INFLUENCE OF TEMPERATURE, TIME, AND SODIUM TRIPOLYPHOSPHATE ON THE SOLUBILITY OF COLLAGEN AND SALT-EXTRACTABLE MEAT PROTEINS

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CHAPTER I

INTRODUCTION

In food systems, an understanding of a protein's response to heat processing is paramount in order for certain desirable product qualities to be realized. In muscle foods, soluble and insoluble myofibrillar proteins and sarcoplasmic proteins are responsible for both fat binding, water binding, and the heat-induced cohesiveness evident in further processed meats (Fukazawa, Hashimoto, and Yasui, 1961; Nakayama and Sato, 1971; Samejima, Takahashi, and Yasui, 1976; MacFarlane, Schmidt, and Turner, 1977). These properties are demonstrated in products ranging from hamburger and sausage to restructured and intact muscle foods.

Protein coagulation and gelation are manifestations of a very complex set of reactions classified under the rather broad heading of protein denaturation (Tumerman, 1974). Denaturation involves changing the protein, both chemically and/or physically, so that it retains little if any of its natural properties. One manner of inducing denaturation is by the application of heat. Heat denaturation involves imparting thermal energy to a point that causes the inherent order of the biological macromolecules to revert to a more

stable state of disorder or randomness. This disordering of the structure involves disrupting secondary stabilizing forces of the molecule such as hydrogen bonds, salt-bridges, van Der Waals forces, and possibly a reorganization of disulfide linkages (Tumerman, 1974). A reorientation of hydrophobic amino acids alters the solute properties of the protein and results in precipitation or coagulation of the proteinaceous material (Shimada and Matsushita, 1980).

How each protein type (i. e. stromal vs. myofibrillar) manifests heat denaturation can be related to its amino acid composition and more specifically its primary structure (Shimada and Matsushita, 1980). Each amino acid has a different pKa as well as other potentially different characteristics such as sulfhydrl and imidazole groups that influence structural uniqueness (Stryer, 1981). The interplay between pH and charge on the protein molecule are related to individual amino acid pKa and subsequently protein solubility (Kinsella, 1979). Therefore, it is understandable why different combinations of these amino acids would result in molecules that possess such dissimilar characteristics. Other environmental factors such as temperature and ionic strength must also be considered in protein denaturation.

The functionality of either salt-soluble muscle proteins or bovine corium collagen in food systems revolves around how these constituents will behave in a very complex environment. Certain preprocessing factors such as state

and type of rigor development and storage conditions will influence the solubility of meat proteins. Adjuncts that are added to maximize the utility of the product such as NaCl and polyphosphates also change protein behavior. Therefore, if we consider the presence of polyphosphates and NaCl(pH and ionic strength) in light of protein-protein, protein-solvent, and protein-lipid interactions (Acton, Zeigler, and Burge, 1983), protein behavior becomes very intriging and fascinating.

Thus, it was the purpose of the research reported in this thesis to study how temperature and time affected the behavior of bovine corium collagen and salt-soluble muscle proteins when either 3% NaCl or 3%NaCl + 0.44% STPP were used as extractants.

CHAPTER II

LITERATURE REVIEW

The solubility of muscle proteins is an extremely important characteristic with respect to product quality because it is these soluble proteins, primarily actin, myosin, and actomyosin, that act as fat emulsifiers and water binders as well as imparting unique textural properties to processed muscle foods after heat processing. Protein types vary with respect to solute properties and response to heat treatment, therefore these factors dictate processing regimes that are designed to maximize raw material performance in a meat system. The purpose of this review is to discuss structural properties of collagen and muscle proteins and their response to heat and solvent effects.

Physical and Chemical Properties of Actin and Myosin

Muscle tissue is composed of three types of proteins that are classified according to their solubility in various concentrations of neutral salt solutions. These classes are myofibrillar, sarcoplasmic, and stromal proteins. Myofibrillar proteins are soluble in strong KCl solutions.

(.5-.6M); sarcoplasmic proteins are soluble in weak KCl solutions (.05-.1M); and the stroma proteins are insoluble after prolonged extraction in strong salt solutions (Szent-Gyorgi, 1960). In skeletal muscle, the approximate percentages for myofibrillar, sarcoplasmic, and stromal proteins are 60.5, 29, and 10.5% respectively (Acton, 1979).

The major myofibrillar proteins with respect to raw material functionality are actin and myosin. Actin and myosin will vary in the form present depending on the degree of rigor development in the post mortem tissue (Macfarlane et al.,1977; Hamm, 1982). Prerigor, the two myofibrillar proteins exist separately as long as the plasticizing effect of ATP is present. However as ATP is depleted, energy is not available to break crossbridges between the two proteins and as a result they associate to form the actomyosin complex (Bodwell and McClain, 1971; Hamm, 1982). These forms are important since actomyosin is inferior to myosin in regard to functional characteristics (Nakayama and Sato, 1971; MacFarlane et al. 1977; Seigel and Schmidt, 1979).

