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COMPOSITION AND GELLING PROPERTIES OF WHEY PROTEIN CONCENTRATES IN MODEL GEL AND FRANKFURTER SYSTEMS AS INFLUENCED BY IONIZED CALCIUM

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ABSTRACT

COMPOSITION AND GELLING PROPERTIES
OF WHEY PROTEIN CONCENTRATES IN MODEL GEL
AND FRANKFURTER SYSTEMS AS INFLUENCED BY IONIZED CALCIUM

By

Allan J. Rose

Four whey protein concentrate (WPC) gels, pH 7.0, containing added sodium tripolyphosphate (NaTPP) or EDTA (0, 10, 50 and 100 mM) were analyzed to determine the effect of ionized mineral concentration (Ca, Na) on rheological and water retention properties. Gel hardness and water retention were maximized at [Ca^{2+}] between 3-6 mM. Cooling temperature, type of buffer, and pH affected overall gelling properties while ionic strength did not. Chicken breast salt-soluble protein (SSP):WPC combination gels (0.3M NaCl, pH 6.5 processed at 65°C and 90°C) increased in gel strength and decreased in gel deformability and expressible moisture as WPC concentration, processing temperature, and NaTPP increased. Turkey frankfurters with 7% WPC (protein basis) experienced yield and textural changes with increasing temperature and NaTPP concentration. Results indicate that WPCs with adjusted [Ca^{2+}] can be used to improve yields and alter texture of processed meats.

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INTRODUCTION

An estimated 80 million tons of whey are produced annually as a by-product of the cheese industry. (Kinsella, 1984; Bottomley et al., 1990). Considered as waste until recently, whey was dumped into sewers for disposal (Bottomley et al., 1990). During the past few years, whey proteins have received increased attention as potential components for human food in the form of whey protein concentrates (WPCs) (Delaney, 1976; Morr, 1979; de Wit, 1984; Harper, 1984; Bottomley et al., 1990). This is due to their high nutritional value and especially to their functional characteristics, namely the ability of whey proteins to form emulsions, foams and heat-induced gels (Kilara, 1984; Mangino, 1984; Melachouris, 1984).

From an economic standpoint, one of the most important functional aspects of WPC is its ability to form heat-induced gels. These gels are capable of holding both water and other non-protein components of food systems (Schmidt et al., 1984; Zirbel and Kinsella, 1988; Bottomley et al., 1990; Kuhn and Foegeding, 1991a). Differences in processing techniques have led to variability in the gelling properties of many commercial WPCs (Sternberg et al., 1976; Huffman, 1988; Morr and Foegeding, 1990; Kuhn and Foegeding, 1991a;

Parris et al., 1991). This variability may be the primary reason for the limited success of WPCs in the food industry (Mangino et al., 1987; Morr and Foegeding, 1990; Kuhn and Foegeding, 1991a; Brandenberg et al., 1992).

Many researchers have studied the role of calcium on gelling properties of WPC (Mulvihill and Kinsella, 1988; Zirbel and Kinsella, 1988). It is accepted that calcium is required at a certain concentration for gelling to take place. However, studying the role calcium plays in WPC gelling properties is difficult because the concentration of calcium is very sensitive and can be affected by milk source, treatment during cheese manufacture, and heat processing. As a result, WPCs contain variable calcium concentrations and inconsistent functionalities.

One way to control the calcium concentration is to take advantage of its easily altered equilibrium between the ionic, protein-bound, and colloidal calcium phosphate forms. This can be accomplished using a sequestrant, like sodium tripolyphosphate (NaTPP), which will bind ionic calcium and effectively remove it from the system. Conversely, if the concentration of calcium is too low, it can be added back in an easily controlled form, like calcium chloride ($CaCl_2$).

In the comminuted meat industry a critical balance exists between the quality and availability of the meat protein source, its functional ability, and ultimately cost. To reduce costs, the industry has examined nonmeat proteins as a replacement for the higher priced meat proteins. One

such nonmeat protein is whey protein concentrate. However, WPC is just one of many nonmeat binders available to the food industry. To compete successfully against such well characterized binders as soy protein, WPCs must have consistent functionality for specific applications. The objectives of this study were:

1) To alter the concentration of free calcium [Ca^{2+}] in a WPC by sequestration using long-chain polyphosphates.

2) To test the use of phosphate treated WPCs in a gelation model system.

3) To test the use of phosphate treated WPCs in a low-fat frankfurter system processed to two different temperature.

LITERATURE REVIEW

2.1. WHEY AND WHEY PROTEINS

Whey is defined as the aqueous phase that is drained from the curd of clotted milk during the manufacture of cheese (McWilliams, 1989). There are two types of whey: sweet whey and acid whey (Table 1). Sweet whey is produced during the rennet coagulation of milk commonly used to make hard and semi-hard cheeses. Acid whey is formed during acid coagulation like that used in the manufacture of cottage cheese. Whey from different sources can have very different compositions even at this early stage of processing (Table 1). For example, even though the protein content of whey remains fairly constant (0.7-0.8% on liquid basis), lactose, milkfat, and minerals can vary significantly (Morr, 1989).

The mineral content of acid and sweet whey is significantly different (Table 2). Acid whey tends to have a greater content of minerals than sweet whey. This seems to be due to the dissolution of the colloidal calcium phosphate during acidification (Morr, 1989). When the casein micelles are broken up, minerals that are bound to proteins (even partially bound) will be solubilized. This is especially noticeable with calcium which is used biologically to link individual casein micelles together.

Table 1. General composition[a] and pH[b] of sweet and acid whey (%)

Component	Sweet whey	Acid whey
Lactose	4.9	4.4
Protein	0.8	0.7
Minerals	0.5	0.8
Milkfat	0.2	0.04
Lactic acid	0.2	0.5
Water	93.4	93.6
pH	5.3-6.6	4.4-5.3

[a] Adapted from Morr, 1989
[b] Adapted from Scott, 1989

Table 2. Mineral composition of whey (mg/100g of whey)

Mineral	Sweet whey	Acid whey
Calcium	55	102
Magnesium	6	9
Sodium	48	47
Potassium	144	142
Phosphorus	47	64
Iron	0.08	0.09
Zinc	0.07	0.33

Adapted from Scott, 1989

Beta-lactoglobulin (ß-lg) is the most abundant of the whey proteins, representing about 50% of the total protein content (Table 3). Beta-lactoglobulin is a typical globular protein existing as a dimer (molecular mass of 36.7 kDa) at room temperature and pH between 5.5 and 7.0 (Swaisgood, 1982). Under more severe conditions (e.g. ≥40°C), ß-lg will dissociate into two identical monomers. Monomeric bovine ß-lg contains 162 amino acid residues five of which are Cys/2 (Cys/2 = half-cystine) (Fox, 1989). Four of these Cys/2 occur as disulfide bonds with one acting as a free thiol. Although the literature is somewhat conflicting, bovine ß-lg appears to contain 10-15% α-helix, 45-50% ß-sheet, 15-20% ß-turns and the rest unordered structure. Several genetic variants of ß-lg exist of which type A and B are the most common.

Alpha-lactalbumin (α-la) is the next most abundant protein representing about 20% of the total protein content of whey. It is the smallest of the whey proteins with a molecular mass of about 14 kDa. This compact, globular protein is comprised of about 26% α-helix, 14% ß-sheet, 60% unordered structure and some ß-turns (Fox, 1989). The 123 residue bovine α-la is stabilized by eight Cys/2 that exist as four intramolecular disulfide bonds. Because of its calcium binding affinity, α-la is considered a metalloprotein. This characteristic is also responsible for imparting much of the heat stability to α-la.

Like the two previous whey proteins, bovine serum

Table 3. Concentration and properties of whey proteins[a]

	Concentration (g/liter)	Percent of total protein	Isoelectric point	Molecular mass (kDa)
β-Lactoglobulin	3.0	50	5.3-5.5	18.3
α-Lactalbumin	0.7	20	4.2-4.5	14.0
Immunoglobulins	0.6	10	5.5-8.3	150-200
Bovine serum albumin	0.3	6	5.1	66.0

[a] Adapted from Morr, 1989; Fox, 1989

albumin (BSA) has been comprehensively reviewed. The 582 residue bovine BSA has a calculated molecular mass of 66,267 Da (Fox, 1989). The native molecule contains 34 Cys/2 residues existing as intramolecular disulfide bonds and one free thiol group at residue 34 (Fox, 1989). This ellipsoidal shaped protein is estimated to contain 55% α-helix, 16% ß-sheet, and about 29% unordered structure. The effect of BSA on the functional properties of whey proteins has been largely ignored because it represents only about 6% of the total protein fraction of whey (de Wit and Klarenbeek, 1984).

The immunoglobulins IgG, IgA, IgM, IgD, and IgE represent about 9% of the protein fraction in whey (Fox, 1989). They are the largest of the whey proteins having molecular masses of about 150, 160, 180, 185, and 200 kDa respectively. Of the five, IgG is by far the most common representing about 80% of the total immunoglobulins. In general, immunoglobulins are tetramers consisting of two heavy polypeptide chains (molecular mass 50-70 kDa) and two light chains (molecular mass about 22 kDa) held together by disulfide bonds. Like BSA, the highly complex, heat sensitive Igs have received little attention with regard to functionality due their limited abundance in whey.

The remaining protein fraction is collectively known as the proteose-peptones. This portion consists of polypeptide segments of partially degraded proteins. Although they may constitute 23% of the total protein (Fox, 1989), their

effect on functional properties has been ignored due to the high variability in composition (e.g. molecular masses range from about 4.1-41 kDa).

2.2. WHEY PROTEIN CONCENTRATE PRODUCTION

Many methods have been used to isolate whey proteins. Some of these include heat coagulation, polyphosphate complexing, and gel filtration (Marshall, 1982). Due to the dramatic increase in demand for highly purified WPC (Clark, 1987), these techniques have been largely replaced with ultrafiltration/diafiltration (UF/DF) or ion exchange adsorption (IEA) methods. There are two principle advantages to using one of these two latter techniques: 1) the treatment is less harsh on the protein thus producing a less denatured product and 2) the processes have evolved into relatively rapid purification techniques.

Ultrafiltration is the most frequently used method for concentrating whey proteins. The process fractionates different sized solute components based on molecular sieving through membranes with various pore sizes. Ultrafiltration membranes typically have pore sizes between 1.5 and 100 nm which retain protein (and possibly other macromolecules $\geq$ 1000 kDa) while allowing water, minerals, and lactose to permeate. To accomplish diafiltration, water is added during ultrafiltration to remove additional minerals and lactose. The relatively low temperatures and mild pH produces a highly functional WPC product containing 50-70%

protein.

Ion exchange adsorption techniques are based on the amphoteric nature of proteins. Regardless of the equipment, the process involves four basic steps: 1) the whey is pumped into the ion exchanger where it is adjusted to a particular pH and adsorbs with the ion exchanger; 2) lactose and minerals are washed out of the reactor with water; 3) the proteins are desorbed from the ion exchanger by adjusting the pH; 4) the proteins are removed from the reactor using water (Morr, 1989). This WPC of IEA can be quite different than the WPC from UF/DF (Table 4). For example, the protein concentration is often ≥ 90% while the mineral, lactose, and pH are greatly reduced.

The outcome of these processes is a commercially available, concentrated form of whey protein. No standard of identity has been established for WPC, thus products vary widely in composition and subsequently in functional properties, especially gelling ability. To add to the confusion are similar products like whey protein isolate (WPI) which experience additional purification and have different gelling properties. Despite the differences, these widely variable products have found many applications (Table 5).

2.3. FOOD PROTEINS AND GELATION

From a commercial standpoint, protein gels are vitally important in food systems. They constitute an important

Table 4. Composition of various whey protein concentrates prepared by ultrafiltration/diafiltration (UF/DF) or ion exchange adsorption (IEA)[a]

	pH	Protein (%)	Lactose (%)	Calcium (mmols/kg solids)	Phosphate (mmols/kg solids)
UF/DF					
Normal UF	7.0	55.8	26.4	0.16	0.12
Neutral UF/DF	6.7	73.9	5.4	0.15	0.13
Acid UF/DF	3.2	73.2	13.4	0.04	0.11
IEA					
Spherosil 'QMA'	4.0	75.9	4.5	0.13	0.06
Spherosil 'S'	6.0	90.4	0.1	0.10	0.10
Vistec	8.1	86.4	0.4	0.05	0.12

[a] Adapted from Morr, 1989.

Table 5. Applications of whey protein concentrates in food products[a]

General Products	Related Products	Functional Demands
Beverages	Chocolate drink	Colloidal stability
	Soft drink	Solubility
Confectionery	Frappé	High whipping power
	Meringue	Foam stability at high temperatures
Desserts/dressings	Whipped topping	Whipping ability + fat
	Salad dressing	Emulsifying ability
"New" products	Cheese-like product	Heat setting
	Spreadable product	Emulsification and water binding
Meat products	Ham	High solubility at low viscosity
	Frankfurter	Water- and fat-binding
Bakery products	Bread	Dough formation
	Cake	Fat-binding and heat setting

[a] Adapted from deWit (1989)

functional role especially in the area of stabilizing and texturizing various food products.

Defining a gel has proven to be difficult. Some researchers, for instance, have found it convenient to define gels based on their molecular structure and their ability to bind liquid (Kinsella, 1976). Others have employed a more textural approach by defining gels based on their solid/liquid nature (Clark and Lee-Tuffnell, 1986). For the purposes of this paper, the definition given by Hermansson (1979) is most appropriate: "gels are three dimensional matrices or networks in which polymer-polymer and polymer-solvent interactions result in immobilization of a large amount of water by a small amount of protein".

Ferry (1948) explained protein gelation as a two step process. In the first step, the protein is partially unfolded to expose the functional groups and allow them to interact. This partial unfolding can be induced by several means including pH changes, enzyme addition, mechanical agitation, and, most commonly, heat. The second step of the process involves the aggregation of the partially unfolded proteins into a crossed linked matrix.

Many forces are involved in forming and stabilizing protein gels. In addition, different forces can dominate based on environmental characteristics (e.g. pH). In any case, it has been determined that for a gel to form, the attractive forces must displace the repulsive forces (Schmidt, 1981). That is, if there is not enough attraction

between the partially unfolded proteins, they will remain in solution. These forces are broken up into four categories: covalent bonds, hydrogen bonds, hydrophobic interactions, and electrostatic interactions.

2.4. WHEY PROTEIN CONCENTRATE GELATION

The factors affecting the functional properties of WPC, including gelation, have been divided into two groups: intrinsic factors and extrinsic factors (Kinsella and Whitehead, 1985). The intrinsic factors are those related to the inherent properties of the proteins forming the gel matrix (amino acid composition, molecular mass, size and shape, sulfhydryl groups). Extrinsic factors include the environmental conditions that exist during the process of gelation (pH, temperature, ionic strength, minerals, and non-protein solids).

2.4.1. INTRINSIC FACTORS

The proteins in whey that possess gelling properties are ß-lactoglobulin (ß-lg) and bovine serum albumin (BSA) (Ziegler and Foegeding, 1990). As previously mentioned, ß-lg is at a much higher concentration in WPCs and is therefore considered the primary gelling protein. Studies are highly conflicting as to the minimum protein concentration needed to form a gel. Values range from as low as 1.0-1.2% (Sternberg et al., 1976) to 7.5-8.0% (Harper, 1992). This variation is most likely the result of compositional differences in the WPC as well as the non-

standardized methods used for preparation and evaluation. Regardless, whey protein gels are very concentration dependent.

Intermolecular cross-linking of peptides is necessary for gelation to occur. Differences observed in functional properties of proteins reflect different types of cross-links mediated by the number and type of interactive sites on the protein molecule (Kinsella and Whitehead, 1989). Bonds most often associated with WPC gelation are of three types: disulfide bonds, hydrophobic interactions, and ionic bonds (Morr, 1978; Kohnhorst and Mangino, 1985).

Much work has been done regarding the relationship of the sulfhydryl content of WPC to its gelling properties. Schmidt et al. (1979) found a maximum gel hardness at a cysteine concentration of 9.7 mM. Kim et al. (1987) attributed the strength of WPC gels to the sulfhydryl content of the protein fraction (especially ß-lactoglobulin). Conversely, Kohnhorst and Mangino (1985) concluded that the sulfhydryl content of WPC did not vary enough to explain changes in gel strength. Similar results were found by Shimada and Cheftel (1988) as they observed that the number of disulfide bonds did not increase as the protein concentration increased. Consequently, it has been concluded that although disulfide bonds are important for stabilizing WPC gels, the various rheological properties observed in different gels is not a function of the sulfhydryl content.

Hydrophobic interactions influence the folding of proteins into their native structures. Likewise, when proteins gel, the structure of the final gel is largely determined by hydrophobic forces (Kinsella and Whitehead, 1989). Mangino et al. (1987) studied the effects of various heat processing treatments on the functionality of WPCs. They determined that WPC made from heat-treated milk had a lower gel strength than WPC made from milk receiving no heat treatment (12% protein, pH 6.5). They found the decrease to be significantly correlated (R=-0.845) to a decrease in protein hydrophobicity. In an earlier study, Kohnhorst and Mangino (1985) developed a mathematical model for predicting the strength of gels made from WPC based on the amount of hydrophobicity. Their results showed a positive relationship between hydrophobicity and gel strength (R=0.78).

The contribution of hydrogen bonds to the formation of protein gels is not clear. Nevertheless, within internal hydrophobic regions with pH values below the pI where the carboxyl groups are highly protonated, H-bonds may contribute significantly to the stability of various gel structures (Kinsella, 1984). Parris et al. (1991) used Fourier transform infrared spectroscopy (FTIR), to study the effect of heat treatment on whey protein denaturation. They determined that heat treatment (85°C, 30 min.) resulted in formation of two bands (1684 and 1613 cm^{-1}) in the amide I region of ß-lg at frequencies usually associated with

intermolecularly hydrogen bonded ß-sheets. Conversely, they found more heat-induced aggregation in sweet whey than acid whey even though less intermolecular hydrogen bonding occurs. They indicated that this may be due to less specific hydrophobic or ionic interactions. Similar results were found by Katsuta and Kinsella (1990) in a study of the effects of temperature on the activation energies (ΔHa) of WPI gels. Activation energies describe the forming and breaking of noncovalent cross-links in gels. The results showed ΔHa decreased as temperature was increased (15°-75°C). This suggests that noncovalent structures other that hydrophobic interactions (i.e. hydrogen bonds) are involved in stabilizing the gel structure.

The last of the stabilizing forces is that of electrostatic interactions. These are the ionic associations occurring between the negative-charged and the positive-charged amino acid residues (Kinsella, 1984). Their probable stabilizing ability comes from attractive and repulsive forces found with charges located on the polypeptide chain. Under gelling conditions, there is a balance between the attractive forces necessary to maintain structure and repulsive forces needed to keep the proteins from associating too tightly and collapsing. Because of the extreme sensitivity of this interaction to environmental conditions (i.e. pH, heat, minerals), much of the work done to examine this force has focused on altering the gelation environment. The special case of calcium effects on

gelation will be addressed later.

2.4.2. EXTRINSIC FACTORS

The effect of temperature on the gelling properties of WPCs is by far the most measurable. Temperature influences occur during both the processing of WPCs and upon heating during ingredient applications. Heat can directly influence gelation by causing structural changes in the protein. Researchers have classified heat-induced protein changes as reversible and irreversible (deWit and Klarenbeek, 1984). In addition, heat can influence gelation by altering the composition of the non-protein components (mineral, fat, lactose). These changes occur during WPC production and will be addressed in another section.

Reversible changes occur during low to moderate heat treatments commonly used during the manufacture of WPC (pasteurization between 60 and 70°C) due to reversible denaturation of α-la. Mangino et al. (1987) studied the effects of heat processing on WPC functionality. They found that WPCs made from pasteurized milk were lower in gel strength than those made from unpasteurized milk. These results were attributed mainly to structural changes in the protein.

Irreversible changes may occur at higher temperatures due to protein denaturation. deWit and Klarenbeek (1984) studied the denaturation temperatures of whey proteins using differential scanning calorimetry (DSC). Transition

temperatures of 78°, 72°, and 64° were observed for ß-lg, BSA, and IgG respectively, at pH 6.0 in 0.07 M phosphate buffer. Mangino (1984) discussed how these higher temperatures can affect the formation of protein gels. He indicated that heat can affect both the rate of protein denaturation and rate of protein-protein interactions. Adverse effects are typically found at temperatures where protein aggregation proceeds more rapidly than protein unfolding. When this occurs, a precipitate forms without sufficient water immobilization (Mangino, 1984). The sensitivity of whey proteins to denaturation is affected by other environmental factors. These factors include pH, non-protein composition (lactose, fat, minerals), protein concentration, ionic strength, and calcium concentration.

As suggested earlier, pH has a major influence on gelling characteristics of WPC. This influence can be in the form of structural changes of protein but is most often a result of changes in ionic interactions. In either case, the intrinsic factors discussed previously are affected. Zirbel and Kinsella (1988) reported that gel hardness decreased as the pH increased from 6.0 to 9.0. Other researchers reported similar findings (Schmidt et al., 1978; Shimada and Cheftel, 1988). Xiong (1992) studied the influence of more neutral pH values on the thermal aggregation of whey proteins. Using the technique of differential change in optical density (Clark and Ross-Murphy, 1987), it was shown that lower pH facilitated

protein-protein aggregation between pH values of 5.5 and 7.5. Dunkerley and Hayes (1980) agreed with this data reporting that gel strength decreased as the pH was increased from 4.69 to 7.86. Harwalkar and Kalab (1985), working with ß-lg at pH 2.5, formed gels that immobilized large amounts of water and were inelastic. deWit (1981) studied gelation of ß-lg over a wide pH range. Opaque gels were formed at pH 6.0, whereas completely transparent gels were formed below pH 3.5 and above pH 7.0. Dunkerley and Zadow (1988) also studied WPC gels over a large pH range. They described gels as smooth below pH 3.0, smeary pastes between pH 4 and 7, and rubbery above pH 7.0.

Shimada and Cheftel (1988) suggested hydrophobic interactions were major contributors to gel formation at neutral pH. Harwalkar and Kalab (1985) suggested that near the isoelectric point (pI), protein-protein interactions were mostly due to an equal concentration of positive and negative charges, thus facilitating aggregation. Hillier et al. (1980) found intermolecular disulfide bonds increased as the pH increased. Others have found disulfide bonds to be important for gel formation at pH values above 7.0 (Dunhill and Green, 1965; deWit, 1981). Harwalkar and Kalab (1985) suggested at pH values below about 3.0, proteins became positively charged and the repulsive forces became dominant.

