from these model system gels indicate WPC may be useful to alter the textural characteristics of processed

meats. Addition of WPC to comminuted poultry meat products may reduce the rubbery texture of these products. Whey protein concentrate with low solubility may improve the yield of low-fat processed meat products which are usually higher in water. Whey protein concentrates with high-protein solubility form firm gels at 90°C which hold large quantities of water. Thus, they may be useful for improving the texture and yield of meats processed to high temperatures such as canned meats and shelf-stable dinners.

4.8 Frankfurter Batter Model System

4.8.1 Effect of Whey Protein Concentrate on Yield

At 70°C, cooked yield for the model system frankfurters with 3.5% added WPC did not differ significantly ($P < 0.05$) from the 97.9% cooked yield of the control, regardless of WPC solubility (Table 24). Cooked yield for all model system frankfurters processed at 90°C was significantly less than the cooked yield of model system frankfurters cooked at 70°C ($P < 0.05$). Yield values at a processing temperature of 90°C were significantly higher ($P < 0.05$) for model system frankfurters with 3.5% added WPC 98 (93.2%) and WPC 80 (94.3%) than for the control.

Ensor et al. (1987) added 1.75%, 2.0% and 3.5% WPC to knockwurst batter and cooked them in a smokehouse to 82°C. They reported no difference in cooked yield compared to an all-meat control. Thompson et al. (1982) found no significant difference in yield between all-meat

Table 24. Yield data for model system chicken frankfurters prepared with 3.5% whey protein concentrates (WPC) of different protein solubility^a

Processing Temperature (°C)	WPC Treatment ^b	Cooked Yield (%)	Reheat Yield (%)
70°C	Control	97.9 ^c	86.1 ^c
	WPC 98	97.4 ^c	85.7 ^c
	WPC 80	97.7 ^c	86.8 ^c
	WPC 47	97.6 ^c	85.1 ^c
	WPC 41	98.3 ^c	84.9 ^c
90°C	Control	91.0 ^f	86.2 ^c
	WPC 98	93.2 ^{de}	89.3 ^c
	WPC 80	94.3 ^d	86.4 ^c
	WPC 47	91.7 ^{ef}	86.8 ^c
	WPC 41	92.5 ^{def}	87.6 ^c

^a WPC was substituted for chicken meat on a protein basis.

^b Numbers indicate percent protein solubility of WPC treatments.

^{c-f} Means within columns followed by the same superscript are not significantly different ($P < 0.05$).

frankfurters and frankfurters supplemented with 10% whey concentrate when the products were heat processed to 89°C.

No significant differences in reheat yield of model system frankfurters were seen among treatments at 70° or 90°C (Table 24). Reheat yield for model system frankfurters heated at 90°C was slightly higher (87.5%) than for model system frankfurters cooked at 70°C (85.6%), though not significantly different ($P > 0.05$).

Results of trials adding 7% WPC to model system frankfurters are considered preliminary, as only one replicate was tested. Model system frankfurters with 7.0% added WPC exhibited higher cooked yields when cooked to 70°C (96.1%) than when cooked to 90°C (91.0%) (Table 25). It appears that model system frankfurters with 7.0% added WPC 41 exhibited higher cooked yields (97.7%) than the control (95.9%), although this could not be verified statistically since only one replicate of the 7% WPC substitution experiment was run. At 90°C, WPC-supplemented model system frankfurters averaged a 91% cooked yield while the control exhibited a cooked yield of 87.8%. The WPC 98- and WPC 80-substituted model system frankfurters exhibited the highest yield at 92.3% and 92.5%, respectively when heat processed to 90°C (Table 25). The WPC's may have formed gels which trapped more of the water and fat. Reheat yield of model system frankfurters substituted with 7.0% WPC averaged 83.3% at a processing temperature of 70°C and 84.5% at a processing temperature of 90°C (Table 25).