Myosin constitutes approximately 55% of the total contractile element (Bodwell and McClain, 1971). Myosin has a molecular weight of 500,000 and is described in terms of light meromyosin (LMM) and heavy meromyosin (HMM) in discussions of ATPase activity and mechanisms of contraction (Bendall, 1969). Heavy meromyosin is more complex than light meromyosin because it contains a portion of the tail region of the total myosin molecule and also the two

globular heads that possess both the actin binding sites and ATPase activity. The latter components may be obtained by papain digestion. The products of papain digestion are the S-1 and S-2 subfragments of the protein with the S-1 subfragment representing the globular heads. The S-1 and S-2 subfragments represent an actual length of 570 A with a MW of 100,000 (Bendall, 1969; Bodwell and McClain, 1971). Light meromyosin has a total average length that varies from 600 to 900 A and it possesses a coiled-coil structure that is the result of two identical alpha-helical molecules. LMM's molecular weight is 150,000 and it makes up approximately 71% of the tail portion of the molecule (Bendall, 1969).

The most significant or noticeable difference between HMM and LMM is that HMM has a much lower alpha-helical content than LMM. The alpha-helical content of light meromyosin is approximately 105% compared to 48% for the globular portion of heavy meromyosin (Harrington, 1979). This inequality is a reflection of the tertiary structure differences imposes on fibrous and globular proteins as a result of the imino acid proline (Stryer, 1981).

Actin is a contractile protein found in muscle and it exists in a fibrous or polymeric form. The monomeric globular actin (MW 47000) is spherical in shape, however as it polymerizes it becomes fibrous actin with molecular weights as varied as the number of monomeric subunits comprising the molecule. As compared to myosin, the

alpha-helical content of G-actin is low (29-30%) and its diameter is 55 A. Actin, as it exists in the sarcomere, consists of two strands of the polymeric form wound around one another to form a super helix (Bendall, 1969).

The amino acid composition of myosin and actin varies with respect to the content of polar amino acids in that 17% of the residues for myosin are basic and 18% are acidic whereas actin contains approximately 11.4% free acid groups and 12% basic groups. The total number of polar side chains for myosin and actin, respectively, are 38% and 33% (Bodwell and McClain, 1971). The striking difference between actin and myosin is the proline content. Actin contains twice as many proline residues as myosin with the majority of the imino acids for myosin being present in the globular head portion of the molecule (Bodwell and McClain, 1971). The steric restrictions placed on the molecule as a result of the presence of proline dictate in this case whether it is a fibrous or a globular constituent of the protein.

Physical and Chemical Properties of Collagen

Collagen may represent up to 20-25% of the total protein (Bodwell and McClain, 1971) and it exists throughout the body in tissues such as skin, tendon, bone, cornea, basement, and muscle (Dutson, 1976; Bornstein and Traub, 1979). Collagen is the end product of a combination of many biochemical alterations that occur on the tropocollagen

molecule, known collectively as post-translational modification (Prockop, Berg, Kiwirikko, and Uitto, 1976; Duston, 1976; Bornstein and Traub, 1979; Stryer, 1981; Aberle and Mills, 1983). Post-translational modification includes hydroxylation of proline and lysine residues, glycosylation of some hydroxyl groups on lysine with galactosyl or glucosylgalactosyl side chains, and synthesis of disulfide bonds (Prockop et al., 1976; Stryer, 1981; Aberle and Mills, 1983).

The basic structural unit that is constant for all collagen types is the triple helix (Aberle and Mills,1983). Tropocollagen is a triple stranded superhelix with a mass of approximately 285 kdal, a length of 3000 A, and a diameter of 15 A (Piez, 1966; Stryer, 1981). The amino acid and carbohydrate composition of the polypeptide chains making up the tropocollagen molecule vary, thus giving rise to the different collagen types found throughout the body. These types are designated as Types I, II, III, IV (Piez, 1976; Privalov, 1982). The chemical properties of Type I are most significant relative to potential uses of hide collagen as a food since it predominates in skin collagens (Bornstein and Traub,1979; Stryer, 1981; Aberle and Mills, 1983).

Type I collagen is composed of two identical $\alpha 1$ chains and a third chain, $\alpha 2$. Type I and type III collagens have the lowest hydroxylysine content and thus the lowest carbohydrate content since the carbohydrate moiety binds to the hydroxylysine residue (Stryer, 1981).