Much work has been done with respect to the effect of ionic species and concentration on the functional properties of WPCs: aggregation (Varunsatian et al., 1983; Xiong,

1992), denaturation (Varunsatian et al., 1983), solubility (deWit and Klarenbeek, 1984), and gelation (Schmidt et al., 1979; Mulvihill and Kinsella, 1988; Foegeding et al., 1992). In general, the influence of ions on WPC gelation seems to be dependent primarily on valence (Foegeding, 1992). For instance, above the pI where proteins possess an overall negative charge, binding of cations is effective in neutralizing the net negative charge. Likewise, below the pI anions can help to neutralize the net positive charge. Ionic species change the forces involved in the gelling process by binding to protein molecules with opposite charges.

One commonly overlooked environmental factor is that of protein concentration. Mangino (1984) discussed protein concentration with respect to how it determines both the likelihood of gelation and to the characteristics it imparts on the finished gel. Simply put, as protein concentration increases, the likelihood of intermolecular cross-links increases over intramolecular cross-links. Therefore, a minimum protein concentration is required for gelation. Increasing the protein concentration beyond this point increases cross-linking and produces firmer gels. More recently, Steventon et al. (1991) used percolation analysis to study the effect of protein concentration on WPC gelation. Their results were similar in that the probability of gelation increased as the probability of intermolecular cross-linking increased with increasing

protein concentration. Katsuta and Kinsella (1990) used creep compliance and activation energy data to analyze the temperature and protein concentration effects on whey protein gels. By studying WPI gels ranging from 10 to 15% total protein, they were able to show that the nature of the cross-links in the gel structures varied with protein concentration (i.e., as protein concentration increased, more rigid cross-links were formed, probably due to increased intermolecular covalent disulfide bonds).

Recently, it has been shown that components other than protein may affect gelation. As discussed, functional characteristics are largely dependent on the physicochemical properties of proteins in their native and denatured states (i.e., protein conformation, pH, type and concentration of ionic species, and temperature). In turn, these properties are governed by components that may be altered with processing. In general, these components include lactose (Delaney, 1976; Pappas, 1991), lipids (Kohnhorst and Mangino, 1985; Mangino et al., 1987; Xiong et al., 1991), milkfat globule membrane (Xiong and Kinsella, 1991), ratio of ß-lg:α-la:BSA (Kohnhorst and Mangino, 1985; Kim et al., 1987), and especially the mineral content (Varunsatian et al., 1983; Kuhn and Foegeding, 1991b). Among the minerals, many researchers have studied the influence calcium has on the gelling properties of protein (Johns and Ennis, 1981; Mulvihill and Kinsella, 1988; Zirbel and Kinsella, 1988).

2.4.3 CALCIUM AND WPC GELATION

Calcium is a mineral found in large quantities in whole milk and in milk products, including whey protein concentrate. Much work has been done to study the relationship between calcium and protein gelation, however, the influence of calcium on functional properties is still highly ambiguous. In any case, gelling characteristics have been shown to be influenced by different concentrations of calcium.

It is well known that calcium is required at a certain concentration for gelling to take place. Schmidt et al. (1979) determined that gel hardness of dialyzed WPCs increased to a maximum as $CaCl_2$ was increased to 11.1 mM. In contrast, Dunkerly and Zadow (1984) determined that hardness of WPC gels gradually decreased with added calcium. These two studies directly conflict each other as to the amount of calcium needed to optimize the gel system. Despite this conflict, the authors agreed that most commercial WPCs contained calcium at a concentration different from that necessary to optimize gel properties.

Recent studies have attempted to standardize the gelling properties of WPCs and WPIs by removing most components except protein, and then adding back different components to the system, one at a time, to evaluate the effects. Among these components calcium has received considerable attention. Johns and Ennis (1981) used texture profile analysis to examine rheological properties of whey

protein concentrates to study the effect of replacing calcium ions with sodium ions on heat-induced gels. Results indicate that a 100% replacement of calcium ions with sodium ions resulted in a significant increase in gel hardness, cohesiveness, gumminess, springiness and chewiness. Calcium replacement of 33% or 67%, however, resulted in no significant change in gel properties.

Kuhn and Foegeding (1991a) dialyzed three different commercial WPCs and one WPI. After dialysis and calcium replacement to equal concentrations, they found that shear stress of gels was still significantly different. Brandenberg et al. (1992) using three commercial WPCs and one WPI found even larger differences in stress when they removed the low molecular weight components and replaced each system with equal calcium (0.1 & 0.2 M) before heating.

One possible explanation for differences reported in the literature may be due to the form of calcium in the protein system. Calcium is found in three forms in whey protein: 1) protein bound, 2) complexed with lactose, phosphate, and possibly some lipids and other minerals (called colloidal calcium phosphate), and 3) ionized [Ca^{2+}] (Demott, 1969; Muldoon et al., 1972; Hazell, 1985). The equilibrium between these three forms is easily altered.

Ionized calcium usually accounts for about 10 to 30% of total calcium in whey (Demont, 1968). Ionized calcium can react when equilibrium conditions are altered due to changes in phosphate concentration (i.e. addition of sodium

tripolyphosphate) and temperature. Thus, the equilibrium of calcium in the colloidal and protein bound states is altered, changing the functional characteristics of the protein (Augustin, 1991).

The question remains: what is the mechanism by which calcium affects the functionality of whey proteins? One theory suggests that above the isoelectric pH of the protein, calcium creates a net repulsion between molecules. This relationship would then encourage hydrophobic interactions (Kinsella and Whitehead, 1989). Another theory proposes that calcium, acting as a divalent cation, cross-links between two neighboring anionic protein molecules. This cross-linking is therefore an integral part of the formation of the gel matrix (Mulvihill and Kinsella, 1988). Still others have implied the influence to be a combination of the above two theories (Harper, 1992).

In an attempt to unravel the puzzle, Foegeding et al. (1992) correlated circular dichroism spectroscopy and DSC data to the rheological properties of ß-lg. They added $CaCl_2$ and NaCl at 20 mM and 100 mM concentrations, respectively, to pure ß-lg sols and observed their behavior throughout the gelling process. It was found that sodium and calcium cations produced significantly different gels. They concluded that, based on circular dichroism data, the differences found in the gelation process were not a result of cation-associated differences during the denaturation process but rather these cations had a differential effect

during the aggregation process. Xiong (1992) explained that calcium, being a divalent cation, possesses two positive charges and thus the ability to form an ionic bridge between two adjacent carboxyl groups of neighboring polypeptides. Sodium, however, only has one positive charge and thus cannot form a bridge.

2.5. FOOD GRADE PHOSPHATES AND GELATION

Food grade phosphates exist in many forms and have many names but are generally categorized into 5 groups: 1) orthophosphates (existing as monomers), 2) pyrophosphates (existing as dimers), 3) polyphosphates (existing as polymers with more than two P atoms, 4) cyclic phosphates, and 5) miscellaneous phosphates (those that fit no other group) (Molins, 1991). The two most commonly used classes of phosphates in the food industry are orthophosphates and polyphosphates.

The first class, orthophosphates, are used primarily as buffering agents, while the second class, long-chain polyphosphates, have several uses. The two primary uses of long-chain polyphosphates are as metal ion sequestrants and as polyelectrolytes. As a polyelectrolyte, phosphate binds to the positive sites on a protein and may change functional properties. In the dairy industry, polyelectrolyte and sequestrant properties are used to alter emulsification characteristics and protein aggregation. In the processed meat industry, the polyelectrolyte property is of greatest

concern. Phosphates are used to raise the pH of the meat and increase the solubilization of muscle proteins. In doing so, water binding capacity and overall yields are significantly increased.

More specifically are the effects of phosphates on the extractability of actin and myosin. Binding of meat pieces in the manufacture of processed meat products, regardless of animal species, depends on the extraction of salt-soluble proteins within the meat, specifically actin and myosin (Molins, 1991). Siegel et al. (1978) studied the role of phosphates with respect to their ability to extract these proteins. They used 0.5% of a phosphate mixture (NaTPP and sodium phosphate glass) alone and in combination with 0%, 1%, 2% and 3% NaCl to show that phosphates were the crucial ingredients (not NaCl) for determining the relative ratios of extracted actin and myosin. In addition, the authors asserted that optimal extraction occurred at 2% NaCl and 0.5% phosphate.

Nakai and LiChan (1985) determined that the strength of WPC gels can be increased by adding polyphosphates. They suggested this may be due to calcium chelation by phosphate. de Rham and Chanton (1984) showed that the solubility of whey protein could be altered by manipulating the various concentrations of calcium, magnesium, citrate, and phosphate. This change in solubility ultimately led to changes in gelling properties.

2.6. MUSCLE PROTEINS AND GELATION

Muscle proteins are most commonly classified into three categories based on solubility: sarcoplasmic, myofibrillar and stromal (Molins, 1991). Sarcoplasmic, or water soluble proteins, are the proteins that surround the myofibrils in the intact muscle tissue. They make up approximately 30% of the total protein depending on the tissue and are referred to as water soluble because of their high solubility in water or low ionic strength salt solutions. The second class of muscle proteins are the myofibrillar proteins. They make up about 50-55% of the total protein in muscle. Myofibrils are involved in muscle contraction. They are referred to as salt-soluble proteins (SSP) due to their high solubility in low to moderate ionic strength salt solutions (ionic strength $\approx$ 0.3). The third group of muscle proteins are the stromal, or insoluble, proteins, due to their insolubility. These are primarily the connective tissue proteins and their function is essentially one of support. They make up the remainder of the muscle proteins.

Grabowska and Sikorski (1976) summarized the gelling ability of sarcoplasmic, myofibrilar and stromal protein from cod fish muscle. In general, they discovered sarcoplasmic and stromal proteins to be very poor gel-formers while myofibrillar proteins formed excellent gels. This superior gel-forming ability of the myofibrils is typically attributed to the presence of myosin which constitutes about 50-55% of the myofibrilar protein fraction

(Smith, 1988).

Many researchers have described myosin as a molecule containing two globular "heads" attached to a rod-like "tail" (Ziegler and Acton, 1984; Stone and Stanley, 1992). Myosin has an estimated molecular mass of about 500,000 daltons (Foegeding, 1992). The molecule is made up of 2 heavy chains and 4 light chains. The heavy chains each have a molecular mass of approximately 200,000 daltons. Most of the heavy chain exists in a α-helical structure but forms a globular "head" near the amino terminus. The light chains, each with molecular masses of about 20,000 daltons, are associated with the heavy chain "heads" (Bandman, 1987). Another myofibrillar protein thought to be involved in gelation is actomyosin which is a complex of myosin and actin (actin is another myofibrilar protein constituting about 20-25% of the total myofibrilar protein fraction). Alone, actin produces weak "curd-like" aggregates. When mixed with myosin, however, actin acts as a cross-linking agent and adds increased gel firmness.

Many researchers have used differential scanning calorimetry (DSC) to study thermal transitions of proteins during the denaturation process. The technique can be used to identify transition temperatures (Tm) at which protein conformational changes are detected by absorption of thermal energy. Xiong et al. (1987) identified three Tm in their study of native beef, pork, lamb and chicken thigh muscles. They ascribed Tm of 57-60°C to myosin, 66-67°C to

sarcoplasmic and connective tissue, and 78-80°C to actin but found that Tm were species and pH dependent. At pH 5.6, chicken muscle exhibited five Tm at 57, 62, 67, 72, and 79°C. They attributed the 57°C Tm to myosin, the 79°C Tm to actin and the 67 and 72°C Tm to sarcoplasmic proteins. The 62°C Tm was the result of combined denaturation of connective tissue, sarcoplasmic proteins, and myosin or actomyosin.

Yamamoto et al. (1988) suggested that myosin gelling properties are related to the length of the native myosin filament. Gelling of short filaments resulted in coarsely aggregated gels with low rigidity while long myosin filaments formed more rigid gels upon heating.

Ziegler and Foegeding (1990) presented a general picture of myosin gelation. The process begins with partial unfolding of the myosin molecule between 35-40°C. Unfolding is followed by aggregation or intermolecular association stabilized by covalent and noncovalent interactions. Myosin heavy chain is the main subunit involved in gelation.

Studies have shown that myofibrillar proteins isolated from poultry breast muscle formed stronger more deformable gels than those isolated from leg muscle (Foegeding, 1987; Xiong and Brekke, 1989). This difference was once thought to be caused by variations in myosin isoforms found in the different muscle tissues. Recently, however, Dudziak et al. (1988) demonstrated that myosin and actomyosin isolated from turkey breast and leg muscles have similar Tm, but very

different rheological properties. Ziegler and Foegeding (1990) suggested there were differences in the aggregation process resulting in variation in gelling properties.

Stone and Stanley (1992) described the gelation of myofibrillar protein in stages based on temperature. The first stage, called the "setting" stage occurs between 30°C to 40°C. During this stage, hydrophobic interactions between neighboring myosin tail sections form the support for the initial gel structure. The second stage, called the "network softening" stage, occurs between about 40°C to 60°C. During this stage, gels appear to lose some rigidity. This is attributed to the dissociation of myosin from actin. The third stage, called the "gel strengthening" stage, occurs above 60°C. During this stage, gel strength is attributed to the aggregation of myosin head regions. Dispute exists about the type of interaction involved but evidence supports the involvement of sulfhydryl and/or hydrophobic interactions.

2.7. MIXED PROTEIN GELS

For the sake of simplification, many researchers have chosen to study protein functionality using single component "model" systems in which the element is first isolated then studied. This has certainly been the case for the study of protein gelation. However, from a physical standpoint, foods are multicomponent systems and as such are very complex. Most are comprised of many different compounds,

some of which gel (proteins and complex carbohydrates), some of which do not gel (lipids, minerals, water), but both affect how the other functions. For this reason it is often necessary to study protein functionality, particularly protein gelation, using a multicomponent system.

Ziegler and Foegeding (1990) discussed multicomponent gels based on the thermodynamic compatibility of the components in the system. They theorize that the potential for these components to interact is the basis for the mechanism of gelation and the type of gel ultimately created. They subsequently grouped multicomponent gels into one of five categories:

1. Single-phase filled gel where the filler remains soluble in the gel matrix.

2. Two-phase filled gel where the filler exists as dispersed particles or as a secondary gel matrix.

3. Complex gel without copolymerization where the "nongelling" constituent associates in a random manner with the primary gelling network.

4. Complex gel with copolymerization where two or more proteins copolymerize to form a single network.

5. Interpenetrating polymer network (IPN) where both gelling networks are continuous.

The effects of partially insolubilized WPCs on chicken breast SSP gels have been studied by Beuschel (1992b) and Hung (1992). Their results indicate that the gelling properties of various whey protein concentrates greatly influence the gelling properties of chicken SSP proteins.

Beuschel (1992b) reported that many factors influenced how WPC reacted with SSP proteins in a mixed protein system. It was found that at temperatures commonly used in meat processing (65-71°C), WPCs with high solubilities (> 80%) improved water-holding properties, but not textural properties. Higher processing temperatures of 90°C were needed before highly soluble WPC improved hardness and deformability of SSP:WPC combination gels. Conversely, WPCs with reduced solubility (< 47%) were effective at increasing gel hardness and deformability at 65°C suggesting that lower solubility WPCs may be better suited for enhancing textural characteristics for comminuted meat products.

Hung (1992) found that addition of WPC to SSP resulted in many changes in the dynamic moduli and microstructure of heat-induced gels. Whey protein concentrate with high solubility (> 80%) had a better gelling ability than low solubility WPCs (< 47%). The 27% soluble WPC formed insoluble aggregates rather than gels. At temperatures below those needed for whey protein gelation, less soluble WPCs absorbed water, occupied the interstitial spaces of the

SSP matrix, and increased gel elasticity. Conversely, highly soluble WPCs increased elasticity more effectively at temperatures above those needed for whey protein gelation by forming a "coupled network" or "phase separated network" with chicken SSP.

Study of microstructure revealed that combination gels containing 27% and 41% soluble WPCs were composed of large denatured whey protein aggregates that interfered with SSP crosslinking. Combination gels containing 98% and 80% soluble WPCs were comprised of a fibrous network of SSP at 65°C and a globular network of WPC at 90°C.

2.8. RHEOLOGY OF VISCOELASTIC MATERIALS

2.8.1. SMALL DEFORMATION STUDIES

Like all polymer structures, protein gels exhibit viscoelastic behavior. Viscoelastic protein gels are "solid-like" in that they demonstrate linear stress behavior up to experimentally measurable strains. From a theoretical and mathematical standpoint, this linear behavior is inherently superior for determining fundamental properties of viscoelasticity. Methods used to determine this type of linear viscoelastic behavior can be broken up into two groups: static and dynamic methods.

Static testing includes two types of tests, creep and stress relaxation. With creep, an instantaneous and constant stress is applied to the material and strain is measured as a function of time. With stress relaxation, an

instantaneous and constant strain is applied to the material and stress required to maintain that strain is measured as a function of time. Dynamic testing involves subjecting the material to an oscillatory deformation and measuring the size and phase lag of the resulting stress. In either case, the properties determined are fundamental and considered independent of the equipment used.

2.8.2. LARGE DEFORMATION STUDIES

Although small deformation studies may be easier to describe theoretically, the reaction of materials to large deformations and failure have been shown to correlate better with the gel strength evaluated in the mouth. As a result, food scientists have found it equally important to study viscoelastic behavior based on what the consumer perceives as food texture. Indeed, to give a complete viscoelastic description of a gel involves both small and large deformation studies.

Empirical properties ultimately determined under large deformation are fracture shear stress and fracture shear strain. These values are defined as hardness and deformability of the material, respectively. Other common names given these properties are shear stress and shear strain at failure.

A number of geometric shapes have been successfully tested in evaluating failure properties of gels (e.g. cylinder, strip, etc.). There are, however, four basic

"forces" used to analyze these geometries: extensional, compressive, simple shear, and torsional. More than one of these forces may be acting on the material at any given time. The inherent advantages and disadvantages of each method will be discussed in their respective sections.

2.9. INSTRUMENTAL ANALYSIS

Borwankar (1992) classified rheological instrumental measurements of foods into three categories: fundamental measurements, empirical measurements and imitative measurements.

2.9.1. FUNDAMENTAL METHODS

The objective of fundamental analysis is to obtain data for the material tested that describes its fundamental properties. The values are, ideally, independent of the instrument used. Subsequently, these methods are useful tools for experimentally interpreting gel structural properties. In general, small deformation studies are used for fundamental analysis because they yield well defined viscoelastic properties like viscosity and elasticity.

2.9.1.1. DYNAMIC TESTING

The best example of fundamental viscoelastic analysis in foods is known as dynamic mechanical spectroscopy. As previously mentioned, this technique involves subjecting the material to oscillatory deformation and measuring the magnitude and phase lag of the resulting stress (Ziegler and

Foegeding, 1990). For perfectly elastic solids, the stress is in-phase with the applied strain. For perfectly viscous fluids, the stress is precisely 90° out-of-phase. Therefore, phase angles between 0° and 90° represent viscoelastic materials like protein gels.

Two fundamental properties are evaluated with dynamic testing. The first of these is called the "storage modulus" (G′). This is a measure of the ability of the material to "store" the applied energy and thus defines the elasticity of the material. The second of these properties is known as the "loss modulus" (G′′). This is a measure of the tendency of the material to "loose" the applied energy as heat and thus represents the viscous component of the system.

Dynamic analysis is a very powerful tool for measuring viscoelastic properties of protein gels for three basic reasons. First, it offers a means by which fundamental gelling properties can be determined. Also, the method can show time/temperature effects on the gelling mechanism. Lastly, it may be a very useful technique for elucidating protein structures and become a good predictor of other gelling properties.

Tung (1978) gave a short review of fundamental tests and how they might be used to help predict functional properties like gelation. The review emphasized the importance of rheology for helping to define the physicochemical nature of proteins. In general, it was concluded that dynamic and other fundamental testing is a

valuable tool for studying protein gel rheology.

The most common reason for using the dynamic method for protein gels analysis is its ability to evaluate viscoelasticity throughout the gelation process. For instance, Paulson and Tung (1989) used dynamic testing to determine rheological changes in meat emulsions containing non-meat proteins. Using this technique, they showed that replacing meat proteins with non-meat proteins altered the gelling temperature, time of gelation, and elasticity of the resulting frankfurters. Overall, the method was sensitive to changes in the gel system and was an excellent method of analysis for this type of product. Likewise, Xiong and Kinsella (1991) used dynamic testing to show how the type of fat influenced the rheological properties of milk based gels. They determined the viscoelastic properties as a function of gelation temperature and percent fat. Once again, the method was sensitive to changes in the gel system and was a valuable tool for analysis.

Another reason for dynamic analysis is the fundamental parameters it yields. For example, in a recent review by Steventon et al. (1991) the dynamic method was used to determine the accuracy of mathematically modeling the gelation of WPCs based on the percolation theory. The method proved to be useful because fundamental properties were obtained (G′ & G″). This was important because fundamental analysis helped to eliminate unnecessary variables that would have been produced using empirical

analysis.

2.9.1.2. STRESS RELAXATION

Another example of fundamental analysis is the method known as stress relaxation. With this test, the material is subjected to an instantaneous strain and the stress required to maintain this strain is measured as a function of time. Data gathered are typically expressed in terms of the stress relaxation modulus:

$$G=f(t)=\frac{stress(t)}{strain}=\frac{\sigma}{\gamma_{constant}} \qquad (1)$$

where:

$G=f(t)$ = stress relaxation modulus which is a function of time

$\gamma_{constant}$ = instantaneous and constant strain

σ = stress required to maintain constant strain

One of the greatest motivations for using stress relaxation is to allow for complete evaluation of a gel system. Essentially, this means that part of the gel is used for rheological studies, another part is used to evaluate water holding capacity, and another is used to examine the microstructure. Thus, a complete study can be carried out on the relationship between structural, functional, and rheological properties of the gel system.

Nakayama and Sato (1971) used stress relaxation to

compare the binding quality of meat based on the different ratios of myosin in the total myofibrillar protein fraction. In addition to viscoelastic behavior, other data, like water holding capacity, needed to be determined on the same sample. They determined that processing conditions like time, temperature, and salt concentration influenced myofibrillar protein gelation. Likewise, in a study by Sone et al. (1983), stress relaxation was used to correlate the microstructure of protein gels with rheological properties. They found this method to be very useful in determining fundamental viscoelastic properties and relating them to gel matrix microstructure.