Table 25. Yield data for model system chicken frankfurters prepared with 7.0% whey protein concentrates (WPC) of different protein solubility^a

Processing Temperature (°C)	WPC Treatment ^b	Cooked Yield (%) ^c	Reheat Yield (%) ^d
70 °C	Control	95.9 (0.5)	82.4 (0.0)
	WPC 98	94.6 (1.3)	83.7 (0.4)
	WPC 80	96.0 (1.2)	84.6 (1.3)
	WPC 41	97.7 (0.9)	83.6 (2.0)
	WPC 27	96.1 (0.7)	81.1 (0.1)
90 °C	Control	87.8 (2.2)	85.5 (1.3)
	WPC 98	92.3 (0.8)	84.3 (1.0)
	WPC 80	92.5 (1.4)	84.9 (2.2)
	WPC 41	91.7 (1.6)	86.0 (2.5)
	WPC 27	90.5 (0.9)	83.5 (0.2)

^a WPC was substituted for chicken meat on a protein basis.

^b Numbers indicate percent protein solubility of WPC treatments.

^c Values are means of eight analyses for one replicate of WPC with standard deviations in parenthesis.

^d Values are means of duplicate analysis for one replicate with standard deviations in parenthesis.

4.8.2 Effects of Whey Protein Concentrate on Proximate Composition

The proximate composition of model system frankfurters with 3.5% added WPC are presented in Table 26. Batters were formulated to contain 12% protein, 30% fat, 56% water and 2% salt. Moisture and fat did not differ significantly ($P > 0.05$) among treatments at cook temperatures of 70° and 90°C.

Moisture content averaged 56.3% when cooked to 70°C and 56.0% when cooked to 90°C. Fat content was 27.1% for model system frankfurters cooked at 70°C and 26.5% when cooked at 90°C. Model system frankfurters containing 3.5% WPC 80 or WPC 47 were significantly higher in protein (13.3 and 13.4%, respectively) than the control (12.4%) when cooked to 70°C. The reason for this difference was not understood. Protein content of model system frankfurters cooked at 90°C, which averaged 13.8%, was not significantly different among treatments ($P > 0.05$).

Model system frankfurter batters with 7.0% added WPC were formulated to contain 12% protein, 25% fat, 63% moisture and 2% salt. Protein content of model system frankfurters heated to 70°C varied little among treatments and averaged 12.6% (Table 27). However, moisture and fat content of WPC-containing model system frankfurters averaged 61.7% and 21% respectively, while the control contained 59.4% moisture and 23.6% fat. Thus, WPC may be helpful in retaining moisture at the expense of fat during processing.

Solubility of WPC treatments did not greatly affect protein, moisture and fat of model system frankfurters

Table 26. Proximate composition of model system chicken frankfurters prepared with 3.5% whey protein concentrates (WPC) of different protein solubility^a

Processing Temperature (°C)	WPC Treatment ^b	Protein (%)	Water (%)	Fat (%)
70 °C	Control	12.4 ^C	55.9 ^C	28.1 ^C
	WPC 98	13.0 ^{de}	56.2 ^C	27.3 ^C
	WPC 80	13.3 ^{cd}	56.7 ^C	26.3 ^C
	WPC 47	13.4 ^{cd}	56.3 ^C	26.4 ^C
	WPC 41	13.1 ^{de}	56.3 ^C	27.3 ^C
90 °C	Control	13.6 ^{cd}	56.2 ^C	26.9 ^C
	WPC 98	13.7 ^{cd}	55.8 ^C	26.4 ^C
	WPC 80	13.9 ^C	56.5 ^C	25.9 ^C
	WPC 47	13.8 ^C	55.6 ^C	26.9 ^C
	WPC 41	13.8 ^C	55.5 ^C	26.6 ^C

^a WPC was substituted for chicken meat on a protein basis.

^b Numbers indicate percent protein solubility of WPC treatments.