The amino acid composition of the alpha helix is rather unique in that a repeatable formula $(GLY\text{-}X\text{-}Y)_{340\pm2}$ occurs (Alberle and Mills, 1983). Glycine comprises about 33% of the total complement of amino acids with the imino acids proline and hydroxyproline making up 12% and 10% respectively. Hydroxylysine is present in low concentration (11%) and tyrosine, histidine, and the sulfur containing amino acids represent less than 1% (Bodwell and McClain, 1971; Piez, 1976).

Heat Denaturation of Myofibrillar Proteins and Collagen

The chemical and physical changes that occur in muscle proteins upon heating are profound (Hamm and Deatherage, 1960; Hamm, 1966; Paul, Buchter, and Wierenga, 1966; Trautman, 1966; Tumerman, 1974). Alterations in protein molecular structure on exposure to heat are seen on a gross scale as precipitation or an insolubilizing effect of heat. In processed meats as well as in intact muscle, heat related changes that result in gelation, coagulation, aggregation, precipitation, or flocculation, determine both textural and palatability traits. The response of individual proteins to heat application differ and this response is dependent on inherent stabilizing forces such as hydrogen bonding, van Der Waals forces, electrostatic interaction, and hydrophobic bonding (Tumerman, 1974; Stryer, 1981; Privalov, 1982).

In studies on the heat-induced changes that occur in

proteins, the midpoint at which the conformational transition of A (natured) to B (denatured) is 50% complete is used to indicate the structural stability of biological macromolecules such as proteins and deoxyribonucleic acid. This parameter is termed Tm, pHm, or Cm, depending on whether the independent variable measured is temperature, pH, or salt concentration (von Hippel and Schleich, 1969).

Myofibrillar Proteins

Variations in amino acid composition and thus in stabilizing forces are reflected in the complex protein system of post-mortem muscle. Cheng and Parrish (1979) observed heat-induced changes in myofibrillar proteins using SDS-polyacrylamide gel electrophoresis and they found that alpha-actinin becomes insoluble at 50 C, followed by myosin at 55 C; actin at 70-80 C, and tropomyosin and troponin at 80 C. Wright, Leach, and Wilding (1977), in differential scanning colorimetric studies of muscle tissue assigned transition temperatures of 60, 67, and 80 C to myosin, sarcoplasmic proteins, and actin respectively. These varying degrees of resistance to thermal denaturation are expected since the respective proteins vary relative to amino acid composition and sequence. The subunits of myosin also exhibit different Tm's and efforts have been made to discuss various properties of heat denaturation relative to these subunits (Samejima et. al., 1976). These workers demonstrated that the myosin total rod exhibits transition

temperatures of 47 C and 55 C. Thermodynamic studies on myosin, myosin rod, and the products of tryptic digestion also suggested that variations in structural stability are reflections of protein function in ante-mortem muscle (Samejima, Morita, Takahashi, and Yasui, 1972; Stabursvik and Martens, 1980; Privalov, 1982) i. e. the hinge region in addition to its susceptibility to trypsin is also less resistant to heat (Privalov, 1982).

It is well documented that environmental factors such as solvent properties (pH and ionic strength), temperature, and mechanical treatment can result in protein behavioral characteristics as varied as the environment to which they are exposed (Young, 1974; Goodno and Swenson, 1975; Stabursvik and Martens, 1980). Young (1974) reported that the degree of heat damage, as reflected by a loss in emulsifying capacity, below pH 8.5 resulted in the inability of actomyosin to bind fat. However, above pH 8.5 the emulsifying capacity of actomyosin was much better. Even though this improvement in functionality existed, it was not enough to override the heat damage that was sustained. Stabursvik and Martens (1980) demonstrated that by changing the pH from 5.4 to 8.7 the more heat-stable myosin component, light meromyosin, (65 C) became the less heat-stable one (52 C) while the less heat-stable component, heavy meromyosin, (52 C) became the more heat-stable one (65 C). Actin denaturation, however, is less sensitive to pH change as evidenced by the small peak attributed to

denaturation (77 C) at pH values between 5.4 and 6.1 (Stabursvik and Martens, 1980).