This method is especially suited for the evaluation of pre-formed gels. For instance, Skinner and Rao (1986) used stress relaxation to determine linear viscoelastic behavior of seven commercial brands of frankfurters. The objectives of the study were to obtain normal stress relaxation data for frankfurters and to determine a rheological model representing the physical behavior.

Foegeding (1992) used this method to determine the effect of protein concentration on the rheological properties of whey protein isolate. The objective was to evaluate gelling properties based on fracture and nonfracture strains. Fracture strains were determined using torsion testing (discussed later). Nonfracture strains were evaluated using stress relaxation. Using stress relaxation, it was determined that there is a strong relationship

between strain at failure and fundamental viscoelastic properties of whey protein isolate gels.

In every study, stress relaxation worked well and gave the desired fundamental data. This method seemed especially suited for evaluating fundamental properties of commercially formed gels (i.e. frankfurters) and for correlating various properties like rheological, water holding, and microstructural.

2.9.1.3. CREEP COMPLIANCE

The third type of fundamental instrumental analysis is the creep compliance method. With this procedure, the material is subjected to an instantaneous and constant stress and the strain is measured as a function of time. Results are typically given in terms of the creep compliance modulus:

$$J=f(t)=\frac{strain(t)}{stress}=\frac{\gamma}{\sigma_{constant}} \qquad (2)$$

where:

$J=f(t)$ = creep compliance modulus which is a function of time

$\sigma_{constant}$ = instantaneous and constant stress

γ = stress over period of time

In addition, some researchers measure the recovery of strain upon removal of the stress.

Because of the similarity of the stress relaxation and

the creep compliance methods, it would be expected that they would be used on products of similar physical nature. As a result, this method is suited for evaluating fundamental properties of previously formed gels (i.e. frankfurters) and for correlating various properties like rheological, water holding, and microstructural.

Along with stress relaxation, Skinner and Rao (1986) used creep compliance to determine linear viscoelastic behavior on the same commercial brands of frankfurters. Again, the objectives of the study were to obtain normal creep data for frankfurters and to determine a rheological model for representing the physical behavior.

Okamoto et al. (1990) used the creep method to determine fundamental properties of pressure induced gelation of various proteins. This data was then used to compare to heat-induced gels of the same proteins. Once again, dynamic testing was inappropriate because the instruments used do not have the capacity to form pressure-induced gels.

Katsuta and Kinsella (1990) found the method particularly useful for determining the effect of temperature on viscoelastic properties of whey protein gels. The method was used because fundamental properties were determined on pre-formed gels over the temperature range of 25°C to 65°C.

2.9.2. EMPIRICAL METHODS

Like fundamental methods, the purpose of empirical measurements is to obtain various rheological data for food materials. However, unlike the fundamental methods, empirical measurements yield rheologically subjective data that is dependent on the instrument and conditions. Despite this drawback, the relative ease at which data is collected using empirical instruments makes this type of measurement invaluable to the food industry for maintaining product quality control and in process equipment design.

For the most part, empirical rheological properties are determined using large deformation and fracture studies. Theoretically, this may include forces of simple shear, torsion, compression, and tension. Many problems have been encountered using tension, however, that make the methodology impractical (e.g. problems attaching gels of any geometry to instruments capable of "stretching" the sample to failure). Therefore, the methods of shear, torsion, and compression are the methods of choice.

2.9.2.1. COMPRESSION

The technique most commonly used for large deformation studies in protein gel rheology is compression. With this test, a gel sample, usually of cylindrical geometry, is compressed a certain distance at a certain speed between a driving plate and a transducer plate. Results of this method are typically given in terms of fracture shear stress

and fracture shear strain although other terms like force at failure and deformation at failure have also been used.

The motivation for using the compression method may be one of convention. When texture testing began, a technique was needed to fracture the material in a practical manner. Compressing gels was easier than shearing and stretching them. Over the years it has since become a standard method for evaluating protein gel properties. Some recent compression studies are listed in Table 6.

Miura and Yamauchi (1984) used this method to evaluate the effect of protein-lipid-water interactions on the rheological behavior of soybean gels. Their objective was to study the crosslinks responsible for gelation and how they were affected by various lipid concentrations. They found the compression method to be suitable and that hardness of heat-induced soybean protein gels increased as the level of added lipid increased from 1% to 4%. O'Riordan et al. (1989) used compression to examine alkaline gelation of plasma proteins to assess the effects of various reagents on the rheological properties of these proteins. They found compression to be an appropriate method for evaluating gel fracture studies. They determined that there is a critical balance between protein-protein and protein-solvent interactions with respect to formation of homogeneous gels.

Chung and Lee (1990) used the method to describe the relationships between physicochemical properties and textural properties of mixed protein gels. Proteins used

Table 6. Protein gels tested using the compression method

Type of protein	Source
Frankfurter	Huang and Robertson (1977)
Fish	Hamann and Webb (1979)
Soybean	Miura and Yamauchi (1984)
Surimi	Lee and Chung (1989)
Plasma	O'Riordan et al. (1989)
Surimi	Chung and Lee (1990)
Globular	Wang and Damodaran (1990)
Alginate, κ-carrageenan, agar	Chai et al. (1991)
WPC	Kuhn and Foegeding (1991b)
Locust bean, κ-carrageenan	Mirza and Lelievre (1992)

ranged from WPC, soy protein isolate, α-lactalbumin, egg white, milk protein isolate, and wheat gluten and they were all mixed individually into fish based surimi gels. Their objective was to study amino-acid composition as it relates to gel-forming properties. Using compression, they learned that understanding the physicochemical properties of individual proteins in mixed protein systems can help optimize the textural properties of such formulated products. They also found that cooking time and temperature significantly affected the gelation behavior of nonfish protein. Wang and Damodaran (1990) used the method to determine the effect of disulfide bond reducing agents on thermal gelation properties of globular proteins. Their results suggested that the hardness of globular protein gels at a particular protein concentration is fundamentally related to the average molecular weight of the protein. Kuhn and Foegeding (1991b) used compression to evaluate whey protein gel rheology. The objective of their study was to determine how dialyzable compounds and total calcium influenced shear stress and shear strain of heat-induced gels. Using this technique they determined that calcium plays a key role in gelation even in the presence of other minerals like NaCl. They suggested that a minimal concentration of calcium was necessary for optimal whey protein concentrate gelation.

Huang and Robertson (1977) used compression analysis to study the texture of commercial frankfurters. Previous work

suggested that compression tests were dependent on crosshead speed and size of the compressive plungers. Their investigation was aimed at determining the best combination of plunger speed and size for frankfurter evaluation. It was determined that all combinations of crosshead speed and plunger size were sensitive enough to differentiate between the frankfurter samples. However, the combination of 0.635 cm diameter plunger at a crosshead speed of 5.08 cm per min gave the least mean square error and was recommended for reproducability.

Lee and Chung (1989) evaluated the performance of the compression method in discriminating surimi gels prepared from a wide range of starting materials. It was determined that compression testing could be used to reliably compare gels as long as moisture levels, chopping times, cooking temperatures, and sample sizes remained constant.

In a study by Hamann and Webb (1979), compression was used to evaluate material properties of fish gels. The objective was to try and correlate the data gathered to data from a trained sensory panel. Results indicated that the maximum compressive cell force was a good predictor of sensory springiness, firmness, cohesiveness, and gel strength. They cautioned, however, that only heat coagulated fish pastes were tested and a application to other protein sources and gels should not be assumed.

Mirza and Lelievre (1992) used the compression technique to study the gelling properties of kappa

carrageenan and locust bean gum gels. Their objective was to compare the compression method to the torsional technique (described later) using highly brittle and highly elastic gels. They found that failure properties from the two tests were in agreement provided the appropriate sample dimensions were used.

2.9.2.2 PENETRATION

A second method of empirical analysis used in protein gel rheology is that of penetration. Sometimes called puncture testing, this method uses a compression device (e.g. Instron) that is rod-like instead of a plate-like to decrease the surface area in contact with the sample. Depending on the thickness of the rod and the size of the sample, some compressive forces acting on the sample may be changed to shear forces.

Penetration is quite similar to compression. Equipment, techniques, and properties for each method are the same, except for the size of the plunger. As previously stated, the surface area of the plunger is relatively small compared to the sample so shearing force is measured rather than compressive. Relative size, however, lends itself to ambiguity in results so much of the work using penetration has been done to determine the effects of plunger characteristics.

As previously discussed, Huang and Robertson (1977) used various plunger sizes and crosshead speeds to evaluate

shear and compressive forces in their investigation of penetration testing of frankfurters. In general, they found that overall force measurements increased as crosshead speed and plunger diameter increased. They also observed an increase in skin toughness with decreasing plunger diameter. Their results suggest that textural measurements using penetration are strongly dependent on plunger size and crosshead speed.

Lee and Chung (1989) evaluated the performance of this method in discriminating surimi gels prepared from a wide range of starting materials. The objective was to compare penetration testing with compression testing. They concluded that compression testing is more useful for measuring the overall binding of the gel matrix (cohesiveness), while penetration is much better for determining the overall density of the gel matrix (hardness).

Another common reason for conducting penetration tests has to do with the samples tested. An excellent way of making reproducible protein gels is by cooking them in screw-capped test tubes. Although this method retains water while heating, extruding the sample for testing can be quite difficult and may damage the sample. Penetration tests can be performed on the sample directly in the tube and thus offers a method of evaluation.

Hickson et al. (1982) used the penetration method for characterizing rheological properties of heat-induced bovine

plasma and egg albumin gels to compare elasticity and rigidity of the two gel systems. In general, they determined that at the same protein concentration and treatment condition, bovine plasma proteins form a harder, more elastic gel than egg albumen. Similarly, Foegeding et al. (1986) used this method to describe the effect of heating rate on myosin, fibrinogen and albumin protein gels prepared in 16 x 100 mm tubes. There objective was to compare rheological properties of the different proteins. Results suggest that differences in the gelling temperature and overall hardness illustrate that individual plasma proteins differ in functionality.

Kim et al. (1987) used penetration analysis to evaluate rheological properties of whey protein concentrates to relate protein composition of whey protein concentrate (β-lactoglobulin, α-lactalbumin, and bovine serum albumin) to functional properties including heat-induced gelation. It was determined that ß-lactoglobulin was significantly correlated with gel strength of the WPC. Schmidt et al. (1978) used penetration to study rheological properties of heat-induced whey protein concentrate gels to determine the effect of dialysis on the hardness. In general, it was found that dialysis of WPC resulted in "stronger, more cohesive, less springy, more gummy, more chewy and more translucent gels". In both of the above studies, the gels were made in screw-capped test tubes and tested directly in the tubes.

2.9.2.3. TORSION

For purposes of this text, torsion testing is discussed in the empirical methods section. (primarily due to large deformation analysis). It should be noted, however, that torsion testing is quite often considered a fundamental test. This fundamental nature also defines the intrinsic advantages that torsion testing has that make it superior to other failure methods: 1) volume changes occurring in the sample geometry during testing are negligible, 2) failure occurs even in highly deformable gels, and 3) compressive, tensile, and shear stresses are equal so the material will fail under the stress for which it possess the least strength (Montejano et al., 1983).

With this technique, the idea is to shape the gel into a known and reproducible geometry and then twist the sample with a machine that measures torque (i.e. viscometer). Montejano et al. (1985) showed that the parameters of true shear stress (τ_{max}) and true shear strain (γ_{max}) are significantly correlated to various texture profile analysis parameters (discussed later).

Although sound in theory, practical applications have been limited due to the lack of technology. Within the past decade, however, equipment for torsion testing has advanced significantly. As a result, a great deal of research has ensued most of which has concentrated on testing and applying the theories.

Montejano et al. (1985) used torsion testing for

rheological analysis of eight different heat-induced protein gels including samples made from egg white, fish, turkey, beef and pork. Results were compared with data collected using texture profile analysis (TPA, discussed later) and sensory data. The authors reported that torsion failure tests can be used to reliably predict sensory characteristics of the gels in the study.

Kim et al. (1986) used the torsion method to describe the structural failure properties of two surimi gels to determine the effect of freeze-thaw abuse on gel-forming properties. Results indicated that cyclic freezing and thawing lowered gel strength and deformability of both gel samples. Kuhn and Foegeding (1991a) used the method to evaluate rheological properties of heat-induced whey protein isolate gels to study the effect of salts on gelling properties. In general, they found a significant difference in gels formed with monovalent versus divalent cations.

2.9.3. IMITATIVE METHODS

The third type of measurement found in food rheology is that of imitative methods. The purpose of these methods is to objectively simulate what happens under real circumstances (i.e. chewing in the mouth). These methods can be quite useful due to the complex nature of food texture.

2.9.3.1. TEXTURE PROFILE ANALYSIS

The best example of this type of method is the Texture Profile Analysis (TPA) technique developed at General Foods (Friedman et al., 1963). Like typical compression, the sample is subjected to large deformation and failure. In addition to the stress and strain measurements, however, sample properties continue to be measured as the stress is removed from the gel (compression plates pull apart) and a second compression cycle is run on the already fractured structure. The idea is to imitate the first two bites on this material in the mouth.

Analysis of the subsequent force-time curve resulted in the development of seven properties that have been found to highly correlate with similar sensory data. The following names have been adopted to correlate with terminology used in sensory analysis (Szczesniak, 1975; Bourne, 1978):

FRACTURABILITY - is defined as the force at the first significant break in the curve.

HARDNESS - defined as the peak force during the first compression cycle.

COHESIVENESS - defined as the ratio of the positive force during the second compression to that during the first compression.

ADHESIVENESS - defined as the negative force area for the first bite, representing the work necessary to pull the compressing plunger away from the sample.

SPRINGINESS - defined as the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite.

GUMMINESS - defined as the product of hardness x cohesiveness.

CHEWINESS - defined as the product of gumminess x springiness.

Like simple compression, texture profile analysis has become a standard method for examining gel properties. For years it has been considered the best method for objectively evaluating sensory characteristics in various food systems. Indeed, the incentive for conducting this test is still to objectively simulate the mastication process.

Zirbel and Kinsella (1988) used the technique to examine properties of gels made from whey protein isolate to determine factors affecting the various rheological characteristics. In general, they found gel hardness, springiness, and cohesiveness increased with heating temperature and springiness and cohesiveness increased with

increasing pH. Also, they concluded that pH and heating temperature were interrelated.

Singh et al. (1985) used texture profile analysis to evaluate the influence of various cooking treatments on texture properties of frankfurters to develop a methodology for objectively analyzing textural parameters. In general it was found that TPA was a good method for evaluating the texture properties of hardness, brittleness and elasticity. Other properties like cohesiveness, gumminess and chewiness could also be effectively evaluated provided processing temperatures were closely monitored. In a similar study by Klettner (1989), TPA was again used to test the rheological properties of frankfurters to establish the relationship between measured firmness values and sensory impression. Unlike other some other studies, it was found that instrumental and sensorial properties didn't always correlate. It was suggested that further study is needed on the measurement characteristics of the instruments as compared with sensory properties.

Like compression, texture profile analysis is a standard method. As such, studies are often conducted to compare other methods to TPA. In any case, the motivation is the same: accurate objective properties.

Okamoto et al. (1990) used the method to compare food protein gels produced by heat and pressure. Results indicate significant differences between pressure- and heat-induced gels of every food protein examined. In general,

pressure-induced gels had a higher deformability and hardness while possessing a lower adhesiveness than the heat-induced gels. They concluded that the gelling mechanism is different between the two methods of inducing gelation.

MATERIALS AND METHODS

3.1. Whey Protein Concentrates

Five experimental whey protein concentrates (WPC) were prepared and characterized by Beuschel (1992a). Foremost Whey Products (Clayton, WI 54701) donated liquid WPC from ultrafiltration of Parmesan cheese whey. The WPC was heat treated to produce protein with different solubilities (0.1 M NaCl, pH 7.0; Morr et al., 1985). The control WPC received no heat treatment and had a solubility of 98%. Heat treatments of 78.2°C/30s, 92.2°C/30s and 126.7°C/30min resulted in WPC with solubilities of 47%, 41% and 27%, respectively. An 80% soluble WPC was obtained by mixing 98% and 47% soluble WPC. The 98%, 80%, 47%, and 27% soluble WPC were examined in this study.

Three commercial WPC were obtained from three sources: 1) DMV USA: RT-75, Lot #1885, P.O. Box 1628, La Crosse, WI 54602-1628 (WPC A); 2) Danmark Protein: LPD-80, P.O. Box 594, Worthington, OH 43085 (WPC B); 3) New Zealand Milk Products Inc: Alacen 878, Petaluma, CA 94952. (WPC C).

3.2. Preparation of Whey Protein Concentrate Gels

3.2.1. Preparation of Hydrating Solutions

Standard hydrating solutions were made by dissolving 0.1 M 3-[N-Morpholino]propane-sulfonic acid (MOPS) buffer with deionized-distilled water (DD-H_2O) in a beaker. The pH was adjusted (if necessary) using a pH meter (Model 145, Corning Inc., Corning, NY) with either 1.0 M NaOH or 1.0 M HCl. Solutions were then transferred to a volumetric flask and made to the mark with DD-H_2O. Other components (i.e. sodium tripolyphosphate (NaTPP), NaCl, ethylenediamine tetraacetic acid (EDTA), and/or calcium chloride ($CaCl_2$)) were dissolved similarly in the standard hydrating solution to produce solutions needed for each experiment. This was done before final dilution and pH adjustments were carried out.

Sodium tripolyphosphate (Sigma Chem Co., St. Louis, MO 63155) was added to the standard hydrating solution at 10, 50 and 100 mM concentrations. These solutions were used during the study of gelling properties of the commercial WPCs and the experimental partially insolubilized WPCs. These were also the concentrations used in the study of cooling temperature on gelling properties. In the experiments examining buffer and pH effects, MOPS buffer was removed and the NaTPP concentrations remained the same.

During the study of ionic strength on WPC gels, NaCl was added to the standard solution at 30, 150 and 300 mM concentrations (concentrations equalling the ionic strength

of NaTPP). Ethylenediamine tetraacetic acid was added at 10, 50 and 100 mM concentrations to equal the concentrations of NaTPP used. Calcium chloride ($CaCl_2$) was added to the standard hydrating solution at concentrations of 5, 10, 25, 50 and 100 mM. All reagents including NaTPP, EDTA, and $CaCl_2$ were reagent grade or better.

3.2.2. Preparation of Protein Slurry

Whey protein concentrate was weighed into a 250 mL beaker to achieve the desired final concentration for a total of 125 g of slurry (slurries ranged from 10% to 18% protein concentration). The protein was hydrated with the appropriate buffer solution described previously. This consisted of initial drop by drop addition and mixing of slurry until the protein was virtually hydrated. All but 2-3 mL of the remaining solution was then added. The slurries were then stirred at ambient temperature using a 2.5 cm magnetic stirrer for 1 hr. The beaker was placed on a plastic mat to prevent heating. The pH was monitored and adjusted (if necessary) using either 1.0 M NaOH or 1.0 M HCl.

After 1 hr, the slurries were transferred into a desiccator for degassing (The desiccator connected to an aspirator via a rubber tube to form a vacuum chamber). Solutions were degassed under the following conditions: 1) The pressure of the vacuum was released every 5 min until the foam did not exceed about 5 cm above the level of the

solution (usually 1 time for commercial WPC and 2 or 3 times for the experimental WPC). 2) The vacuum was then maintained until the "head" of foam disintegrated. Total degassing time for commercial WPC was about 25-30 min and for the experimental WPC about 1 hr. Upon effective degassing, slurry pH was again checked and adjusted if necessary. The appropriate buffer solution was then added to achieve the correct final concentration (w:w).

3.2.3. Heat-Setting of Protein Slurry

Approximately 14 cm long glass tubes (Pyrex brand, 22 mm O.D., 19 mm I.D., 1.5 mm thick) were sprayed with silicone ("Pro-Magic" Food Grade Silicone Spray, Midco Products Co., Inc., Maryland Heights, MT 63043) to prevent sticking. One end of the tube was closed using a #3 rubber stopper. Protein slurry was then transferred into the tube until about 6-9 mm from the top. The open end was then closed with another #3 rubber stopper while squeezing the stopper to allow trapped air to escape from the head space. Tubes were then placed vertically in a wire holding rack.

Slurries were heated in a 90°C water bath for 15 min. Due to the large size of the water bath, the addition of tubes caused a temperature drop of 1°C. Tubes were immediately cooled in 20°C water bath to an internal temperature of 25°C. Internal temperature was monitored using a thermocouple inserted into a sample tube. The thermocouple sample tube was processed identically with the

other tubes but was not used for any rheological measurements. Tubes were removed from the 20°C water bath and allowed to equilibrate overnight at room temperature for testing.

3.3. Extraction of Chicken Salt-Soluble Protein

Chicken salt-soluble protein (SSP) was extracted as described by Beuschel (1990) with some modifications. Source and extraction of chicken muscle remained the same except 0.3 M NaCl was used in place of 0.6 M NaCl and 0.05 M Na phosphate buffer. Extracts were adjusted to pH 7.0 using 0.1 M NaOH after stirring for 20 min All additional steps remained the same except the final NaCl concentration was adjusted to 0.3 M rather than 0.6 M. Solution containing 0.3 M NaCl (pH 6.5) was added to give a final protein concentration of 8% (w:w).

3.4. Preparation of Whey Protein Concentrate:Salt-Soluble Protein Gels

3.4.1. Preparation of Hydrating Solutions

Buffer solutions were made by dissolving NaTPP and/or NaCl with DD-H_2O at four times the concentration needed in the final slurries (1% and 2% for NaTPP and/or 1.2 M for NaCl respectively). The pH was adjusted to 6.5 using either 1.0 M NaOH or 1.0 M HCl. Whey protein concentrate was then hydrated using the appropriate solution at one quarter of the final weight. Therefore, all slurries contained NaCl at 1.2 M concentration and NaTPP at either 0, 1.0, or 2.0%

concentration. The balance of the total weight was then achieved using DD-H_2O, thus yielding final slurry concentrations of 0.3 M NaCl and 0, 0.25, and 0.50% NaTPP.