^{c-e} Means within columns followed by the same superscript are not significantly different (P < 0.05).

Table 27. Proximate composition of model system chicken frankfurters prepared with 7.0% whey protein concentrates (WPC) of different protein solubility^a

Processing Temperature (°C)	WPC Treatment ^b	Protein ^c (%)	Water ^c (%)	Fat ^c (%)
70 °C	Control	13.0 (0.0)	59.4 (0.2)	23.6 (0.3)
	WPC 98	12.9 (0.3)	62.8 (0.7)	19.6 (0.7)
	WPC 80	12.1 (0.2)	61.0 (0.1)	21.4 (0.3)
	WPC 41	12.4 (0.1)	61.3 (0.7)	21.7 (0.6)
	WPC 27	12.6 (0.0)	61.7 (0.3)	21.4 (0.6)
90 °C	Control	14.0 (0.1)	58.2 (0.3)	23.5 (0.1)
	WPC 98	12.5 (0.5)	59.9 (0.8)	23.3 (0.1)
	WPC 80	12.6 (0.0)	59.5 (1.0)	22.0 (1.4)
	WPC 41	12.9 (0.1)	59.3 (0.9)	23.0 (0.1)
	WPC 27	12.6 (0.6)	60.5 (0.3)	21.7 (1.3)

^a WPC was substituted for chicken meat on a protein basis.

^b Numbers indicate percent protein solubility of WPC treatments.

^c Means represent duplicate analysis of one replication of WPC.

cooked to 90°C (Table 27). Model system frankfurters with added WPC averaged slightly higher moisture (59.8% vs. 58.2%) and slightly lower protein content (12.7% vs. 14%) than the control. Fat content for all model system frankfurters cooked to 90°C varied little and averaged 22.7%.

4.8.3 Effect of Whey Protein Concentrate on Hardness, Cohesiveness and Springiness

Values for hardness, cohesiveness and springiness did not differ significantly ($P > 0.05$) among model system frankfurters with 3.5% added WPC, regardless of cooking temperature or WPC solubility (Table 28). Though differences may have existed, they probably were not detected because of the large sample-to-sample variation. The batter was very viscous which resulted in many air bubbles in the model system frankfurters. This was probably one source of variation. However, some trends were observed which will require further study. These trends agree with model system gel results and will be discussed here.

At 70°C, hardness ranged from 40.7 N for the WPC 98-substituted model system frankfurter to 50.7 N for the control frankfurter. As WPC insolubilization increased, hardness of model system frankfurters cooked at 70°C increased. This is consistent with the SSP:WPC model system gels. Hardness varied from 48.1 N for the WPC 47-added treatment to 59.4 N for the WPC 98-substituted model system frankfurters processed to 90°C. The treatment with the 98%

Table 28. Texture profile parameters of model system chicken frankfurters prepared with 3.5% whey protein concentrates (WPC) of different protein solubility^a

Processing Temperature (°C)	WPC Treatment ^b	Hardness ^c (N)	Cohesiveness ^c	Springiness ^c (%)
70°C	Control	50.7	0.29	72.7
	WPC 98	40.7	0.28	72.5
	WPC 80	43.7	0.25	72.1
	WPC 47	45.3	0.27	72.1
	WPC 41	45.5	0.27	72.0
90°C	Control	50.3	0.26	71.7
	WPC 98	59.4	0.25	71.7
	WPC 80	58.4	0.25	71.5
	WPC 47	48.1	0.28	71.0
	WPC 41	58.5	0.24	69.9

^a WPC was substituted for chicken meat on a protein basis.

^b Numbers indicate percent protein solubility of WPC treatments.

^c Hardness, cohesiveness and springiness did not vary significantly between temperatures and among treatments ($P > 0.05$).

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WPC-added exhibited the firmest texture. This is also consistent with the SSP:WPC gel study. Though not significant, model system frankfurters cooked to 90°C were slightly harder than those cooked to 70°C.