3.4.2. Preparation of Combination Protein Slurry

Slurries of WPC A consisting of 8, 16, and 24% (w:w) protein were prepared in each of the buffers discussed in section 3.4.1. The method of slurry preparation was as described earlier with the degassing step eliminated. These WPC slurries were then combined 1:1 (w:w) with 8% SSP to obtain combination slurries of 4% SSP and either 4%, 8%, and 12% whey protein. Control slurries consisting of SSP (4% w:w) and WPC (12% and 16% w:w) alone were also produced using the same buffers. The pH of each slurry was again measured and adjusted (if necessary) by the method described earlier (using 0.1 M NaOH or HCl). All slurries were stored at 4°C overnight.

3.4.3. Heat-Setting of Combination Protein Slurry

After standing at 4°C overnight, the slurries were degassed. Slurries were placed individually into a vacuum mixer (Stephan Universal Machine, UMC 5 Electronic, Columbus, OH 43228) for degassing. The vacuum pump was turned on until the slurry approximately doubled in volume. The slurry was then mixed at 200 RPM to remove trapped air and reduce the volume (about 5-10 sec). This was repeated until a full vacuum was pulled and the slurry no longer increased in volume.

Approximately 14 cm long glass tubes (Pyrex brand, 22 mm O.D., 19 mm I.D., 1.5 mm thick) were sprayed with silicone ("Pro-Magic" Food Grade Silicone Spray, Midco Products Co., Inc.) to prevent sticking. One end of the tube was closed using a #3 rubber stopper. Due to the highly viscous nature of the slurries, a 60cc plastic syringe (Model# 9663, Luer Lok, Beckton Dickinson & Co., Rutherford, New Jersey 07070) was used to insert the slurries into the tubes (again, until about 6-9 mm from the top). The open end was then closed with an another #3 rubber stopper. Slurries were heated in either a 65°C or a 90°C water bath for 15 min and cooled as previously described.

3.5. Evaluation of Model Gel Systems

3.5.1. Stress and Strain at Failure

Stress and strain at failure was determined using a torsion technique (Hamann, 1983). A torsion gelometer system (Gel Consultants, Inc. Raleigh, NC 27612) consisting of a milling machine (Model #91) and a viscometer (Brookfield, Model DV1, Brookfield Engineering Laboratories, Inc. Stoughton, MA 02072) was used. Sample cores (29 mm x 19 mm) were cut using the template and cutter provided with the system. The ends of the samples were fastened to plastic disks using cyanoacrylate adhesive (Wonder Bond Plus, Borden, Inc., Columbus, OH 43215). The center section of each sample cylinder was then milled to 10 mm

diameter using the milling machine. Samples were twisted in the viscometer at a speed of 2.5 rpm. The following equations were used to calculate stress and strain at failure (Hamann et al., 1991):

Stress at Failure

$$\tau = \textit{viscosity reading} \times 1.581 \quad (3)$$

where: τ = stress at failure

1.581 = stress equation constant

viscosity reading = reading on viscometer at failure

Strain at Failure

$$\gamma = [0.150 \times \textit{time}_{(sec)}] - [0.00818 \times \textit{viscosity reading}] \quad (4)$$

where: γ = strain at failure

0.150 = uncorrected shear strain constant

time (sec) = time it takes for sample to fracture

0.00818 = corrected strain equation constant

viscosity reading = reading on viscometer at failure

$$TSS = \ln\left[1 + \left(\frac{\gamma^2}{2}\right) + \gamma\left(1 + \left(\frac{\gamma^2}{4}\right)^{0.5}\right)\right] \quad (5)$$

where: TSS = true shear strain

γ = strain at failure

3.5.2. Expressible Moisture

Two methods of determining expressed moisture were used: The Beuschel method and the Kocher method. The Beuschel method is that described by Beuschel et al. (1992a) and remained unmodified for this study. The Kocher method was developed by Kocher and Foegeding (1992) and was modified for this investigation.

Samples of WPC gel were used to standardize the Kocher method (0.1 M MOPS buffer, 10 mM NaTPP, pH 7.0). Cores of WPC gel were cut to a length of 10 mm using the template and cutter mentioned earlier in the evaluation of stress and strain at failure. A coffee straw (3.0 mm I.D.) was then used to cut a smaller sample lengthwise from the core. The final core sample was 10 mm x 3 mm. Cores were placed into the inner portion of micro-centrifuge tubes (Whatman Centrifugal Ultrafilters, Series 7000, Catalog# 6610N7168, Hillsboro, OR 97123). The micropore filter from each inner tube was removed using concentrated sulfuric acid (≈ 12 N) to allow for easier passage of moisture. Samples were spun at 162, 365, 650, and 1000 x g (1000, 1500, 2000, and 2500 RPM, respectively) for 10 min using a swinging bucket centrifuge (IEC Model K Centrifuge, Daman/IEC Division, Needham Heights, MA 02194) to determine the force at which an appreciable amount of moisture was expressed with as little gel deformation as possible. Secondly, the time necessary to reach equilibrium at 365 x g was examined. Centrifugation times of 2.5, 5, 7.5, 10, 20, 30, 45, 60, and

75 min were used. The final conditions for evaluating expressible moisture using this method were 365 x g for 10 min.

3.6 Preparation of Frankfurters

3.6.1 Frankfurter Formulations

Frankfurters were produced as described by Hung (1992) with modifications. Ground turkey drumstick meat (Bil Mar Foods, Inc., Zeeland, MI 49464), pork fat (Meat Lab, Michigan State University, East Lansing, MI 48823), DD-H_2O, NaCl, NaTPP, and WPC were used in the formulations. The control frankfurters were formulated to contain 12% protein, 15% fat, 69% moisture, and 2% NaCl. Sodium tripolyphosphate was substituted for 12.5% or 25% NaCl on a weight basis (0.25% and 0.5% of total formulation, respectively). Whey protein concentrate was substituted for 7.0% drumstick meat on a weight basis. Frankfurter formulations are given in Table 7.

3.6.2. Ingredient Preparation

Sodium chloride and NaTPP were dissolved in DD-H_2O as described earlier in buffer preparations. Solutions were made at twice the component concentration needed. Solutions were ultimately added to the batter as half the total volume of moisture. The balance of moisture in the batter was achieved by addition of DD-H_2O.

Whey protein concentrate was hydrated using the NaCl/NaTPP solutions. This WPC slurry was then stored at

Table 7. Formulation for low fat turkey frankfurters with 7.0% whey protein concentrate (WPC) A substituted for meat and 0.25% and 0.5% sodium tripolyphosphate (NaTPP) substituted for NaCl

Ingredient (without WPC)	Formulation A	B	C
Turkey drumstick meat (g)	513.4	513.4	513.4
Water (g)	180.6	180.6	180.6
Fat (g)	86.1	86.1	86.1
NaCl (g)	20.0	18.0	16.0
NaTPP (g)	0.0	2.0	4.0
WPC (g)	0.0	0.0	0.0

Ingredient (with WPC)	Formulation A	B	C
Turkey drumstick meat (g)	477.4	477.4	477.4
Water (g)	180.6	180.6	180.6
Fat (g)	86.1	86.1	86.1
NaCl (g)	20.0	18.0	16.0
NaTPP (g)	0.0	2.0	4.0
WPC (g)	35.9	35.9	35.9

4°C overnight. Pork fat was ground twice through a Kitchen Aid (Model K5-A, Troy, OH 45374) equipped with 4 mm diameter grinder plate and stored in the freezer. Ground turkey meat was also stored in the freezer but was thawed in a 4°C cooler overnight before using.

3.6.3. Batter Preparation

Turkey meat, half of the total DD-H_2O (4°C) and the NaCl/NaTPP solutions (with and without WPC A) were chopped in a Hobart bowl chopper (Model 84181 D, Troy, OH 45374) at a speed of 3450 rpm for 3 min in a 4°C cooler. The batter was transferred to a vacuum mixer (Stephan Universal Machine, UMC 5 Electronic) where the pork fat and remaining water (in the form of ice) were added. The batter was chopped at a speed of 900 RPM, with a vacuum of 2.07 kg/cm^2 (11.6 psi) for 6 min. Batter temperature was maintained under 4°C by circulating cold water through the jacketed mixer bowl. Each batter treatment was produced in triplicate.

3.6.4. Frankfurter Preparation

Batter was stuffed with a hand stuffer into pre-weighed, silicone sprayed, 50 mL polycarbonate centrifuge tubes, re-weighed and capped. Six tubes from each treatment were heated to internal temperatures of either 71.1°C or 90°C in a 75.1°C or a 94°C water bath, respectively. Upon reaching the desired temperature, tubes were cooled immediately in an ice bath to an internal temperature of

25°C. Frankfurters were immediately removed from the tubes, weighed, placed in plastic bags, and stored in a 4°C cooler overnight for further testing.

3.7. Evaluation of Frankfurters

3.7.1. Cook Yield

Cook yield was determined as described by Beuschel (1990) by dividing the weight of each cooked, drained frankfurter by the weight of the uncooked batter and multiplying by 100. Six frankfurters were evaluated for each treatment.

3.7.2. Severe Reheat Yield

Severe reheat yield was determined as described by Beuschel (1990). Twenty gram samples were cut from each frankfurter and placed in 100 mL of 95°C distilled H_2O for 10 min. Samples were removed, cooled for 5, and reweighed. Severe reheat yield was calculated by dividing final weight by initial weight and dividing by 100. Each treatment was evaluated in quadruplicate.

3.7.3. Stress and Strain at Failure

Stress and strain at failure was determined using torsional analysis described earlier. Ten frankfurter samples were evaluated for each treatment.

3.7.4. Expressible Moisture

Expressible moisture was determined by the Kocher method described earlier with modifications. Centrifuging

for 30 min at 365 x g was insufficient to reach expressible moisture equilibrium. Further study revealed that 45 min at 365 x g was required to completely express free moisture. Each frankfurter treatment was evaluated in quadruplicate.

3.8. Chemical and Analytical Analysis

3.8.1. Protein

Protein concentration was calculated for WPC and frankfurters following AOAC (1990) 920.105 and 981.10, respectively. The conversion factors of 6.38 and 6.25 were used for the WPC and frankfurter samples, respectively. Samples of WPC were examined in quadruplicate while frankfurter treatments were examined in triplicate.

3.8.2. Moisture

Moisture analysis of WPC was evaluated following AOAC (1990) 927.05. Each WPC was examined in triplicate. Moisture analysis of frankfurters was determined following the AOAC method (1990, 950.46 (b)). Frankfurter treatments were determined in duplicate.

3.8.3. Fat

Fat analysis of WPC was determined as described by the AOAC method (1990, 932.06). Each WPC was analyzed in duplicate. Fat analysis of frankfurters was determined following the AOAC method (1990, 960.39). Frankfurter treatments were determined in duplicate.

3.8.4. Minerals

3.8.4.1. Ionic Mineral Concentration

Ionic calcium and ionic sodium concentrations were determined on WPC slurries before heat treatment at room temperature (25°C) using ion selective electrodes. The calcium ion system consisted of a calcium selective electrode (Model #93-20, Orion Research, Inc., Boston, MA 02129) and a reference electrode (Model 90-01, Orion Research, Inc.). Calcium standard solution (1000 ppm Calcium Standard, lot# H508 KJCK-P, Mallinckrodt Specialty Chemicals C., Paris, KY 40361) was used to make standard solutions of 1, 10, and 100 ppm Ca^{2+} in DD-H_2O. Ten milliliters of each standard and every WPC slurry was pipetted into 50 mL beakers. Ionic strength adjuster (saturated $AgCl_3$ solution) was added to each beaker at 2% (v:v) concentration (0.2 mL) with stirring. The ion concentration was measured using a pH meter (Corning model 145, Corning Inc., Corning, NY) in the millivolt reading position.

Sodium ion concentration was determined similarly using a combination electrode (Model 13-620-503, Sodium Combination Electrode, Fisher Scientific, Pittsburgh, PA 15219). Sodium standard solution (1000 mM sodium standard, prepared from dried analytical grade NaCl) was used to make standard solutions of 1, 10, and 100 mM Na^+ in ionic strength adjuster (triethanolamine, #T-1377, Sigma Chemical Co.). One milliliter of slurry was diluted with 9 mL of

ionic strength adjuster to give a 1:10 dilution. The ion concentration was measured using a pH meter (Corning Model 145, Corning Inc.) in the millivolt reading position. Calcium and sodium ion concentrations for combination SSP:WPC gels were measured using the same techniques.

3.8.4.2. Total Mineral Concentration

Mineral composition was determined using atomic absorption/emission spectroscopy. A wet ashing technique was used for sample preparation. Approximately 1.0 g samples were weighed accurately into acid washed, 250 mL Erlenmeyer flasks. Twenty milliliters of nitric acid and 5 mL perchloric acid were transferred to each flask. Each flask was then placed on a hot plate, covered with a watch glass and digested at 125°C to 150°C. Samples were closely monitored for 1.5 - 2.0 hr while digestion occurred. After cooling and dilution to 100 mL, total calcium, magnesium, potassium, and sodium were determined using a Smith-Hjefke (Model 4000, Garland CA 90245) atomic absorption/emission spectrophotometer. Total phosphorus was determined colorimetrically (Gomorri, 1942). All WPC samples, SSP extractions, and frankfurter batter treatments were analyzed in duplicate.

3.8.5. Nonprotein Nitrogen Analysis of Whey Protein Concentrates

Nonprotein nitrogen (NPN) of WPC was determined as described by Beuschel (1990). Nonprotein nitrogen was

expressed as percent of total weight and was calculated as follows:

$$\%NPN = \frac{[Amount\ of\ Supernatant\ Kjeldahl\ Nitrogen]}{[Amount\ of\ WPC]} \quad (6)$$

$$[Amount\ of\ Supernatant\ Kjeldahl\ Nitrogen] = \frac{(ml\ HCl) \times (1.4007) \times (N\ HCl)}{sample\ weight_{(grams)}} \quad (7)$$

where: mL HCl = milliliters of ≈ 0.1 N HCl used for titration

1.4007 = molecular mass of nitrogen expressed in grams/100 mL

N HCl = normality of HCl

Sample weight = weight of sample

$$[Amount\ of\ WPC] = [WPC_{(g/ml)}] \times [sample\ weight_{(grams)}] \quad (8)$$

where: $[WPC]_{(g/ml)}$ = ≈ 3.0 grams WPC/70 mL

$Sample\ weight_{(grams)}$ = weight of 10 mL aliquot analyzed

Each WPC sample was analyzed in triplicate.

3.8.6. Whey Protein Concentrate Solubility

Solubility of WPCs was determined as described by Beuschel (1990) with modifications. After centrifugation,

the supernatant was poured through #1 Whatman filter paper and 5 mL aliquots were analyzed for protein by the method described earlier. Solubility was reported as a percent and calculated as follows:

$$\%Solubility = \frac{[supernatant\ protein_{(mg/ml)}]}{[total\ protein_{(mg/ml)}]} \times 100 \qquad (9)$$

$$[supernatant\ protein_{(mg/ml)}] =$$

$$\left(\frac{(ml\ HCl)\times(1.4007)\times(N\ HCl)}{sample\ weight_{(grams)}} - \%NPN\right) x\ 6.38 \qquad (10)$$

where:

mL HCl	=	milliliters of ≈ 0.1N HCl used for titration
1.4007	=	molecular mass of nitrogen expressed as a percentage
N HCl	=	normality of HCl
Sample weight	=	weight of 5 mL aliquot analyzed (grams)
%NPN	=	% Nonprotein nitrogen (expressed as decimal)
6.38	=	Kjeldahl conversion factor for dairy proteins

$$[total\ protein_{(mg/ml)}] =$$

$$\left(\frac{\%P}{6.38-\%NPN}\right) x \left[6.38\ x \left(\frac{orig.\ wt.}{50ml}\right)\right] x\ 100 \qquad (11)$$

where: %P = % protein in WPC (expressed as decimal)

%NPN = % nonprotein nitrogen (expressed as decimal)

6.38 = Kjeldahl conversion factor for dairy proteins

orig. wt. = original weight of WPC (grams)

50 mL = original dilution volume

Solubilities of WPCs were determined in triplicate.

3.8.7. Lactose

Lactose concentration of WPC was calculated colorimetrically using an enzyme assay kit (Boehringer-Mannhein, Catalog #176303, Mannheim, West Germany). Each WPC was analyzed in duplicate.

3.8.8. Electrophoresis of Whey Protein Concentrates

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the total protein of WPC A, B, and C. Samples were prepared in 25 mL glass vials by dissolving enough WPC in 20 mL of sample buffer (0.625 M tris (hydroxymethyl) aminomethane (Tris) buffer, 2% SDS, 10% glycerol, 1% ß-mercaptoethanol, and a few grains of bromophenol blue, pH 7.2) to give a final protein concentration of 2 mg/mL, then heated in boiling water for 10 min.

Electrophoresis was accomplished using polyacrylamide gels on a Bio-Rad Mini-Protean II Dual Slab Cell (Model

1000/500 Power Supply, Bio-Rad Laboratories, Richmond, CA 94804). The stacking gel (4% acrylamide) consisted of 1.33 mL acrylamide (from 30% acrylamide stock solution), 5.0 mL Tris buffer (0.25 M Tris, pH 6.8), 10 µl of 99% N,N,N′,N′-Tetramethylethylenediamine (TEMED), 0.4 mL of 1% ammonium persulfate, and 3.26 mL DD-H_2O. The resolving gel (14% acrylamide) consisted of 4.33 mL acrylamide (from 30% acrylamide stock solution), 5.0 mL Tris buffer (0.75 M Tris, pH 8.8), 10 µl TEMED, 0.4 mL of 1% ammonium persulfate, and 0.26 mL DD-H_2O. The electrode buffer consisted of 0.025 M Tris, 0.192 M Glycine, and 0.1% SDS (pH 8.3).

Each lane of the gel was loaded with 10 or 20 µg of protein. Molecular weight standards (Sigma Kit for Molecular Weights, MW-SDS-70L 14,000-70,000, Sigma Chemical Co.) were run simultaneously to serve as references. The power supply held the current constant at 220 mA until the tracking dye reached the bottom of the resolving gel. Gels were stained overnight in 9:45:45 acetic acid:water:methanol solution containing 0.4% Commassie Brilliant Blue. A solution consisting of 3:2:35 (v:v:v) acetic acid:methanol:water was used to destain the gels.

3.8.9. Calculation of Ionic Strength

Ionic strength calculations for NaTPP and NaCl gels were accomplished by the method described by Trout and Schmidt (1986) using the following formula:

$$I = 0.5[[Phos_1](Z)^2 + [Na^{+1}] + [H^+] + [Cl^-]] \qquad (12)$$

where: I = ionic strength

Z = the charge of the phosphate molecule calculated by:

$$Z = [Na^+]/[Phos]] - (H^+/[Phos]) \qquad (13)$$

$[Phos_1]$ = molal phosphate concentration

[Phos] = molar phosphate concentration

H+ = number of moles of HCl to titrate the sample to pH 7.0

$[Na^+]$ = sodium ion molar concentration

$[Na^+_1]$ = sodium ion concentration

Cl^- = moles of chloride ion introduced by HCl addition

A sample calculation for 100 mM NaTPP sols is given in Appendix 3.

3.9. Statistical Analysis

A completely randomized block design was used for all experiments. Mean square error, analysis of variance, and separation of means were performed by the statistical software program MSTATc (1989).

3.9.1. Whey Protein Concentrate Gels

Three replicates of all WPC gel systems were analyzed. A two-way analysis of variance (replicate x treatment) was

performed on stress, strain, and expressible moisture data at each sodium tripolyphosphate concentration. Interaction was significant at P > 0.05. Means were separated with Tukey's significant difference test using the mean square error term.

3.9.2. Salt Soluble Protein: Whey Protein Concentrate Combination Gels

Three replicates of all SSP:WPC combination gel systems were analyzed. A 3 x 2 x 4 factorial (replicate x temperature x treatment) was used to analyze variance in stress, strain, and expressible moisture data for all gels. Interaction was significant at P > 0.05. Means were separated with Tukey's significant difference test using the mean square error term.

3.9.3. Frankfurters

Three replicates of all frankfurter systems were analyzed. Variance of the 7% WPC substituted model system turkey frankfurters was analyzed using a 3 x 2 x 3 factorial (replicate x temperature x treatment). Interaction was significant at P > 0.05. Means were separated with Tukey's significant difference test using the mean square error term.

RESULTS AND DISCUSSION

4.1. Modification of Expressible Moisture Procedure

Expressible moisture of WPC A gels (12% protein (w/w)) in 0.1M MOPS buffer, pH 7.0) as a function of increasing centrifugal force was examined (Table 8). Whey protein concentrate gels were not deformed at 365 x g and an appreciable amount of total projected moisture (≈ 73%) was expressed. This became the centrifugal force used for further investigation.

Expressible moisture for WPC A gels (12% protein (w/w)) in 0.1M MOPS buffer, pH 7.0) as a function of time at 365 x g are shown in Table 9. Centrifuging the gels with this force for 30 min expressed nearly the same moisture (30.9%) as that expressed after 90 min (31.5%). As a result, centrifugation conditions for expressible moisture of WPC gels was modified to 30 min at 365 x g. Results showed that SSP:WPC combination gels behaved the same, but frankfurters required a centrifugation time of 45 min at 365 x g.

4.2. Whey Protein Concentrate Composition

4.2.1. Proximate Composition

The proximate composition of the four WPCs are given in Table 10. All four WPC samples were greater than 90%

Table 8. Expressible moisture (%) of WPC A gels (12% protein (w/w)) made in 0.1M MOPS buffer, pH 7.0) as a function of centrifugal force for 10 min

Force (x g)	Expressible moisture (%)
162	14.42 ± 1.59
365	23.54 ± 1.86
650	28.86 ± 2.08
1000	32.15 ± 1.59

Table 9. Expressible moisture (%) of WPC A gels (12% protein (w/w)) made in 0.1M MOPS buffer, pH 7.0) as a function of centrifugation time at 365 x g

Time (min)	Expressible moisture (%)
2.5	8.3 ± 1.04
5.0	16.1 ± 1.77
7.5	18.6 ± 2.60
10.0	21.5 ± 2.13
20.0	27.1 ± 1.52
30.0	30.9 ± 1.33
45.0	31.1 ± 1.59
60.0	31.3 ± 1.03
75.0	31.5 ± 1.23
90.0	31.5 ± 1.32

Table 10. Proximate composition (%) and protein solubility (%) of whey protein concentrates (WPCs)

Characteristic	WPC A	WPC B	WPC C	WPC D
Protein	74.3	76.6	56.3	73.6
Moisture	6.2	5.7	6.1	4.5
Fat	6.6	7.0	4.3	6.1
Ash	2.9	2.6	3.4	4.4
Nonprotein nitrogen[a]	2.5	2.1	4.3	3.5
Lactose	6.8	7.3	27.3	8.3
Solubility	92.5	94.0	96.9	96.6

[a] expressed as percent protein

soluble in 0.1 M NaCl (pH 7.0) suggesting that little protein had been denatured during WPC production. Composition differed slightly with the exception of WPC C which was higher in lactose (27.3%) and lower in protein (56.3%). This is because Beuschel (1992a) used a whey source that had not been delactosed.