Ensor et al. (1987) added 1.75%, 20% and 3.5% WPC to knockwurst and found that hardness values were significantly greater than the control, but found no difference among the ratio of WPC addition. Parks and Carpenter (1987) reported that increased substitution of non-fat dry milk (NFDM) or milk protein hydrolysate (MPH) for bull meat in a frankfurter batter decreased hardness values. Thompson et al. (1982) also found that addition of succinylated whey concentrate (SWC) or whey concentrate (WC) decreased firmness of frankfurters over controls.

Cohesiveness did not vary significantly ($P > 0.05$), regardless of temperature or treatment (Table 28). Values for cohesiveness averaged 0.27 and 0.26 for model system frankfurters cooked to 70°C and 90°C, respectively. Ensor et al. (1987) reported an interaction between cohesiveness and the temperature at which the compression test was performed. When the test was conducted at 3.5°C, WPC-supplemented model system frankfurters were more cohesive than the control; however, at 60°C no differences in cohesiveness were observed.

Springiness did not differ significantly ($P > 0.05$) between WPC-supplemented model system frankfurters and control frankfurters or among individual WPC treatments at

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cooking temperatures of 60°C or 90°C (Table 28).

Springiness for all model system frankfurters averaged 72.3% and 71.2% at 70° and 90°C, respectively. Ensor et al. (1987) also reported that the addition of WPC to frankfurters had no effect on the recovery of the product between the compression cycles.

Since only one replicate of model system frankfurters containing 7.0% WPC was prepared, no statistical comparisons could be made (Table 29). However, some general observations can be discussed. The average hardness values of the WPC-added model system frankfurters was half that of the control, whether heated to 70°C (16.4 vs. 34.6 N) or 90°C (16.8 N vs. 32.1 N). This is consistent with the findings of Comer (1979), who added milk proteins to comminuted meat products. Parks and Carpenter (1987) also found that substituting milk protein hydrolysate or nonfat dry milk for bull meat in a frankfurter formulation decreased hardness values of the cooked frankfurters. Others (Thompson et al., 1982; Ensor et al., 1987) have reported that adding WPC to comminuted meats increased firmness. Our results indicate that 7.0% WPC substitution may be too high to produce a frankfurter with acceptable texture.

Cohesiveness of model system frankfurters did not appear to vary greatly with respect to addition of 7.0% WPC or WPC solubility (Table 29). Cohesiveness values for all treatments averaged 0.24 at 70°C and 0.23 at 90°C.

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Table 29. Texture profile parameters of model system chicken frankfurters prepared with 7.0% whey protein concentrates (WPC) of different protein solubility^a

Processing Temperature (°C)	WPC Treatment ^b	Hardness ^c (N)	Cohesiveness ^c	Springiness ^c (%)
70 °C	Control	34.6 (12.5)	0.25 (0.03)	73.3 (6.7)
	WPC 98	19.0 (7.8)	0.22 (0.01)	59.0 (2.0)
	WPC 80	15.5 (2.7)	0.22 (0.01)	64.0 (1.0)
	WPC 41	11.8 (3.7)	0.25 (0.01)	65.7 (1.5)
	WPC 27	19.2 (3.2)	0.26 (0.01)	67.3 (1.5)
90 °C	Control	32.1 (9.0)	0.24 (0.02)	71.3 (1.2)
	WPC 98	18.0 (1.6)	0.23 (0.02)	68.0 (1.7)
	WPC 80	16.7 (3.2)	0.21 (0.01)	63.7 (1.2)
	WPC 41	14.7 (3.0)	0.24 (0.04)	63.3 (2.3)
	WPC 27	17.8 (1.7)	0.25 (0.01)	67.0 (1.0)

^a WPC was substituted for chicken meat on a protein basis.

^b Numbers indicate percent protein solubility of WPC treatments.

^c Values of the means of triplicate analysis with standard deviations in parenthesis.