Unlike proximate composition, mineral composition of WPCs was variable (Table 11). Calcium ranged from 55.1 mM (WPC D) to 101.7 mM (WPC A). Sodium ranged from 100.0 mM (WPC A) to 547.4 mM (WPC D). Phosphorus, potassium, and magnesium were also variable, ranging from 118.6 mM to 320.0 mM, 36.9 mM to 257.5 mM, and 4.1 mM to 20.7 mM, respectively. Small differences in mineral composition, like those between WPC A and WPC B, are likely caused by small differences in raw materials (i.e. milk). Larger differences in composition (WPC C), are likely caused by differences in techniques of WPC manufacture or possibly by deliberate manipulation by the manufacturer (WPC D).

The rational for choosing these WPCs was based on the variable mineral composition. Whey protein concentrates B and C were chosen due to their relatively high concentration of calcium and low concentration of sodium. Conversely, WPC D was chosen because of its low concentration of calcium and high concentration of sodium. Whey protein concentrate A was chosen because it was experimentally produced and highly characterized by Beuschel et al. (1992a and 1992b) and Hung (1992).

Table 11. Mineral composition (mM) of whey protein concentrates (WPCs)

Mineral	WPC A	WPC B	WPC C	WPC D
Calcium	101.7	93.2	92.5	55.1
Sodium	100.0	116.1	135.5	547.4
Phosphorus	120.4	118.6	129.8	320.0
Potassium	152.3	142.4	257.5	36.9
Magnesium	13.6	17.9	20.7	4.1

4.2.2. Electrophoresis of Whey Protein Concentrates

Electrophoresis was carried out on WPCs A, B, and C using 20 μg of protein/lane (Figure 1). Proteins with molecular masses of 70.9, 66.2, 57.3, 27.4, 22.2, 18.0, and 14.6 kilodaltons (kDa) were identified. The 66.2 kDa protein was probably bovine serum albumin (BSA), which has a reported molecular mass of 66.3 kDa (Fox, 1989). The proteins with molecular masses of 70.9, 57.3, 27.4, and 22.2 kDa are likely monomeric forms of immunoglobulins. de Wit and Klarenbeek (1984) stated that immunoglobulins consist of a heavy chain of about 50-70 kDa and a light chain consisting of about 22 kDa. Proteins with molecular masses of 18.0 and 14.6 kDa are likely β-lactoglobulin (β-lg) and α-lactalbumin (α-la), which have reported molecular masses of 18.4 and 14.2 kDa, respectively (de Wit and Klarenbeek, 1984). Results indicate that the protein compositions of WPCs A, B, and C do not vary much with respect to each other.

4.3. GELATION OF COMMERCIAL WHEY PROTEIN CONCENTRATES

4.3.1. EFFECT OF SODIUM TRIPOLYPHOSPHATE ON THE CALCIUM ION CONCENTRATION OF WHEY PROTEIN CONCENTRATE SOLS

Calcium ion concentrations ($[Ca^{2+}]$) for sols prepared from three commercial WPCs (A, B, and D) and one experimental WPC (C) at four sodium tripolyphosphate (NaTPP) concentrations (0, 10, 50, and 100 mM) are shown in Table 12. In each case, $[Ca^{2+}]$ decreased significantly with

Figure 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whey protein concentrate (WPC) showing the relative concentration of ß-lactoglobulin (ß-lg), α-lactalbumin (α-la) and bovine serum albumin (BSA) when applied at 20 µg protein\lane on a 14% acrylamide gel (1: low molecular weight standard; 2: WPC A; 3: WPC B; 4: WPC C)

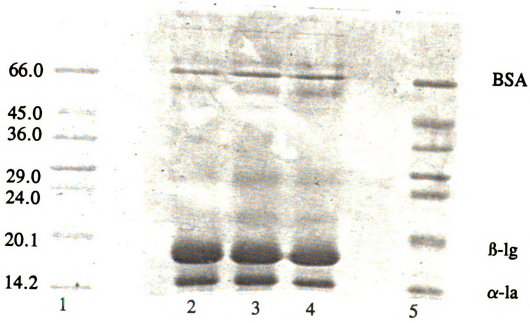
66.0
45.0
36.0
29.0
24.0
20.1
14.2
1
2
3
4
5
BSA
ß-lg
α-la

Table 12. Ionic calcium concentration ([Ca^{2+}] in mM) of whey protein concentrate (WPC) sols in 0.1M MOPS buffer, pH 7.0, and 0-100 mM sodium tripolyphosphate (NaTPP)

NaTPP (mM)	Ionic Strength	WPC A[b]	WPC B[b]	WPC C[c]	WPC D[a]
0	0	17.45[d]	16.49[d]	13.24[d]	1.86[d]
10	0.016	2.87[e]	2.80[e]	5.39[e]	0.32[e]
50	0.080	0.19[f]	0.17[f]	0.17[f]	0.05[f]
100	0.160	0.05[g]	0.06[g]	0.01[g]	0.02[g]

[a] 10% protein concentration (w/w)
[b] 12% protein concentration (w/w)
[c] 18% protein concentration (w/w)
[d-g] Means within the same column followed by the same letter do not differ significantly ($P < 0.05$).

increasing concentration of NaTPP. Whey protein concentrates A, B, C, and D had significantly different initial [Ca^{2+}] ranging from 1.86 mM to 17.45 mM but had final [Ca^{2+}] that were not significantly different ranging from 0.01 mM to 0.06 mM at 100 mM NaTPP.

Reduction in [Ca^{2+}] was expected due to the calcium ion sequestering ability of NaTPP. Other phosphates like sodium orthophosphate and sodium hexametaphosphate with different chain lengths and structures also affect gelling properties of WPCs. Preliminary experiments were performed with these phosphates and are given in appendices 1 and 2.

In addition, protein concentration appeared to have a negligible effect on the final [Ca^{2+}] (NaTPP concentration of 100 mM) when increased from 10% (w:w) in WPC D to 18% (w:w) in WPC C.

Ionic strength values shown were calculated as illustrated in appendix 3. In each subsequent table, addition of 0, 10, 50, and 100 mM NaTPP will also equal ionic strength values of 0, 0.016, 0.080, and 0.160, respectively.

4.3.2. EFFECT OF SODIUM TRIPOLYPHOSPHATE ON THE STRESS AT FAILURE OF WHEY PROTEIN CONCENTRATE GELS

Stress at failure for gels prepared from three commercial WPCs (A, B and D) and one experimental WPC (C) at four NaTPP concentrations (0, 10, 50, and 100 mM) are given in Table 13. Gel hardness increased to a maximum at 10 mM

Table 13. Stress at failure (kPa) for whey protein concentrate (WPC) gels in 0.1M MOPS buffer, pH 7.0, and 0-100 mM sodium tripolyphosphate (NaTPP) heated at 90°C for 15 min and cooled in a 20°C water bath to an internal temperature of 23°C

NaTPP (mM)	WPC A[e]	WPC B[e]	WPC C[f]	WPC D[d]
0	11.8[b]	5.7[b]	25.8[b]	14.5[ab]
10	22.1[a]	11.4[a]	36.3[a]	13.5[bc]
50	13.6[b]	6.9[b]	17.6[d]	11.6[c]
100	11.8[b]	8.8[ab]	20.8[c]	16.7[a]

[a-d] means within the same column followed by the same letter do not differ significantly ($P < 0.05$).
[d] 10% protein concentration (w/w)
[e] 12% protein concentration (w/w)
[f] 18% protein concentration (w/w)

NaTPP in WPC A, B and C. These gels represented about a 100% increase in hardness for WPCs A & B and about 50% increase in hardness for WPC C. Sodium tripolyphosphate did not improve the hardness of gels made with WPC D. Regardless of protein concentration or initial [Ca^{2+}], hardness was maximized within the narrow [Ca^{2+}] range of 1.86 mM in WPC D to 5.39 mM in WPC C. These results suggest that a certain concentration of [Ca^{2+}] is necessary for optimum gel hardness. Our results are consistent with Mulvihill and Kinsella (1988) who found that [Ca^{2+}] of 10 mM was necessary to optimize strength of ß-lactoglobulin gels. They suggested the primary effect of [Ca^{2+}] involves electrostatic cross-linking of protein molecules by Ca^{2+}. They further suggested that at sub-optimal [Ca^{2+}] electrostatic cross-linking was weak resulting in weaker gels and at excessive [Ca^{2+}], cross-linking attraction was too great and the gel matrix collapsed. In their study of mineral salt effects on whey protein gelation, Kuhn and Foegeding (1991a) found a similar concentration of 20 mM added $CaCl_2$ necessary to optimize hardness of heat-induced WPI gels. They suggested that alteration of the [Ca^{2+}] was an effective way of modifying the rheological properties.

4.3.3. EFFECT OF SODIUM TRIPOLYPHOSPHATE ON THE STRAIN AT FAILURE OF WHEY PROTEIN CONCENTRATE GELS

Strain at failure for gels prepared from three commercial WPCs (A, B and D) and one experimental WPC (D) at

four sodium tripolyphosphate (NaTPP) concentrations (0, 10, 50, and 100 mM) was inconsistent (Table 14). Maximum strain at failure occurred at 0 mM NaTPP for WPCs A and C and 100 mM NaTPP for WPCs B and D.

Gel deformability ranged from 1.48 to 1.57 when NaTPP concentration was 10 mM for WPCs A, B, and C and NaTPP concentration was 0 mM for WPC D. These NaTPP concentrations resulted in the same narrow [Ca^{2+}] range of 1.86 mM to 5.39 mM found to maximize gel hardness. Like stress, strain at failure was sensitive to divalent cationic effects of calcium.

Results differ from those found in the literature. Mulvihill and Kinsella (1988) found that [Ca^{2+}] of 15 mM resulted in optimal deformability of ß-lactoglobulin gels. They suggested that at the point of optimum ionic calcium, ionic bridges between protein molecules were strongest and remained intact under stress. Conversely, Kuhn and Foegeding (1991a) observed a sharp increase in WPI gel deformability as $CaCl_2$ increased reaching a maximum at 100 mM $CaCl_2$. They suggested that differences in the literature are likely due to differences in the gelling systems examined and the sensitivity of shear strain.

4.3.4. EFFECT OF SODIUM TRIPOLYPHOSPHATE ON THE EXPRESSIBLE MOISTURE OF WHEY PROTEIN CONCENTRATE GELS

With the exception of WPC D, addition of 10 mM NaTPP significantly reduced expressible moisture to a minimum (Table 15). This decrease was followed by subsequent

Table 14. Strain at failure (dimensionless) for whey protein concentrate (WPC) gels in 0.1M MOPS buffer, pH 7.0, and 0-100 mM sodium tripolyphosphate (NaTPP) heated at 90°C for 15 min and cooled in a 20°C water bath to an internal temperature of 23°C

NaTPP (mM)	WPC A[e]	WPC B[e]	WPC C[f]	WPC D[d]
0	1.92[a]	1.61[ab]	1.57[a]	1.57[b]
10	1.48[b]	1.53[bc]	1.56[a]	1.46[c]
50	1.65[ab]	1.36[c]	1.23[b]	1.39[c]
100	1.79[a]	1.80[a]	1.47[a]	1.70[a]

[a-c] Means within the same column followed by the same letter do not differ significantly ($P < 0.05$).
[d] 10% protein concentration (w/w)
[e] 12% protein concentration (w/w)
[f] 18% protein concentration (w/w)

Table 15. Expressible moisture (%) for whey protein concentrate (WPC) gels in 0.1M MOPS buffer, pH 7.0, and 0-100 mM sodium tripolyphosphate (NaTPP) heated at 90°C for 15 min and cooled in a 20°C water bath to an internal temperature of 23°C

NaTPP (mM)	WPC A[e]	WPC B[e]	WPC C[f]	WPC D[d]
0	50.2[a]	44.8[a]	33.2[a]	34.7[b]
10	20.3[c]	22.1[c]	29.3[b]	33.6[b]
50	38.8[b]	35.7[b]	29.8[b]	43.0[a]
100	43.8[ab]	42.7[a]	32.8[a]	46.1[a]

Expressible moisture determined as described by Beuschel (1990).

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

[d] 10% protein concentration (w/w)

[e] 12% protein concentration (w/w)

[f] 18% protein concentration (w/w)

increases in expressible moisture which equalled or surpassed the control (0 mM NaTPP) gels. In general, higher expressible moisture coincided with higher strain at failure and lower stress at failure. This makes sense because a higher level of moisture will provide additional lubrication to a gel thus increasing deformability and potentially making a overall softer gel. Beuschel et al. (1992a) reported similar results in their study of WPC solubility as it relates to gelation properties. They too found that increased expressible moisture tended to correspond with increased strain at failure.

Once again minimum expressible moisture occurred within the [Ca^{2+}] range of 1.86 mM to 5.39 mM. Expressible moisture was lowest where gel hardness was highest in WPCs A, B and C. Like stress at failure, results suggest that a certain [Ca^{2+}] was necessary to optimize expressible moisture regardless of protein concentration or initial [Ca^{2+}].

4.3.5. EFFECT OF IONIC STRENGTH ON THE PROPERTIES OF WHEY PROTEIN CONCENTRATE GELS

The effect of ionic strength was tested by preparing gels with NaCl at ionic strengths ranging from 0 to 0.3 to cover the ionic strength range of 0.016, 0.08 and 0.16 observed in 10, 50, and 100 mM NaTPP treated gels, respectively (Table 16). Calcium ion concentration increased slightly from 17.4 to 22.9 mM as ionic strength increased from 0 to 0.3. This was likely the result of an

Table 16. The effect of ionic strength on properties of whey protein concentrate A gels (12% protein (w/w)) made in 0.1M MOPS buffer, pH 7.0, and 0-300 mM NaCl heated at 90°C for 15 min and cooled in a 20°C water bath to an internal temperature of 23°C.

NaCl (mM)	Ionic strength	$[Ca^{2+}]$ (mM)	Stress at failure (kPa)	Strain at failure (dimensionless)	Expressible moisture[a] (%)
0	0	17.4	13.1[a]	1.97[a]	45.7[a]
30	0.03	17.4	13.0[a]	1.99[a]	47.1[a]
150	0.15	19.1	11.8[a]	1.91[a]	46.3[a]
300	0.30	22.9	11.4[a]	1.99[a]	45.2[a]

[a] Expressible moisture determined as described by Kocher and Foegeding (1992) except centrifuged at 365 x g.

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

ionic interaction where added Na^{+} (coming from dissociated NaCl) competed with Ca^{2+} for negative charges on the protein. Since there was no NaTPP to sequester the newly liberated calcium, it simply appeared as increased Ca^{2+}.

Gelling properties (stress & strain at failure and expressible moisture) did not differ significantly with changes in ionic strength suggesting the effect of NaTPP was not due to ionic strength but calcium chelating ability. Stress at failure averaged 12.3 kPa, strain at failure averaged 1.97, and expressible moisture averaged 46.1%.

In a study by Xiong (1992), turbidity measurements were used to determine that ionic strength affected the thermal aggregation of WPCs and whey protein isolates (WPIs) resulting in a change in aggregation temperature or rate. No textural analysis was done, however, to correlate these findings with final gel properties. It is possible, therefore, that although ionic strength appeared to significantly affect the temperature and rate at which whey proteins aggregated, it may not have affected the final textural properties of the resulting gels.

4.3.6. EFFECT OF EDTA ON THE PROPERTIES OF WHEY PROTEIN CONCENTRATE GELS

Ethylenediamine tetraacetic acid (EDTA) is a sequestrant that works much like NaTPP by binding ions like Ca^{2+}. As such, replacing NaTPP with equivalent concentrations of EDTA should produce similar [Ca^{2+}] binding results as seen with added NaTPP.

Stress at failure was greatest in gels treated with 10 mM EDTA. Like the NaTPP treated gels, [Ca^{2+}] decreased significantly as the EDTA concentration increased. Calcium ion concentration decreased from 12.6 mM in control to undetectable at 100 mM EDTA (Table 17). Stress at failure of WPC A gels for each treatment (EDTA vs. NaTPP) were similar as the concentration of sequestrant increased from 0 to 100 mM.

Strain at failure for EDTA and NaTPP treated gels were different. Results are in contrast to those with NaTPP in which an initial decrease in strain resulting with addition of 10 mM NaTPP was followed by subsequent increases in strain as NaTPP increased to 50 and 100 mM. Strain at failure increased as EDTA concentration was increased and reached a maximum of 2.31 at 100 mM EDTA.

Expressible moisture for EDTA and NaTPP treated gels was measured using different techniques (Kocher (1992) and Beuschel (1990) respectively). Although the values cannot be directly compared, they seem to follow a similar trend. Expressible moisture was lowest at 10 mM NaTPP and EDTA and increased as the NaTPP and EDTA increased to 100 mM.

With the exception of strain at failure, these results indicate that NaTPP and EDTA affect the gelling properties of WPC in a similar manner. The two compounds may, however, differentially alter the gelling matrix in a way that affects the deformability of the gel. This would help to explain the inconsistency observed among the strain at

Table 17. The effect of ethylenediamine tetraacetic acid (EDTA) on properties of whey protein concentrate A gels (12% protein (w/w)) in 0.1M MOPS buffer, pH 7.0, heated at 90°C for 15 min and cooled in a 20°C water bath to an internal temperature of 23°C

EDTA (mM)	$[Ca^{2+}]$ (mM)	Stress at failure (kPa)	Strain at failure (dimensionless)	Expressible moisture[a] (%)
0	12.6	12.0[d]	1.49[d]	39.4[d]
10	4.1	22.2[b]	1.58[d]	35.4[e]
50	0.01	16.3[c]	2.02[c]	46.6[c]
100	0	14.7[c]	2.31[b]	50.1[b]

[a] Expressible moisture determined as described by Kocher and Foegeding (1992) except centrifuged at 365 x g.

[b-e] Means within the same column followed by the same letter do not differ significantly ($P < 0.05$).

failure data.

Kuhn and Foegeding (1991b) found similar results in their study of WPC and WPI gelation as affected by calcium removal using EDTA, ethyleneglycol-bis-(ß-aminoethyl ether) N,N,N′,N′,-tetraacetic acid (EGTA), and dialysis. In their study, they added 50 mM EDTA and EGTA to three WPCs and one WPI. In every sample, the added sequestrant had either detrimental or insignificant effects on stress and strain at failure for 10% gels produced at pH 7.0. They also found, however, that the [Ca^{2+}] did not significantly change in any case. When dialysis was used to remove calcium, different results were found. Calcium ion concentrations for all systems was significantly reduced and significant increases in gel hardness and deformability were observed for two of the WPCs and the WPI. They suggested that there is probably an optimal concentration of calcium for gel formation, but it depends on the gelling system.

4.3.7. EFFECT OF CALCIUM CHLORIDE ON THE PROPERTIES OF WHEY PROTEIN CONCENTRATE GELS

To further examine the effect of [Ca^{2+}] on WPC gels, calcium chloride ($CaCl_2$) was added to WPC D gels (10% protein (w/w) in 0.1M MOPS buffer, pH 7.0). Whey protein concentrate D was chosen because it had the lowest initial calcium concentration. As expected, the [Ca^{2+}] increased as the concentration of $CaCl_2$ increased from 0 to 100 mM (1.59 mM to 145.7 mM) (Table 18). Between 0 and 25 mM added $CaCl_2$, each mM of $CaCl_2$ produced a 0.5 mM increase in [Ca^{2+}].

Table 18. The effect of $CaCl_2$ on properties of whey protein concentrate D gels (10% protein (w/w)) made with 0.1M MOPS buffer, pH 7.0, heated at 90°C for 15 min and cooled in a 20°C water bath to an internal temperature of 23°C

$CaCl_2$ (mM)	$[Ca^{2+}]$ (mM)	Stress at failure (kPa)	Strain at failure (dimensionless)	Expressible moisture[a] (%)
0	1.59	12.6[bc]	1.40[cd]	31.6[c]
5	3.47	13.2[b]	1.50[c]	31.1[c]
10	5.37	7.1[e]	1.01[e]	30.9[c]
25	12.79	7.6[de]	1.19[de]	46.3[b]
50	43.17	12.4[c]	1.79[b]	47.8[b]
100	145.7	10.1[cd]	1.90[b]	49.7[b]

[a] Expressible moisture determined as described by Kocher (1992) except centrifuged at 365 x g.

[b-e] Means within the same column followed by the same letter do not differ significantly ($P < 0.05$).

With 50 mM added $CaCl_2$, this ratio increased to approximately 1:1 and further increased to 1:1.5 upon addition of 100 mM $CaCl_2$. This data suggests that the protein molecules in solution had many negatively charged sites for the binding of Ca^{2+}. Thus, at lower concentrations of $CaCl_2$ ($\leq$ 25 mM), about half of the dissociated calcium bound to the protein molecules, while at higher concentrations of $CaCl_2$ (> 50 mM), protein molecules became saturated with positive charges until all of the dissociated $CaCl_2$ became Ca^{2+}.

Stress at failure and expressible moisture for these gels were optimized (maximum and minimum respectively) at 5.0 mM $CaCl_2$ which corresponded to 3.47 mM [Ca^{2+}]. This is within the same [Ca^{2+}] range (1.86 - 5.39 mM) at which gel hardness and expressible moisture were optimized in the NaTPP treated WPC gels (Tables 13-15). Further increases in $CaCl_2$ resulted in a general decrease in gel hardness and an increase in expressible moisture. These results are somewhat inconsistent with results of Mulvihill and Kinsella (1988) and Kuhn and Foegeding (1991a). In their studies, addition of a small amount of $CaCl_2$ (about 10 mM) increased gel hardness about 3 to 5 fold. Their experiments, however, were conducted using whey protein sources with lower calcium concentrations (β-lactoglobulin and whey protein isolate respectively). It is likely that WPC D was manufactured to a specific [Ca^{2+}] to optimize gelling properties as addition and removal of [Ca^{2+}] resulted in less than optimal gelling

properties.

Strain at failure was less consistent than stress at failure or expressible moisture. However, gels containing 5 mM added $CaCl_2$ had a strain at failure of 1.50. This value falls within the range of strain data observed with the NaTPP treated gels of 1.48 to 1.57 (Table 14). These results further suggest that $[Ca^{2+}]$ is needed at a certain concentration to optimize WPC gelling properties.

4.3.8. EFFECT OF pH ON THE IONIC CALCIUM CONCENTRATION OF WHEY PROTEIN CONCENTRATE SOLS

The ionic calcium concentration for WPC A sols (12% protein (w/w)) with no added buffer) prepared at pH values of 5.0, 6.0, 7.0, and 8.0 with NaTPP concentrations of 0, 10, 50, and 100 mM was determined (Table 19). As expected, addition of NaTPP decreased $[Ca^{2+}]$ for WPC sols at every pH. In WPC sols of equal NaTPP concentration, $[Ca^{2+}]$ decreased as pH was increased between 5.0 and 8.0. This is the result of changes in the overall charges on the protein. As the pH decreases ($[H^+]$ decreases), charge of the amino acids on the proteins become more positive. As this occurs, less attraction exists between protein and positively charged Ca^{2+}. Thus, less calcium is protein bound and the equilibrium shifts toward more free calcium.

Table 19. Effect of pH on ionic calcium concentration ($[Ca^{2+}]$ mM) of whey protein concentrate A sols (12% protein (w/w)) with no added buffer, and 0-100 mM sodium tripolyphosphate (NaTPP)

NaTPP (mM)	pH 5.0	pH 6.0	pH 7.0	pH 8.0
0	59.3	21.6	13.67	4.60
10	23.6	4.29	2.95	0.84
50	3.74	0.55	0.21	0.05
100	1.63	0.29	0.06	0.02

4.3.9. EFFECT OF pH ON THE GELLING PROPERTIES OF WHEY PROTEIN CONCENTRATE GELS

Gelling properties of WPC A (12% protein (w/w) with no added buffer) prepared at pH values of 5.0, 6.0, 7.0, and 8.0 with NaTPP concentrations of 0, 10, 50, and 100 mM are given in Table 20. In general, gel hardness increased as pH increased from 5.0 to 8.0 at the same NaTPP concentration.

Gels prepared at pH 5.0 at any NaTPP and at pH 6.0 without NaTPP were too weak to measure. However, as NaTPP concentration increased from 10 to 100 mM, gel strength increased at pH 6.0 from 5.85 to 9.64 kPa . At pH 7.0 measurable gels were produced at all NaTPP concentrations with maximum gel strength of 26.7 kPa at 10 mM NaTPP. All NaTPP concentrations produced very rigid, nearly transparent gels at pH 8.0 with the maximum hardness of 34.4 kPa observed at 50 mM NaTPP.

These results are in partial agreement with those by Beuschel (1990) who found that gel hardness remained unchanged between pH 6.0 and 7.0 but then increased significantly as pH was increased to 8.0. The results are also in agreement with Morr and Foegeding (1990) who studied the effect of pH on functional properties of three WPIs and eight WPCs. There was a general trend for gel hardness of each system to increase with increasing pH.

These results are inconsistent with those of Zirbel and Kinsella (1988) who found that gel hardness was greatest at pH between 6.0 and 6.4 and gradually decreased as pH

Table 20. Effect of pH on the gelling properties of whey protein concentrate A gels (12% protein (w/w)) in 0-100 mM sodium tripolyphosphate (NaTPP)

NaTPP (mM)	Stress at failure (kPa)	Strain at failure (dimensionless)	Expressible moisture[a] (%)
		pH 5.0	
0	TWTM	TWTM	49.6^{b}
10	TWTM	TWTM	45.5^{bcd}
50	TWTM	TWTM	44.6^{cd}
100	TWTM	TWTM	42.8^{cd}
		pH 6.0	
0	TWTM	TWTM[e]	46.7^{bc}
10	5.85^{g}	1.62^{cd}	43.4^{cd}
50	8.76^{fg}	1.90^{ab}	44.4^{cd}
100	9.64^{f}	1.89^{ab}	44.9^{cd}
		pH 7.0	
0	10.5^{ef}	1.46^{d}	30.4^{f}
10	26.7^{bc}	1.77^{bc}	11.4^{g}
50	20.4^{f}	1.72^{bc}	36.7^{e}
100	13.4^{e}	1.89^{ab}	43.4^{cd}
		pH 8.0	
0	23.4^{cd}	1.94^{ab}	12.2^{g}
10	26.0^{bc}	1.92^{ab}	9.74^{g}
50	34.4^{a}	1.84^{abc}	33.7^{ef}
100	28.9^{d}	2.07^{a}	41.8^{d}

[a] Expressible moisture determined as described by Beuschel (1990).

[b-h] Means within the same column followed by the same letter do not differ significantly ($P < 0.05$).

TWTM = To Weak To Measure

increased to 8.0. Their study used WPI prepared by ion exchange chromatography which had a lower calcium concentration than WPCs (Morr, 1989). Reduced calcium concentrations may have allowed intermolecular ionic interactions to increase with the changing pH. This is supported by the fact that their hardest gels were found at about pH 6.0. This is nearest the isoelectric point of whey protein isolates and as such would be the pH where the least electrostatic repulsions and greatest protein-protein interactions would occur. If indeed electrostatic interactions dominated, weaker gels should be found with increasing pH as protein repelled each other with negative charges. For gels in this study, electrostatic interactions were clearly less important in determining final gel properties. Support for this comes from the same study (Zirbel and Kinsella, 1988). They noted that gels became less cloudy as pH increased to 8.0. They attributed this to an increase in disulfide bonds above pH 7.0 which make the gel more translucent and elastic.

Gels prepared at pH 5.0 at any NaTPP and at pH 6.0 without NaTPP were too weak to measure. Gel deformability at pH 6.0 significantly increased from 1.62 to 1.90 as NaTPP was increased from 10 to 50 mM. Further increase of NaTPP to 100 mM resulted in no significant change in deformability. At pH 7.0 all NaTPP concentrations produced measurable gels. Deformability increased from 1.46 to 1.77 upon addition of 10 mM NaTPP. Elastic gels were formed at

pH 8.0, however, no significant differences were observed due to NaTPP concentration.

As pH increased, the ability of NaTPP to alter gel deformability decreased until pH 8.0 where addition of NaTPP to 100 mM resulted in no significant change in strain at failure from the control gels. This is likely the result of an increase in disulfide bonds which tend to dominate gel structures ≥ pH 8.0 and are not affected by [Ca^{2+}].

As previously mentioned, the increase in deformability with increasing pH is likely due to an increase in disulfide bonds which are more resistent to breakage than ionic bonds. This is most noticeable in the control gels and gels made with 10 mM NaTPP where initial [Ca^{2+}] are significantly different at different pH values. At NaTPP concentrations of 50 and 100 mM, increases in pH resulted in no significant change in gel deformability, probably because [Ca^{2+}] were nearly equal thus not contributing differently to gel network stabilization.

These results are in partial agreement with those of Beuschel (1990) who observed a decrease in gel deformability with an increase in pH from 6.0 to 7.0 but an increase in deformability with an increase to pH 8.0. Morr and Foegeding (1990) observed similar results to Beuschel (1990) as five WPCs and all three WPIs experienced the same decrease at pH 7.0 and increase at pH 8.0. However, four of their WPCs displayed increases in deformability with increasing pH. They concluded that differences among WPC

preparations are due to variations in processing conditions used during manufacture.

The pH had significant effects on expressible moisture of WPC A gels. At pH 5.0, expressible moisture decreased significantly from 49.6% to 44.6% as the NaTPP concentration increased from 0 mM to 50 mM. There was no significant difference in expressible moisture at any NaTPP concentration for gels at pH 6.0. At pH 7.0 expressible moisture decreased from 30.4% at 0 mM NaTPP to a minimum of 11.4% at 10 mM NaTPP. Increasing NaTPP to 50 mM and 100 mM resulted in significant increases in expressible moisture to 36.7% and 43.4%, respectively. Expressible moisture of gels made at pH 8.0 increased as NaTPP increased from 10 mM to 100 mM.

A higher pH tended to decrease expressible moisture at the same NaTPP concentration. Expressible moisture decreased from 46.7% to 12.2% in the control gels when pH was increased from 6 to 8. Gels with 10 mM and 50 mM NaTPP exhibited a significant decrease in expressible moisture between pH 6.0 and 7.0. Expressible moisture of gels with 100 mM NaTPP did not increase when pH was increased between 5.0 and 8.0. These results suggest that [Ca^{2+}] plays a role in the binding of water to the gel since equal [Ca^{2+}] resulted in equal expressible moisture regardless of pH.

These results are different from those of Beuschel (1990) who found no significant increase in expressible moisture with an increase in pH from 6.0 to 8.0. The most

likely explanation for this variation was given by Morr and Foegeding who suggested that different processing conditions of WPC result in varying functional properties.

Similar [Ca^{2+}] were necessary to optimize gelling properties of WPC A at various pH values. Stress at failure were optimal within a [Ca^{2+}] range of 0.05 mM to 2.95 mM. Expressible moisture were optimal within a [Ca^{2+}] range of 0.84 mM to 4.29 mM. Results indicate that gelling properties of WPC A were optimized within a narrow range of [Ca^{2+}] at pH values between 5.0 and 8.0. These results further suggest that a certain [Ca^{2+}] is necessary to optimize gelling properties of WPC and also that optimal [Ca^{2+}] may be altered with variations in WPC manufacture.

4.3.10. EFFECT OF COOLING TEMPERATURE ON THE PROPERTIES OF WHEY PROTEIN CONCENTRATE GELS

Rheological and expressible moisture properties of WPC A gels (12% protein (w/w) no added buffer, pH 7.0) at four NaTPP concentrations (0, 10, 50 and 100 mM) and two cooling temperatures (0°C and 20°C) are shown in Table 21. Few differences were observed in gels cooled in water baths at 0°C and 20°C to 23°C. An exception to this occurred with gel hardness at a NaTPP concentration of 10 mM, where gels cooled in a 0°C waterbath were significantly harder than those cooled in a 20°C waterbath.

From an experimental standpoint, gels cooled at these two temperatures were different. Gels cooled in the 0°C water bath were more difficult to work with. Approximately

Table 21. The effect of cooling temperature on properties of whey protein concentrate A gels (12% protein (w/w)) made without buffer, pH 7.0, and 0-100 mM sodium tripolyphosphate (NaTPP) heated at 90°C for 15 min

NaTPP (mM)	Stress at failure (kPa)	Strain at failure (dimensionless)	Expressible moisture[a] (%)
cooled in 0°C water bath to internal temperature of 23°C			
0	12.3[d]	2.09[b]	44.7[bc]
10	26.3[b]	1.68[def]	20.0[d]
50	12.8[d]	1.68[def]	38.1[c]
100	13.4[d]	2.00[bc]	43.8[bc]
cooled in 20°C water bath to internal temperature of 23°C			
0	11.8[d]	1.92[bcd]	50.2[b]
10	22.1[c]	1.48[f]	20.3[d]
50	13.6[d]	1.65[ef]	38.8[c]
100	11.8[d]	1.79[cde]	43.8[bc]

[a] Expressible moisture determined as described by Beuschel (1990).

[b-f] Means within the same column followed by the same letter do not differ significantly ($P < 0.05$).

50% of all samples were lost due to cracks in the center of the gel. This occurred due to more rapid cooling (≈ 90 sec from 90°C to 23°C) that changed the rate at which intermolecular bonding could take place. As a result, fewer gels were available for analysis, standard deviations were high, and statistical significance may have been affected. Conversely, gels cooled in a 20°C waterbath experienced a longer cooling period (≈ 405 sec from 90°C to 23°C) which resulted in more uniform gels with few cracks and less sample loss (about 10%).

4.3.11. EFFECT OF BUFFER ON THE PROPERTIES OF WHEY PROTEIN CONCENTRATE GELS

Rheological and expressible moisture properties of WPC A gels (12% protein (w/w)) prepared with and without 0.1M MOPS buffer (pH 7.0) at four concentrations of NaTPP (0, 10, 50 and 100 mM) are shown in Table 22. Addition of 0.1M MOPS buffer (an amine buffer) to maintain pH affected several gelling properties as compared to DD-H_2O.

Control gels in 0.1 M MOPS buffer had a significantly higher [Ca^{2+}] (17.45 mM) than those made without buffer which (13.67 mM). This is likely due to a binding reaction between MOPS buffer with WPC which resulted in Ca^{2+} having fewer sites at which to bind and effectively increased the [Ca^{2+}]. The [Ca^{2+}] difference between MOPS buffer treatments became insignificant as NaTPP increased from 10 mM to 100 mM.

Hardness of WPC A gels decreased significantly from

Table 22. The effect of additional buffer (0.1 M MOPS) on properties of whey protein concentrate A gels (12% protein (w/w)) made with and without MOPS buffer, pH 7.0, heated at 90°C for 15 min and cooled in a 20°C water bath to an internal temperature of 23°C

NaTPP (mM)	$[Ca^{2+}]$ (mM)	Stress at Failure (kPa)	Strain at Failure (dimensionless)	Expressible Moisture[a] (%)
		WITH 0.1 M MOPS BUFFER		
0	17.45	11.8^{d}	1.92^{b}	50.2^{b}
10	2.87	22.1^{c}	1.48^{cd}	20.3^{f}
50	0.19	13.6^{d}	1.65^{bcd}	38.8^{d}
100	0.05	11.8^{d}	1.79^{b}	43.8^{c}
		WITHOUT MOPS BUFFER		
0	13.67	10.5^{d}	1.46^{d}	30.4^{e}
10	2.95	26.7^{b}	1.77^{bc}	11.4^{g}
50	0.21	20.4^{c}	1.72^{bcd}	36.7^{d}
100	0.06	13.4^{d}	1.89^{b}	43.4^{c}

[a] Expressible moisture determined as described by Kocher and Foegeding (1992) except centrifuged at 365 x g.

[b-g] means within the same column followed by the same letter do not differ significantly ($P < 0.05$).

26.7 kPa to 13.6 kPa in 0.1 M MOPS buffer in the presence of 50 mM NaTPP concentration. Deformability of WPC A gels was similar in buffer and DD-H_2O. The only significant effect on strain at failure was observed at 0 mM NaTPP where gels in DD-H_2O were significantly less deformable (1.46) than gels in 0.1 M MOPS buffer (1.92). Gels made with 0.1 M MOPS buffer expressed 50.2% moisture while gels made in DD-H_2O expressed 30.4% moisture at 0 mM NaTPP concentration. The same results were observed at 10 mM NaTPP as gels made with 0.1 M MOPS buffer expressed 20.3% moisture while gels made with DD-H_2O expressed 11.4% moisture. Upon further addition of NaTPP to 50 and 100 mM, both gel systems increased to nearly equivalent expressible moisture levels. Changes in rheological properties were probably due to interactions between the WPC and the buffer which affected the gelling ability of the protein.

4.4. GELATION OF EXPERIMENTAL PARTIALLY INSOLUBILIZED WHEY PROTEIN CONCENTRATES

4.4.1. EFFECT OF SODIUM TRIPOLYPHOSPHATE ON THE CALCIUM ION CONCENTRATION OF WHEY PROTEIN CONCENTRATE SOLS

Calcium ion concentrations for experimental partially insolubilized WPC sols (27, 47, 80 and 98% solubilities) at four NaTPP concentrations (0, 10, 50 and 100 mM) did not differ significantly between protein solubilities (Table 23). As expected, [Ca^{2+}] decreased significantly with increasing concentration of NaTPP from an average of 12.32 mM at 0 mM NaTPP to an average of 0.13 mM at 100 mM NaTPP.

Table 23. Change in ionic calcium concentration ([Ca^{2+}] in mM) of whey protein concentrate (WPC) sols (18% protein concentration (w/w)) in 0.1M MOPS buffer, pH 7.0, and 0-100 mM sodium tripolyphosphate (NaTPP)

NaTPP (mM)	Protein solubility			
	27%	47%	80%	98%
0	12.77[a]	10.93[a]	12.34[a]	13.24[a]
10	5.35[b]	5.42[b]	6.15[b]	5.39[b]
50	0.61[c]	0.76[c]	0.58[c]	0.17[c]
100	0.26[d]	0.18[d]	0.06[d]	0.01[d]

[a-d] Means within the same column and row followed by the same letter do not differ significantly ($P < 0.05$).

Results show that [Ca^{2+}] decreased at about the same rate regardless of protein solubility.

4.4.2. EFFECT OF SODIUM TRIPOLYPHOSPHATE ON THE STRESS AT FAILURE OF WHEY PROTEIN CONCENTRATE GELS

Stress at failure for experimental partially insolubilized WPC gels (18% protein concentration (w/w) in 0.1M MOPS buffer, pH 7.0) at four NaTPP concentrations (0, 10, 50, and 100 mM) are given in Table 24. Stress of 27% soluble WPC gels increased about 25% from 14.1 kPa to 17.3 kPa as NaTPP was increased from 10 mM to 50 mM. Significant increases were also observed for gels made from 80% and 98% soluble WPCs at 10 mM NaTPP. Gel strength increased from 14.2 kPa to 19.5 kPa and 25.8 kPa to 36.3 kPa, respectively.

As discussed earlier, gel hardness was maximized within [Ca^{2+}] of 1.86 mM to 5.39 mM. At higher WPC solubilities of 80 and 98%, gel strength was optimal within or near this range with [Ca^{2+}] of 6.15 and 5.39 mM and stress at failure of 19.5 kPa and 36.3 kPa, respectively. At lower solubilities of 27% and 47%, gel strength was higher below the range at [Ca^{2+}] of 0.61 and 0.18 mM, respectively. Stress at failure was highest at 10 mM NaTPP in WPC of 98% solubility.

These results are consistent with those of Beuschel et al. (1992) who studied the same WPC gels prepared at 90.0°C in 0.1 M NaCl, 0.1 M Na phosphate buffer, pH 7.0. They too found that gel stress increased significantly from 19.3 kPa to 73.7 kPa as WPC solubility increased from 47% to 98%.

Table 24. Stress at failure (kPa) for whey protein concentrate (WPC) gels (18% protein concentration (w/w)) in 0.1M MOPS buffer, pH 7.0, and 0-100 mM sodium tripolyphosphate (NaTPP) heated at 90°C for 15 min and cooled in a 23°C water bath to an internal temperature of 20°C

	Protein solubility			
NaTPP (mM)	27%	47%	80%	98%
0	13.6[b]	2.75[b]	14.2[b]	25.8[b]
10	14.1[b]	3.24[ab]	19.5[a]	36.3[a]
50	17.3[a]	2.82[b]	14.2[b]	17.6[d]
100	15.4[ab]	4.11[a]	16.3[ab]	20.8[c]

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

4.4.3. EFFECT OF SODIUM TRIPOLYPHOSPHATE ON THE STRAIN AT FAILURE OF WHEY PROTEIN CONCENTRATE GELS

Strain at failure for experimental partially insolubilized WPC gels (18% protein concentration (w/w) in 0.1M MOPS buffer, pH 7.0) at four NaTPP concentrations (0, 10, 50, and 100 mM) was significantly affected by WPC solubility (Table 25). At constant NaTPP concentration, gel deformability decreased with decreasing solubility, from an average among all NaTPP concentrations of 1.46 in the 98% soluble WPC to an average of 0.72 in the 27% soluble WPC. These results are consistent with those of Beuschel et al. (1992a) who determined that gel deformability decreased significantly as WPC solubility decreased from 98% to 27%.

With the exception of the 98% soluble WPC gels, no significant change in strain was observed with increasing concentration of NaTPP within a particular WPC solubility. Strain of 98% soluble WPC gels significantly decreased to a minimum of 1.23 upon addition of 50 mM NaTPP. Results indicate that [Ca^{2+}] was less critical to the deformability of WPC gels as the solubility of the protein decreased.

4.4.4. EFFECT OF SODIUM TRIPOLYPHOSPHATE ON THE EXPRESSIBLE MOISTURE OF WHEY PROTEIN CONCENTRATE GELS

Expressible moisture for experimental partially insolubilized WPC gels (18% protein concentration (w/w) in 0.1M MOPS buffer, pH 7.0) at four NaTPP concentrations (0, 10, 50, and 100 mM) are given in Table 26. Unlike the other gelling properties, little difference was observed in

Table 25. Strain at failure (dimensionless) for whey protein concentrate (WPC) gels (18% protein concentration (w/w)) in 0.1M MOPS buffer, pH 7.0, and 0-100 mM sodium tripolyphosphate (NaTPP) heated at 90°C for 15 min and cooled in a 20°C water bath to an internal temperature of 23°C

	Protein solubility			
NaTPP (mM)	27%	47%	80%	98%
0	0.68[a]	0.71[ab]	1.17[a]	1.57[a]
10	0.74[a]	0.89[a]	1.23[a]	1.56[a]
50	0.75[a]	0.80[ab]	1.13[a]	1.23[b]
100	0.72[a]	0.67[b]	1.17[a]	1.47[a]

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

Table 26. Expressible moisture[a] (%) for whey protein concentrate (WPC) gels (18% protein concentration (w/w)) in 0.1M MOPS buffer, pH 7.0, and 0-100 mM sodium tripolyphosphate (NaTPP) heated at 90°C for 15 min and cooled in a 20°C water bath to an internal temperature of 23°C

	Protein solubility			
NaTPP (mM)	27%	47%	80%	98%
0	27.0[b]	33.7[b]	31.1[b]	33.2[b]
10	27.2[b]	32.8[b]	28.5[bc]	29.3[c]
50	25.2[b]	30.8[b]	26.7[c]	29.8[c]
100	25.7[b]	32.1[b]	29.4[bc]	32.8[b]

[a] Expressible moisture determined as described by Beuschel (1990).

[b-c] Means within the same column followed by the same letter do not differ significantly ($P < 0.05$).

expressible moisture among any WPC solubilities or NaTPP treatments.