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Addition of 7.0% WPC appeared to decrease the springiness of the model frankfurters when compared with the control (Table 29). Frankfurters containing WPC yielded springiness values of 64% and 65% at cooking temperatures of 70°C and 90°C, respectively. This is compared to the all-meat control with springiness values of 73.3% and 71.3% when heated to 70°C and 90°C, respectively.

From this work it appears that WPC can be substituted for meat protein up to 3.5% of the formulation. Whey protein concentrate with high solubility increased the yield of model system frankfurters that were cooked to 90°C. However, adding WPC at 7.0% of the formulation may have adverse effects on the texture of processed meats. As with SSP:WPC model system gels, work with the model system frankfurters suggests that solubilized WPC may increase the firmness of meat products processed at or below 70°C.

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CONCLUSIONS

Protein solubility affected the texture and water-holding capacity of gels made from 20% (w:w) WPC in 0.6M and 0.1M NaCl, 0.1 Na phosphate buffers, pH 7.0. As WPC solubility decreased to 41%, the temperature required to form a measurable gel increased. Strain values for these gels decreased as WPC solubility decreased from 98% to 27%. Gels made from WPC with 27% solubility expressed less moisture than gels made with 98% soluble WPC at 70°C. This was ascribed to the ability of insolubilized WPC to absorb water. At 80° and 90°C, 98% soluble WPC expressed less moisture than less soluble WPC because whey proteins form firmer gels.

Increasing the concentration of 98% soluble WPC from 8% to 16% in combination with 4% chicken breast salt-soluble protein (SSP) did not affect gel strength or expressible moisture (EM) when heated at 65°C, but decreased gel elasticity. At a holding temperature of 90°C, increasing WPC 98 from 8% to 16% increased apparent stress at failure from 46.7 to 134.3 kPa, decreased EM from 17.8% to 5.7% and decreased elasticity of gels prepared in 0.6M NaCl, 0.05 M Na phosphate buffer, pH 7.0.

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The pH of multicomponent gels containing 4% SSP and 12% WPC (w:w) altered the strength, elasticity and EM at heating temperatures of 65° and 90°C. Addition of 98% soluble WPC to 4% SSP formed weak gels at pH 7.0 and 8.0 at 65°C, but the gel formed at pH 6.0 was too weak to measure. Additionally, SSP:WPC 98 combination gels at pH 6.0 heated at 65°C expressed more moisture than the 4% SSP gels alone. It appears that the soluble WPC interferes with the gelation of SSP at pH 6.0. Multicomponent gels containing WPC 41 were stronger and more elastic than gels containing WPC 98 at 65°C for all three pH's.

Multicomponent gels containing WPC 98 or WPC 80 were more than three times stronger at pH 7.0 and 8.0 than at pH 6.0. Gels containing WPC 98 or WPC 80 were less elastic than those containing WPC 41 when heated at 90°C. Elasticity was less for all treatments at pH 6.0 than at pH 7.0 or 8.0.

Expressible moisture for all three treatments was less at pH 7.0 and 8.0 than at pH 6.0 when gels were heated at 90°C. This reflects the tendency of WPC to form firmer gels at high pH's which hold moisture more effectively.

Results of this study indicate that the use of highly soluble WPC to increase firmness and yield of processed meat products is most effective at high processing temperatures, at which whey protein forms a gel. Whey protein concentrate with reduced solubility may increase yield and firmness of

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meats processed near 70°C. Soluble WPC's may be useful in altering the elastic properties of processed meats.

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FUTURE RESEARCH

- (1) Examine the salt-soluble protein:whey protein concentrate (SSP:WPC) gels with electron microscopy to determine how the proteins interact.
- (2) Examine the effects of insolubilized WPC on the yield and texture of traditionally processed frankfurters.
- (3) Examine the effect of insolubilized WPC on the sensory characteristics of frankfurters.

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