The 80% soluble WPC gels displayed a significant decrease in expressible moisture from 31.1% in control gels to 26.7% with addition of 50 mM NaTPP. Gels made from 98% soluble WPC exhibited a significant decrease in expressible moisture from 33.2% in control gels to 29.3% and 29.8% with addition of 10 mM and 50 mM NaTPP, respectively. Results show that expressible moisture was more dependent on [Ca^{2+}] at higher protein solubilities.

With the exception of 98% soluble WPC gels, addition of 0 to 100 mM NaTPP resulted in few changes to the gelling properties of the experimental partially insolubilized WPCs. This is in contrast to the commercial WPCs tested which exhibited significant changes in all gelling properties tested as NaTPP was increased from 0 to 100 mM. These results are best explained by data found by Hung (1992) who studied the microstructure of the experimental WPCs using Scanning Electron Microscopy (SEM). It was found that under the severe heating conditions used to make the lower solubility WPCs, polymerization of protein molecules occurred through the formation of non-reducible covalent bonds. Thus, already being "heat-set", these large denatured protein polymers were not influenced by the comparatively weak ionic interactions experienced upon the addition of NaTPP.

4.5. SALT-SOLUBLE PROTEIN AND WHEY PROTEIN CONCENTRATE COMBINATION GELS

4.5.1. IONIC CALCIUM AND IONIC SODIUM CONCENTRATIONS AS A FUNCTION OF PROTEIN CONCENTRATION AND SODIUM TRIPOLYPHOSPHATE CONCENTRATION

Ionic calcium and ionic sodium concentrations of chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) A combination sols (various protein concentrations) at four NaTPP concentrations (0, 0.25, and 0.5 %) are shown in Table 27. Sodium Tripolyphosphate was added in percent concentrations because this is standard convention in the meat industry. For comparison purposes, 0.25 and 0.5 % NaTPP equal about 6.7 and 13.4 mM of NaTPP, respectively. As expected, an inverse relationship existed between NaTPP concentration and [Ca^{2+}] where an increase in NaTPP resulted in a decrease in [Ca^{2+}].

Free calcium concentration increased as WPC concentration increased. Sols containing no NaTPP increased from 0.17 mM [Ca^{2+}] in 4% SSP and 0% WPC sols to 4.50 mM [Ca^{2+}] in 4% SSP and 4% WPC sols. Further increase of WPC in 4% SSP and 16% WPC sols resulted in [Ca^{2+}] of 32.00 mM.

Chicken salt-soluble protein contributed little to total [Ca^{2+}] (0.17 mM at 0 NaTPP concentration). Conversely, [Ca^{2+}] was significantly higher in 12% WPC sols (26.70 mM) than in 4% SSP and 12% WPC combination sols (17.97 mM). This is probably due to binding of [Ca^{2+}] by the chicken SSP. Therefore, although chicken SSP did not contribute additional calcium to the gel system, its

Table 27. Ionic mineral composition of chicken salt-soluble protein (SSP):whey protein concentrate (WPC) A combination sols (various protein concentrations in 0.3M NaCl, pH 6.5) with 0-0.5% sodium tripolyphosphate (NaTPP)

Ratio %SSP:%WPC	NaTPP treatment (%)	Mineral (mM)	
		Ca^{2+}	Na^{+}
4:0	0	0.17	402.7
	0.25	0.034	420.2
	0.50	0.022	440.7
4:4	0	4.50	430.3
	0.25	2.36	450.4
	0.50	0.76	474.6
4:8	0	14.33	442.7
	0.25	1.82	463.3
	0.50	0.87	483.0
4:12	0	17.97	452.1
	0.25	2.03	471.2
	0.50	1.39	490.0
0:12	0	26.70	386.3
	0.25	7.12	414.9
	0.50	3.54	447.5
0:16	0	32.00	438.2
	0.25	16.70	445.7
	0.50	7.69	478.7

presence reduced the concentration of free calcium.

Calcium ion concentrations for 12% WPC sols were significantly higher (26.70 mM) than [Ca^{2+}] determined earlier for 12% WPC sols (17.45 mM, Table 12) without NaTPP. This occurred because gels in the latter study contained 0.3 M NaCl which competed with [Ca^{2+}] binding sites on the protein and effectively raised the overall [Ca^{2+}].

Sodium ion concentration ([Na^{+}]) had a direct relationship to NaTPP addition. This was observed at every protein concentration where addition of NaTPP resulted in an increase of [Na^{+}]. This was a result of the dissociation of the NaTPP molecule and release of Na^{+} into the solution.

Sodium ion concentration in sols without NaTPP increased from 402.7 mM in 4% SSP sols to 452.1 mM in 12% WPC sols. This was caused by sodium in the WPC. The WPC sols at 0 mM NaTPP contained a [Na^{+}] of 386.3 mM. The only source of sodium is that from added NaCl (300 mM), which means that the additional 86.3 mM of Na^{+} must come from WPC. Thus, an increase of 30 mM Na^{+} was expected with the addition of 4% added WPC.

4.5.2. STRESS AT FAILURE AS A FUNCTION OF PROTEIN CONCENTRATION, COOKING TEMPERATURE, AND SODIUM TRIPOLYPHOSPHATE CONCENTRATION

Stress at failure for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) A combination gels (various protein concentrations in 0.3M NaCl, pH 6.5) at three NaTPP concentrations (0, 0.25 and 0.5 %) are found in

Table 28. At 65°C, hardness of 4% SSP gels did not differ at any NaTPP concentration. Hardness increased (44%) from 5.7 kPa with no added NaTPP to 8.2 kPa at 0.25 % NaTPP in combination gels containing 4% SSP and 4% WPC. This trend continued with 4% SSP and 8% WPC and 4% SSP and 12% WPC combination gels as both increased in hardness (350%) upon addition of 0.25 % NaTPP. There was no difference in hardness at any protein combination or concentration in gels prepared with 0.25 or 0.5% NaTPP. Hardness increased from 4.5 kPa in 4% SSP gels to 12.3 kPa in 4% SSP:8% WPC gels containing 0.25 % NaTPP. Hardness also increased from 4.1 kPa in 4% SSP gels to 13.0 kPa in 4% SSP and 12% WPC combination gels containing 0.5 % NaTPP. At 0 % NaTPP, gels significantly decreased in hardness from 4.4 kPa in 4% SSP gels to 2.9 kPa in 4% SSP and 0% WPC combination gels. This is in agreement with Beuschel (1992b) who found that combination chicken SSP and WPC gels tended to weaken as WPC concentration was increased. There was no gel formation at any concentration or any NaTPP treatment for WPC A control gels (12% and 16% WPC) cooked at 65°C. This is consistent with de Wit et al. (1988) who found that gelation of WPC required temperatures between 67-79°C.

Similar results occurred for combination gels produced at 90°C. Hardness of SSP control gels and 4% SSP:4% WPC combination gels did not change at any concentration of NaTPP. Hardness increased from 12.2 kPa at 0 % NaTPP to 34.9 kPa at 0.25 % NaTPP in 4% SSP and 8% WPC combination

Table 28. Stress at failure (kPa) for chicken salt-soluble protein (SSP):whey protein concentrate (WPC) A combination gels (various protein concentrations in 0.3M NaCl, pH 6.5) with 0-0.5 % sodium tripolyphosphate (NaTPP)

Ratio %SSP:%WPC	NaTPP treatment (%)	Stress at failure (kPa) Temperature 65°C	90°C
4:0	0	4.4^{de}	3.1^{h}
	0.25	4.5^{de}	3.4^{gh}
	0.50	4.1^{ef}	3.5^{gh}
4:4	0	5.7^{d}	8.9^{fgh}
	0.25	8.2^{c}	11.7^{ef}
	0.50	7.3^{c}	11.5^{efg}
4:8	0	2.7^{f}	12.2^{ef}
	0.25	12.3^{ab}	34.9^{b}
	0.50	11.1^{b}	35.0^{b}
4:12	0	2.9^{f}	29.1^{bc}
	0.25	13.2^{a}	66.4^{a}
	0.50	13.0^{a}	62.3^{a}
0:12	0	no gel	6.0^{fgh}
	0.25	no gel	5.5^{fgh}
	0.50	no gel	6.4^{fgh}
0:16	0	no gel	23.3^{cd}
	0.25	no gel	20.9^{d}
	0.50	no gel	19.5^{de}

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

gels. Behavior was similar with 4% SSP and 12% WPC combination gels as hardness increased from 29.1 kPa at 0 % NaTPP to 66.4 kPa at 0.25 % NaTPP.

Whey protein concentrate control gels containing 12% and 16% WPC protein behaved the same as the 4% SSP gels as no change in hardness was observed at any concentration of NaTPP. This is inconsistent with results observed earlier with 12% WPC gels where addition of NaTPP resulted in significant increases in gel hardness (Table 13). This most likely occurred due to the addition of 300 mM NaCl in the SSP and WPC combination gel systems. At this relatively high concentration, NaCl probably dominated the gelling system and reduced the cationic effect of Ca^{2+} on gel hardness.

Gels containing 4% SSP and gels containing 12% WPC had added hardness values of 9.1, 8.9, and 9.9 kPa for 0, 0.25 and 0.5 % NaTPP, respectively. Conversely, 4% SSP and 12% combination gels were harder with stress of 29.1, 66.4, and 62.3 kPa for 0, 0.25 and 0.5 % NaTPP, respectively. Even the 16% WPC gels were significantly weaker than the combination gels with hardness values of 23.3, 20.9 and 19.5 kPa. A probable cause for this was described by Oakenfull (1987) as a phase separated gel network. In this system, the SSP would gel at about 65°C. As the temperature increased, WPC would gel in the interstitial spaces and add to the strength of the system.

These results are consistent with Beuschel et al.

(1992b) who found that 4% SSP and 12% WPC were much harder in combination gels than gels prepared with individual proteins.

4.5.3. STRAIN AT FAILURE AS A FUNCTION OF PROTEIN CONCENTRATION, COOKING TEMPERATURE AND SODIUM TRIPOLYPHOSPHATE CONCENTRATION

Strain at failure for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) A combination gels (various protein concentrations in 0.3M NaCl, pH 6.5) at three NaTPP concentrations (0, 0.25 and 0.5 %) is less consistent than stress at failure (Table 29). With the exception of the 12% and 16% WPC gels, gel deformability of all combination gels and 4% SSP gels prepared at 65°C increased as NaTPP concentration increased from 0 to 0.25 %. With further increase of NaTPP to 0.5 %, the strain of 4% SSP gels and 4% SSP:12% WPC combination gels decreased to 2.50 and 1.84, respectively.

Gel deformability of SSP and WPC combination gels decreased as WPC increased from 4% to 12% and NaTPP concentration remained constant. This is in agreement with Beuschel (1992b) who found that as WPC increased from 4% to 12% in SSP:WPC combination gels, elasticity tended to decrease.

Similar results were observed for gels prepared at 90°C as strain at failure decreased when WPC was added to SSP gels. The only exception was between the 4:8 and 4:12 gels at 0 % NaTPP where strain did not change (0.87 to 0.83).

Table 29. Strain at failure of chicken salt-soluble protein (SSP):whey protein concentrate (WPC) A combination gels (various protein concentrations in 0.3 M NaCl, pH 6.5) with 0-0.5% sodium tripolyphosphate (NaTPP)

Ratio %SSP:%WPC	NaTPP treatment (%)	Strain at failure (dimensionless)	
		Temperature	
		65°C	90°C
4:0	0	2.09^{d}	2.26^{b}
	0.25	2.67^{a}	2.56^{a}
	0.50	2.50^{b}	2.48^{a}
4:4	0	2.34^{c}	1.44^{d}
	0.25	2.50^{b}	1.84^{c}
	0.50	2.48^{b}	1.86^{c}
4:8	0	1.71^{f}	0.87^{k}
	0.25	2.25^{c}	1.22^{gh}
	0.50	2.29^{c}	1.18^{hi}
4:12	0	1.58^{g}	0.83^{k}
	0.25	2.00^{d}	1.05^{j}
	0.50	1.84^{e}	1.00^{j}
0:12	0	no gel	1.42^{de}
	0.25	no gel	1.34^{def}
	0.50	no gel	1.36^{de}
0:16	0	no gel	1.32^{efg}
	0.25	no gel	1.24^{fgh}
	0.50	no gel	1.10^{ij}

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

Like gels prepared at 65°C, deformability of all combination gels and 4% SSP gels increased as NaTPP concentration increased from 0 to 0.25 %. Further increase to 0.5 % did not change deformability at any protein concentration.

Changes in strain with addition of NaTPP were less evident for WPC A control gels of 12% and 16% protein prepared at 90°C. The only decrease in deformability was observed in the 16% WPC, 0.5 mM NaTPP gels. These results are inconsistent with those found earlier in the study of 12% WPC gels where addition of NaTPP resulted in a decrease in gel deformability. Like stress at failure, this is due to the addition of 300 mM NaCl which probably altered the gelling system and reduced the cationic affect of Ca^{2+} on gel deformability.

Strain at failure for 4% SSP gels and 12% WPC gels were significantly different than strain at failure for combination gels (4% SSP:12% WPC). At 90°C, 4% SSP gels displayed a strain of 2.26 at 0 % NaTPP. A lower strain of 1.42 was exhibited by 12% WPC gels at the same NaTPP concentration. In combination, 4% SSP:12% WPC gels displayed a strain of 0.83 at 0 % NaTPP. These results are consistent with Beuschel et al. (1992b) who found 4% SSP:12% WPC combination gels to have significantly lower strain than 12% WPC gels. The 4% SSP gels cannot be compared, however, because Beuschel et al. (1992b) found the gels too weak to measure.

Hamann (1983) stated that elasticity of protein gel

systems is directly related to the number of crosslinks. Based on strain results for control gels, SSP had more crosslinks and higher deformability. Therefore, as WPC concentration increased, it interfered with these crosslinks and produced a greater than additive decrease in gel elasticity. Likewise, as temperature increased, WPC probably formed a network of its own and further disrupted the SSP crosslinks. Sodium tripolyphosphate likely had the duel effect of Ca^{2+} sequestration from WPC and SSP solubilization.

4.5.4. EXPRESSIBLE MOISTURE AS A FUNCTION OF PROTEIN CONCENTRATION, COOKING TEMPERATURE, AND SODIUM TRIPOLYPHOSPHATE CONCENTRATION

Expressible moisture for chicken salt-soluble protein (SSP) and whey protein concentrate (WPC) A combination gels (various protein concentrations in 0.3M NaCl, pH 6.5) at three NaTPP concentrations (0, 0.25 and 0.5 %) is shown in Table 30. At 65°C, expressible moisture of decreased from 66.5% in 4% SSP gels to 36.3% in 4% SSP and 12% WPC combination gels containing 0.5 % NaTPP. Within each type of combination gel and the 4% SSP gels, expressible moisture decreased as NaTPP concentration increased from 0 to 0.25 %. The expressible moisture of gels prepared in 0.25 % and 0.5 % NaTPP were not different. Once again, no gels were formed by 12% and 16% WPC A systems at 65°C.

At each NaTPP concentration, gels decreased in expressible moisture from 4% SSP gel to 4% SSP and 12% WPC

Table 30. Expressible moisture[a] (%) of chicken salt-soluble protein (SSP):whey protein concentrate (WPC) A combination gels (various protein concentrations in 0.3 M NaCl, pH 6.5) with 0-0.5% sodium tripolyphosphate (NaTPP)

Ratio %SSP:%WPC	NaTPP treatment (%)	Expressible moisture (%)	
		Temperature	
		65°C	90°C
4:0	0	66.5^{b}	68.7^{b}
	0.25	59.1^{c}	53.1^{c}
	0.50	58.6^{c}	49.5^{c}
4:4	0	58.3^{c}	53.7^{c}
	0.25	48.5^{e}	44.2^{d}
	0.50	49.9^{de}	44.8^{d}
4:8	0	57.1^{c}	44.6^{d}
	0.25	38.5^{fg}	30.6^{e}
	0.50	40.0^{f}	31.8^{e}
4:12	0	51.1^{d}	31.8^{e}
	0.25	37.2^{gh}	23.4^{f}
	0.50	36.3^{h}	22.6^{f}
0:12	0	no gel	49.1^{cd}
	0.25	no gel	46.7^{cd}
	0.50	no gel	47.2^{cd}
0:16	0	no gel	36.3^{e}
	0.25	no gel	36.0^{e}
	0.50	no gel	35.9^{e}

[a] Expressible moisture determined as described by Kocher (1992) except centrifuged at 365 x g.

[b-h] Means within the same column followed by the same letter do not differ significantly ($P < 0.05$).

combination gels. Within each type of combination gel and the 4% SSP gels, expressible moisture decreased as NaTPP concentration increased from 0 to 0.25 %. Expressible moisture of gels prepared in 0.25 % and 0.5 % NaTPP were not different.

There was no change in expressible moisture for 12% and 16% WPC A gels at any NaTPP concentration, however, expressible moisture decreased as protein concentration of WPC A increased from 12 to 16 %. This is inconsistent with results found earlier in the study of 12% WPC gels where addition of NaTPP resulted in a significant decrease in expressible moisture. Like stress and strain at failure, this variation is the result of the presence of 300 mM NaCl which altered the effect of Ca^{2+}.

Expressible moisture for 4% SSP and 12% WPC A control gels were different than the same proteins in combination gels (4% SSP:12% WPC). At 90°C, 4% SSP gels displayed expressible moisture of 68.7 % at 0 % NaTPP. Lower expressible moisture of 49.1 % was exhibited by 12% WPC at the same NaTPP concentration. In combination, 4% SSP:12% WPC gels displayed a lower expressible moisture of 31.8 %.

Beuschel (1992b) observed similar findings in expressible moisture with changes in WPC concentration. He suggested that WPC was acting as a filler by binding water in the interstitial spaces of the SSP network. Data from this experiment support this idea as increases in WPC and temperature reduced expressible moisture values.

4.6. MODEL SYSTEM TURKEY FRANKFURTERS

4.6.1. EFFECT OF SODIUM TRIPOLYPHOSPHATE CONCENTRATION, COOKING TEMPERATURE, AND WHEY PROTEIN CONCENTRATE ON FRANKFURTER YIELD

Addition of NaTPP changed the cooked yield of model system turkey frankfurters prepared with and without 7.0% WPC A (Table 31). In frankfurters prepared without WPC A, cooked yield increased at both processing temperatures as NaTPP concentration increased from 0 to 0.25 %. There was no difference in cooked yield between 0.25 % and 0.5 % NaTPP for frankfurters processed at 71.1°C. For frankfurters processed at 90.0°C, however, cooked yield decreased from 94.6 % with 0.25 % NaTPP to 93.3% at 0.5 % NaTPP. The cooked yield of frankfurters containing 7% WPC A processed at 71.1°C did not change as NaTPP was increased from 0 to 0.5 %. Cooked yield of frankfurters processed at 90.0°C increased from 93.5 % to 95.3 % with addition of 0 % and 0.5 % NaTPP respectively.

Barbut et al. (1988) observed similar results in their study of reduced NaCl turkey frankfurters (2.5% to 1.5%) with NaTPP replacement (0.4%). Their study revealed that cooked yield reductions caused by decreasing NaCl from 2.0 to 1.5%, could be eliminated by addition of 0.4% NaTPP at the 1.5% NaCl level. These results are also consistent with those of Trout and Schmidt (1984) who found that NaTPP treated (0.125 to 5.0%) comminuted beef rolls did not differ in cooked yield when NaCl concentration varied between 1.0 and 2.0%. They postulated that at ionic strength values

Table 31. Yield of model system turkey frankfurters prepared with and without 7.0% whey protein concentrate (WPC) A and added sodium tripolyphosphate (NaTPP)

Processing Temperature (°C)	NaTPP (mM)	Cooked Yield (%)	Reheat Yield (%)
		WITHOUT WPC A	
71.1°C	0	95.8^{c}	84.2^{f}
	0.25	98.6^{a}	88.1^{e}
	0.50	98.3^{a}	89.0^{d}
90.0°C	0	85.7^{f}	90.5^{c}
	0.25	94.6^{d}	92.3^{b}
	0.50	93.3^{e}	92.6^{ab}
		WITH 7% WPC A	
71.1°C	0	96.9^{b}	89.2^{d}
	0.25	97.4^{b}	90.3^{c}
	0.50	96.9^{b}	90.3^{c}
90.0°C	0	93.5^{e}	92.2^{b}
	0.25	94.6^{d}	92.7^{ab}
	0.50	95.3^{c}	93.1^{a}

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

above 0.1 (easily reached at NaCl concentrations ≥ 0.6 %) solubilization of SSP and binding of water were nearly constant. Therefore, at NaCl concentrations above 1.0%, differences observed in cooked yield with added NaTPP are caused by NaTPP and not by a reduction in NaCl.

Reheat yield of frankfurters processed without WPC at both 71.1°C and 90°C increased with addition of 0.25% NaTPP as compared to the control. Addition of NaTPP from 0.25% to 0.5% caused the reheat yield of 71.1°C processed frankfurters to further increase while those processed to 90.0°C did not change. Frankfurters made with 7% WPC A exhibited similar reheat yield characteristics. At 71.1°C, reheat yield of frankfurters increased as NaTPP increased from 0 to 0.25 %. No differences in reheat yield were observed at 0.25 % and 0.5 % NaTPP. At 90.0°C, the only change in reheat yield was an increase from 92.2 % to 93.1 % as NaTPP was increased from 0 to 0.5 %.

NaTPP tends to increase the solubilization of SSP by increasing pH, ionic strength, and chelating metal ions (Molins, 1991). As the solubilization of the proteins increases, their ability to bind water also increases. Therefore, the increases observed for cooked yield and reheat yield in this study are consistent with those in the literature.

In frankfurters without WPC A, increasing cooking temperature from 71.1°C to 90.0°C resulted in a decrease in cooked yield at every NaTPP concentration. Decreases were

greatest without NaTPP where cooked yield decreased from 95.8 % to 85.7 %. Addition of WPC A to the frankfurter formulation produced similar results as cooked yield decreased with increasing temperature at each NaTPP concentration. It is well known in the meat industry that increases in cooking temperature result in decreases in cooked yields. This is a result of moisture being driven off by heat due to its thermodynamic shift into a vapor.

Reheat yield for frankfurters processed to 71.1°C and 90.0°C exhibited opposite effects with regards to cooked yield. In all frankfurter formulations, both with and without WPC A, an increase in processing temperature from 71.1°C to 90.0°C resulted in an increase in reheat yield. This is a direct result of the moisture driven off when the frankfurters were initially cooked. Because more moisture was driven off initially by the higher cooking temperature of 90.0°C, there was less to lose when the frankfurter was cooked a second time in the determination of reheat yield.

Addition of WPC to the turkey frankfurters affected the cooked yield of each system. The cooked yield of frankfurters processed at 71.1°C without NaTPP increased when 7% WPC A was added. An inverse relationship was observed when the NaTPP concentration increased to 0.25 % as cooked yield decreased from 98.6 to 97.4 % when 7% WPC A was added. This relationship was also exhibited in 0.5 % NaTPP frankfurters as cooked yield decreased from 98.3% to 96.9%.

Cooked yield increased over frankfurters processed to

71.1°C at the 0 and 0.5% NaTPP concentrations but remained unchanged for the 0.25 NaTPP treatment (94.6% to 94.6%).

With the exception of the 90.0°C cook at 0.25 and 0.5 % NaTPP concentrations, reheat yield significantly increased as WPC A was added to the formulation. Beuschel (1990) observed similar results and suggested that added WPC may be binding more of the moisture and fat.

4.6.2. EFFECT OF SODIUM TRIPOLYPHOSPHATE CONCENTRATION AND WHEY PROTEIN CONCENTRATE ON FRANKFURTER BATTER PROXIMATE COMPOSITION

Proximate composition for model system turkey frankfurter batter prepared with and without 7.0% whey protein concentrate (WPC) A and three concentrations of NaTPP (0, 0.25 and 0.5 %) are listed in Table 32. Addition of NaTPP at any concentration did not change the proximate composition of frankfurter batters. Conversely, addition of NaTPP resulted in increases in pH for each batter formulation. For batters without WPC A, pH increased from 6.47 to 6.72 as NaTPP concentration was increased from 0 % to 0.5 %. For batters with WPC A, pH increased from 6.41 to 6.62 as NaTPP concentration was increased from 0 % to 0.5 %. Barbut et al. (1988) observed similar results in their study of reduced NaCl turkey frankfurters (2.5% to 1.5%) with NaTPP replacement (0.4%) as pH increased from 6.23 to 6.44 when NaCl:NaTPP concentrations were changed from 2.0%:0% to 1.5%:0.4%. This was expected due to the ability of NaTPP to increase pH (Molins, 1991).

Table 32. Proximate composition and pH of model system turkey frankfurter batter prepared with and without 7.0% whey protein concentrate (WPC) A and 0-0.5% sodium tripolyphosphate (NaTPP)

NaTPP (mM)	Protein (%)	Moisture (%)	Fat (%)	pH
		WITHOUT WPC A		
0	12.4[b]	69.9[a]	12.4[b]	6.47[d]
0.25	12.5[b]	69.1[a]	11.2[b]	6.62[b]
0.50	12.6[b]	67.9[a]	11.4[b]	6.72[a]
		WITH 7% WPC A		
0	14.6[a]	62.9[b]	13.5[a]	6.41[e]
0.25	15.0[a]	62.5[b]	12.8[a]	6.56[c]
0.50	14.5[a]	61.5[b]	12.7[a]	6.62[b]

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

Unlike NaTPP, WPC A produced significant changes in the proximate composition of each frankfurter batter. Protein composition for all three NaTPP concentrations increased when WPC A was added to the formulation. Each batter increased in fat upon WPC A addition at every NaTPP concentration. Whey protein concentrate A decreased moisture content of each frankfurter batter. The pH of each batter decreased as WPC A was added from 6.47 to 6.41 for 0% NaTPP, 6.62 to 6.56 for 0.25% NaTPP, and 6.72 to 6.62 for 0.5% NaTPP.

Whey protein concentrate was replaced on a weight basis, not on a protein basis. Therefore, protein and fat increased because their concentrations in WPC were greater on a weight basis than in the turkey meat on a weight basis. Moisture content decreased for the same reason (moisture of WPC was less than turkey meat on a weight basis). The pH tended to decrease because the pH of the whey proteins was between 5.5 and 6.0 and the meat system to which it was added was between 6.47 and 6.72.

4.6.3. EFFECT OF SODIUM TRIPOLYPHOSPHATE CONCENTRATION, COOKING TEMPERATURE, AND WHEY PROTEIN CONCENTRATE ON FRANKFURTER PROXIMATE COMPOSITION

Proximate composition for model system turkey frankfurters prepared with and without 7.0% whey protein concentrate (WPC) A and three concentrations of NaTPP (0, 0.25 and 0.5 %) are listed in Table 33.

Within each heating temperature, addition of NaTPP did

Table 33. Proximate composition and pH of model system turkey frankfurters prepared with and without 7.0% whey protein concentrate (WPC) A and 0-0.5% sodium tripolyphosphate (NaTPP)

Heat Treatment (°C)	NaTPP (mM)	Protein (%)	Moisture (%)	Fat (%)	pH
		WITHOUT WPC A			
71.1°C	0	12.9^{bc}	70.6^{ab}	12.2^{d}	6.53^{d}
	0.25	12.6^{c}	71.0^{a}	12.6^{d}	6.66^{b}
	0.50	13.1^{bc}	70.3^{ab}	11.3^{d}	6.74^{a}
90.0°C	0	13.7^{b}	69.1^{c}	14.2^{ab}	6.55^{d}
	0.25	13.2^{bc}	69.8^{bc}	13.1^{abc}	6.66^{b}
	0.50	13.3^{bc}	69.4^{c}	13.1^{abc}	6.75^{a}
		WITH 7% WPC A			
71.1°C	0	15.4^{a}	67.0^{d}	13.4^{abc}	6.55^{d}
	0.25	15.7^{a}	66.4^{de}	14.1^{ab}	6.63^{c}
	0.50	15.7^{a}	66.3^{de}	13.6^{abc}	6.66^{b}
90.0°C	0	15.9^{a}	66.2^{de}	13.5^{abc}	6.55^{d}
	0.25	15.8^{a}	65.4^{e}	14.5^{a}	6.61^{c}
	0.50	15.9^{a}	65.5^{e}	13.6^{abc}	6.67^{b}

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

not change proximate composition of the cooked frankfurters. Conversely, pH values increased 0.21 and 0.20 units for frankfurters without WPC A and 0.11 and 0.12 units for frankfurters with WPC A as NaTPP concentration was increased from 0 % to 0.5 %.

The proximate composition of frankfurters made without WPC A varied with cooking temperature. Protein concentration was unchanged as the cooking temperature was increased from 71.1°C to 90.0°C for all NaTPP concentrations. Moisture decreased as processing temperature increased from 71.1°C to 90.0°C for frankfurters with 0 and 0.25 % NaTPP. There was no difference in moisture between 0.25% and 0.5 % NaTPP. As discussed earlier, this is because more moisture (in the form of vapor) was driven off at higher temperatures. Fat concentration did not change with an increase in cooking temperature, with the exception of frankfurters prepared without NaTPP which increased significantly from 12.2 % to 14.2 %. The pH did not change with an increase in temperature.

There was no change in proximate composition or pH in frankfurters containing 7% WPC A due to cooking temperature or NaTPP concentration. This is probably due to the moisture content. Moisture is the only component likely to be affected by temperatures ($\leq$ 90°C) involved in frankfurter production. Since added WPC tended to decrease the amount of moisture lost during cooking, the other components did

not change either.

Addition of WPC A changed the proximate composition of frankfurters as compared to the control. Protein concentrations at each NaTPP concentration increased upon addition of WPC A. Conversely, moisture decreased upon addition of 7% WPC A at every NaTPP concentration. Addition of WPC A resulted in an increase in the concentration of fat in all frankfurters processed to 71.1°C, but had no effect on the concentration of fat in any frankfurters processed to 90.0°C. Frankfurter pH increased as NaTPP increased while temperature had no effect on final frankfurter pH.

4.6.4. EFFECT OF SODIUM TRIPOLYPHOSPHATE CONCENTRATION, COOKING TEMPERATURE AND WHEY PROTEIN CONCENTRATE ON FRANKFURTER TEXTURAL PROPERTIES

Textural properties for model system turkey frankfurters prepared with and without 7.0% whey protein concentrate (WPC) A and three concentrations of NaTPP (0, 0.25 and 0.5 %) are listed in Table 34. When no WPC was added to the formulation, hardness increased upon addition of 0.25% NaTPP as compared to the control at both processing temperatures. Addition of 0.5% NaTPP caused hardness of 71.1°C processed frankfurters to decrease while those cooked at 90.0°C remained unchanged as compared to frankfurters with 0.25% NaTPP. Effects of NaTPP on strain were similar with the exception that the strain of the 0.5% treated frankfurters cooked at 90.0°C decreased in comparison to those processed with 0.25 % NaTPP. Expressible moisture of

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Table 34. Properties of model system turkey frankfurters prepared with and without 7.0% whey protein concentrate (WPC) A and 0-0.5% sodium tripolyphosphate (NaTPP)

Processing Temperature (°C)	NaTPP (mM)	Stress at Failure (kPa)	Strain at Failure (dimensionless)	Expressible Moisture[a] (%)
		WITHOUT WPC A		
71.1°C	0	16.4^{hi}	1.43^{c}	28.5^{b}
	0.25	25.1^{b}	1.57^{b}	22.5^{f}
	0.50	22.1^{c}	1.45^{c}	23.9^{e}
90.0°C	0	17.0^{ghi}	1.28^{ef}	24.8^{d}
	0.25	19.5^{ef}	1.35^{d}	23.9^{e}
	0.50	18.6^{efg}	1.26^{f}	20.8^{g}
		WITH 7% WPC A		
71.1°C	0	15.1^{i}	1.32^{def}	26.8^{c}
	0.25	17.5^{fgh}	1.35^{d}	22.0^{f}
	0.50	15.3^{i}	1.33^{de}	20.2^{g}
90.0°C	0	20.2^{cde}	1.02^{g}	22.6^{f}
	0.25	21.1^{cd}	1.06^{g}	16.5^{i}
	0.50	19.8^{de}	1.08^{g}	17.7^{h}

[a] Expressible moisture determined as described by Kocher (1992) except centrifuged at 365 x g.

[b-i] Means within the same column followed by the same letter do not differ significantly ($P < 0.05$).

frankfurters with 0.25% NaTPP decreased at both cooking temperatures as compared to control frankfurters. Further addition of NaTPP to 0.5 %, resulted in an increase in expressible moisture from 22.5 to 23.9 % at 71.1°C. Expressible moisture of frankfurters processed to 90.0°C decreased from 23.9 to 20.8 %. It is likely that NaTPP solubilized SSP thus increasing water binding potential.

Hardness of frankfurters containing 7% WPC A processed at 71.1°C increased upon addition of 0.25% NaTPP from 15.1 to 17.5 kPa. Hardness decreased with further addition of NaTPP to 0.5% from 17.5 to 15.3 kPa. When the cooking temperature was increased to 90.0°C, however, no change in hardness was observed at any NaTPP concentration. No change in strain was observed at any concentration of NaTPP for frankfurters processed at either temperature. Expressible moisture decreased at both temperatures upon addition of 0.25% NaTPP. Addition of NaTPP from 0.25% to 0.5% caused the expressible moisture of frankfurters processed to 71.1°C to decrease while frankfurters processed to 90.0°C increased.

With the exception of control frankfurters which remained unchanged, increasing cooking temperature from 71.1°C to 90.0°C resulted in a decrease in hardness of frankfurters without WPC A. Increasing the cooking temperature from 71.1°C to 90.0°C resulted in decreases in strain at every NaTPP concentration. Increasing the cooking temperature resulted in a decreases in expressible moisture

for control frankfurters and frankfurters containing 0.5% NaTPP. Expressible moisture of 0.25% NaTPP frankfurters increased from 22.5 to 23.9 % as the temperature increased from 71.1°C to 90.0°C.

Hardness of frankfurters containing 7% WPC A at all NaTPP concentrations increased as the cooking temperature increased from 71.1°C to 90.0°C. The opposite was true for strain and expressible moisture, as increasing the temperature resulted in decreases in these properties at all NaTPP concentrations. This was probably due to a combination effect of the increased gelling ability of WPC at increased temperatures and the fact that less moisture is in the frankfurter causing firmer, less elastic gels that express less moisture.

Addition of 7% WPC A did not change hardness of control frankfurters processed to 71.1°C. However, addition of 0.25% NaTPP resulted in frankfurters with 7% WPC A to decrease in hardness as compared to frankfurters without WPC A. The difference in hardness between frankfurters with and without WPC A continued as NaTPP was increased to 0.5%. These findings are in agreement with those from Thompson et al. (1982). They reported that addition of succinylated whey concentrate or whey protein concentrate resulted in a decrease in firmness of frankfurters. Strain of 71.1°C processed frankfurters decreased at all NaTPP concentrations as 7% WPC A was incorporated. Expressible moisture for 71.1°C processed frankfurters also decreased as WPC A was

incorporated with the exception of the 0.25% NaTPP treated frankfurters which remained unchanged. This is again a result of the water binding ability of WPCs.

At 90.0°C, addition of 7% WPC A resulted in an increase in hardness from 17.0 to 20.2 kPa for frankfurters prepared without NaTPP. No change in hardness was observed with frankfurters containing 0.25 and 0.5 % NaTPP. Strain and expressible moisture, like those for the 71.1°C frankfurters, were lower at every NaTPP treatment. These data are consistent with those of the SSP and WPC combination gels where added WPC resulted in increased gel hardness and decreased strain and expressible moisture at 90°C.

In general, changes in frankfurter hardness are consistent with those found in the literature. In their study of reduced NaCl turkey frankfurters (2.5% to 1.5%) with NaTPP replacement (0.4%), Barbut et al. (1988) found a significant increase in shear force as NaCl:NaTPP concentrations were changed from 2.0%:0% to 1.5%:0.4%. Likewise, Whiting (1984) observed significant increases in gel strength of beef/pork frankfurters as NaCl was reduced from 2.5% to 1.5% and NaTPP was replaced at 0.25%. In both cases, the increase in gel strength observed was attributed to the increase in protein solubilization caused by NaTPP.

4.6.5. EFFECT OF SODIUM TRIPOLYPHOSPHATE CONCENTRATION AND WHEY PROTEIN CONCENTRATE ON THE MINERAL COMPOSITION OF FRANKFURTERS

Mineral composition for model system turkey frankfurter batter prepared with and without 7.0% whey protein concentrate (WPC) A and three concentrations of NaTPP (0, 0.25 and 0.5 %) are listed in Table 35. Addition of sodium tripolyphosphate did not change the total mineral concentration for model system turkey frankfurters. This compound is made up of sodium (Na) and phosphate (P) and so total Na and P was expected to increase proportionally as the concentration of NaTPP was increased in the formulation. However, 0.5 % NaTPP equates to only about 1 mM of sodium and 3 mM of phosphorus. The ability of the atomic absorption unit to precisely measure these small changes, makes statistical separation of the values unfeasible.

Addition of WPC A resulted in an increase in total calcium (Ca) concentration for model system turkey frankfurters. This was expected because milk proteins contain more calcium than meat proteins. Conversely, the other minerals (Na, K, Mg and P) did not change upon addition of WPC A. This was expected because these minerals are similar in milk and meat.

4.6.6. GENERAL SUMMARY OF FRANKFURTERS

Results indicate that addition of WPC to formulations alter finished frankfurter properties. In general, at commonly used processing temperatures (71°C), WPC may be

Table 35. Total mineral composition of model system turkey frankfurter batter prepared with and without 7.0% whey protein concentrate (WPC) A and 0-0.5% sodium tripolyphosphate (NaTPP)

NaTPP (mM)	Ca (mM)	Na (mM)	K (mM)	Mg (mM)	P (mM)
		WITHOUT WPC A			
0	2.81[b]	538.6[a]	48.5[a]	6.50[a]	37.7[a]
0.25	2.48[b]	545.9[a]	46.0[a]	6.48[a]	40.1[a]
0.50	2.61[b]	530.3[a]	47.3[a]	6.39[a]	49.4[a]
		WITH 7% WPC A			
0	5.92[a]	524.0[a]	47.0[a]	6.32[a]	38.7[a]
0.25	5.79[a]	516.1[a]	46.3[a]	6.37[a]	40.2[a]
0.50	5.42[a]	460.1[a]	47.4[a]	6.38[a]	46.9[a]

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

used to improve water holding properties, but not textural properties. Higher processing temperatures (90°C) are necessary before both texture and water holding properties can be improved.

Sodium tripolyphosphate also tended to improve finished frankfurter properties. Unlike the model gel systems, however, NaTPP did not enhance the affects of WPC and indeed the beneficial aspects of NaTPP appeared to be diminished in its presence. The results indicate that more work needs to be done with respect to the effect of NaTPP on WPC in actual foods rather than model systems.

CONCLUSIONS

Free calcium concentration plays a critical role in determining the gelation properties of WPC. Results suggest that some WPCs may contain more free calcium than needed to optimized gelling properties. Sodium tripolyphospate can be used to reduce free calcium in WPCs and alter gel properties. Studies using NaCl and EDTA suggested that ionic strength and sodium content did not affect the gelling properties. Ionized calcium can be altered to improve the rheological properties and water retention properties of WPC gel systems.

Studies at different pH values demonstrate that [Ca^{2+}], rheological properties, and water retention properties were affected by pH. Cooling WPC gels too rapidly after cooking led to experimental difficulties. Additional amine based buffer was unnecessary to control pH and affected all properties of WPC gels including [Ca^{2+}], stress and strain at failure, and expressible moisture. It is vital to control these environmental variables to get reproducible results.

Addition of NaTPP to combination sols containing SSP and WPC altered the [Ca^{2+}] and [Na^{+}] at every protein concentration. Increases in WPC likewise affected the ionic

mineral concentration at every NaTPP concentration. At cooking temperatures of 65°C and 90°C, addition of NaTPP and/or WPC resulted in harder, less deformable gels that expressed less moisture. In addition, changes in cooking temperature also affected these properties.

Addition of NaTPP to turkey drumstick frankfurters increased cook yield and reheat yield. Increasing processing temperature from 71.1°C to 90.0°C decreased cook yield and increased reheat yield. Affects of added WPC were dependent on the concentration of NaTPP.

Addition of NaTPP did not change the proximate composition of turkey drumstick frankfurter batter. Addition of 7% WPC (on a protein basis) resulted in significant increases of protein and fat concentration and significant decreases of moisture and pH. The same was true for frankfurters made from the batter. In addition, cooking temperature significantly increased fat concentration, decreased moisture concentration, and had no affect on protein concentration and pH.

Sodium tripolyphosphate made firmer, more deformable frankfurters with improved water retention properties in formulations without WPC. When 7% WPC was added to the formulation, NaTPP affected only the water retention properties and left textural properties unchanged. Addition of 7% WPC decreased strain at failure and expressible moisture at both temperatures, while stress at failure decreased at 71.1°C and increased at 90.0°C. Addition of

NaTPP to WPC treated frankfurters did not change any textural or water retention property.

The total mineral concentration of frankfurters with NaTPP did not change. Addition of 7% WPC to the formulation increased total calcium concentration but resulted in no changes for any other minerals.

Results of this study indicate that in comminuted meat emulsions, NaTPP has a greater affect on SSP than WPC by solubilizing it, thereby increasing water retention and affecting texture. Results also suggest that using WPCs as fillers in comminuted meat products like frankfurters can change the textural and yield properties. By incorporating NaTPP and higher processing temperatures, frankfurters can be made to a comparable hardness but with reduced rubberiness.

FUTURE RESEARCH

(1) Examine NaTPP treated, WPC added frankfurters with electron microscopy to determine how the proteins interact with each other and with NaTPP.

(2) Examine the composition and gelling properties of whey protein concentrates in model gel and frankfurter systems processed to retort temperatures.

(3) Examine the effect of NaTPP and WPC on the sensory properties of frankfurters.

Appendix 1. Rheological and expressible moisture properties of 98% soluble experimental WPC (16% protein (w:w) made in 0.1M MOPS buffer, pH 7.0) with added Na orthophosphate (NaP)

NaP (mM)	Force at failure (kPa)	Strain at failure (dimensionless)	Expressible moisture (%)
0	35.3 ± 3.36	0.70 ± 0.061	48.1 ± 4.48
10	26.2 ± 3.01	0.68 ± 0.044	46.1 ± 4.22
50	34.3 ± 2.95	0.59 ± 0.041	36.6 ± 4.01
100	30.8 ± 2.56	0.52 ± 0.030	40.4 ± 3.68
200	33.5 ± 3.34	0.62 ± 0.058	33.9 ± 3.86

Appendix 2. Rheological and expressible moisture properties of 98% soluble experimental WPC (16% protein (w:w) made in 0.1M MOPS buffer, pH 7.0) with added Na hexametaphosphate (NaHMP)

NaHMP (mM)	Force at failure (kPa)	Strain at failure (dimensionless)	Expressible moisture (%)
0	22.0 ± 3.33	1.05 ± 0.077	46.0 ± 4.63
10	18.1 ± 3.01	0.95 ± 0.089	38.9 ± 4.12
50	18.8 ± 2.95	0.93 ± 0.056	40.2 ± 3.65
100	13.3 ± 1.56	0.96 ± 0.067	39.6 ± 4.53
200	80.5 ± 9.48	0.77 ± 0.072	29.6 ± 3.02

Appendix 3. Ionic strength calculation of WPC A sols containing 100 mM NaTPP

$$I = 0.5[[Phos_1](Z)^2 + [Na^{+1}] + [H^+] + [Cl^-]]$$

Since no HCl was added for any NaTPP concentration, the equation reduces to:

$$I = 0.5[[Phos_1](Z)^2 + [Na^{+1}]$$

Z, the charge on the phosphate molecule, is dependent on two things: 1) the ratio of $[Na^+]/[Phos]$ and 2) addition of HCl. Since no HCl was added, Z is dependent only on the ratio of $[Na^+]/[Phos]$ which remains constant with a particular chain length to a particular concentration. For NaTPP, it remains constant at 4.0 to at least 0.1M based on dissociation data given by Trout and Schmidt (1986).

$$[Phos_1] = \text{molal phosphate} = \frac{\text{0.00256 moles phosphate in slurry}}{\text{0.130 kg DD-}H_2O \text{ in slurry}} = 0.0197$$

$$[Na^+_1] = \text{molal sodium} = \frac{\text{0.000853 moles sodium in slurry}}{\text{0.130 kg DD-}H_2O \text{ in slurry}} = 0.00656$$

Thus,

$$I = 0.5[(0.0197)(4.0)^2 + (0.00656)] = 0.16$$

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