The ionic strength of the solvent has a tremendous influence on the stability of proteins in solution. Bivalent cations, monovalent cations, and monovalent anions exert different effects on hydration and solubility. These effects vary with pH and thus the charge on the protein (Schut, 1976). Goodno and Swenson (1975) indicated that the melting temperatures (Tm) for myosin and the myosin rod decrease about 10 C as the KCl concentration was raised from .15 to 2.9 M. They also provided evidence that illustrates the importance of ionic strength in the heat sensitivity of actin. By doubling the concentration of NaCl and KCl, they found that the destabilizing effect of these salts was also doubled. It was also pointed out that the destabilizing effect varies between salts since $CaCl_2$ and NaCl solutions of equal ionic strength caused respective declines in Tmax of 4 and 6.5 C for actin). Water and ions (Cl^-, Na^+, K^+, Ca^{+2}, Mg^{+2}) compete for polar side chains on the protein molecule, thus if this binding would alter the charge on the protein and inturn secondary stabilizing forces then the heat sensitizing effect of salt is accurate. Therefore, as a result of this salt destabilization, muscle proteins coagulate at lower processing temperatures (Quinn, Raymond, and Harwalker, 1980) than their unsalted counterparts. Again this response varies with the kind of salt present.

The interaction between proteins and their aqueous environment is also reflected by the fact that proteins may be more resistant to thermal denaturation insitu than in solution (Hamm, 1966; Paul et al., 1966; Wright, Leach, and Wilding, 1977). The transition temperature for the actin component in muscle corresponds closely to intact muscle actin, whereas those transitions in myofibrils and actomyosin are displaced by 10 Kelvin (Wright et al.,1977). These researchers indicate that it may or may not be correct to assume that dilute protein solutions will behave in the same manner as proteins in their natural environment.

Changes in solubility associated with heating can be used to monitor heat denaturation. Hamm and Deatherage (1960) indicated that from 40-60 C the greatest decrease in solubility occured for both the sarcoplasmic and myofibrillar proteins with greater sensitivity observed in the myofibrillar proteins at 40 C. Paul et al. (1966) observed that with either increasing temperature or increasing time at a given temperature extractable nitrogenous compounds of both the sarcoplasmic and myofibrillar fraction of rabbit muscle decreased when KI or KCl were used as extracting adjuncts. Porteous and Quinn (1979), monitored the heat denaturation of meat and meat plus various combinations of a soy isolate, torula yeast concentrate, and skim milk powder, by heating a diluted extract of each combination at 74 C. The decrease in

Kjeldhal Nitrogen from that of the unheated aliquot represented heat denatured protein. These workers found that the solubilities following heat treatment were greater for each protein source combination than for each source heated alone. This observation suggested an interaction between the individual sources in the mixture.

Collagen

Melting temperature (Tm) and shrinkage temperature (Ts) are used to describe the thermal stability of tropocollagen and intact collagen fibrils respectively (Stryer, 1981). The Ts for collagen is approximately 39 C while the conversion of collagen to gelatin (Tm) occurs at approximately 72 C (Acton, 1979; Privalov, 1982). These two indices of thermal stability are related to imino acid content and interchain crosslinking (Piez, 1966; von Hippel and Schleich, 1969; Tanzer, 1976; Bornstein and Traub, 1979; Privalov,1982).

Collagen denaturation is an extremely endothermic reaction (Privalov, 1982) and depends strongly on the stabilizing effect of hydroxyproline. Early work regarding the influence of amino acid composition on Tm correlates total imino acid composition to thermal stability whereas Burjanadze (1979) proposed a better relationship between Tm and hydroxyproline content at the third position of the triplet, $(GLY\text{-}X\text{-}Y)_{340\pm2}$. This conversion of collagen to gelatin represents a random unwinding of the collagen triple

helix to a random coil form (von Hippel and Schleich, 1969).

Von Hippel and Wong (1963) studied the effect of solvent environment and polypeptide chain composition on the collagen gelatin phase transition. These workers found that the order of effectiveness of various salts was similar for reducing Tm of both soluble native collagen and cooled gelatin. They explained this in terms of similar stabilizing mechanisms. Von Hippel and Schleich (1969) divided the effect of neutral salts into direct mechanisms and indirect mechanisms, i.e. direct bonding of ions to specific sites on the protein or a competitive reorganization of the water structure, respectively.

In reference to pH effects, Dick and Nordwig (1966) demonstrated that Ts increased from 32 C at pH 0.95 to 39 C at pH 7.00. They stated that this effect is due to repulsive forces of surplus positive charges and the resulting dissociation of side chain carboxyl groups. Stabursvik and Martens (1980) described the denaturation of connective tissue collagen using differential scanning calorimetry. Thermograms revealed that Tmax shifts from 67 C to 70 C as the pH changes from 5.4 to 6.1. Thus, as pH increased the thermal stability of collagen increased based on these data.

Phosphate Effects on Myofibrillar Proteins and Collagen

The primary aim of mechanical treatment, comminution,

and the addition of NaCl and polyphosphates in the further processing of raw material in the meat processing industry revolves around protein solubility. Kinsella (1979) emphasized this point, indicating that proteins with low solubility indices have limited functional properties and thus more limited uses. Solubilizing this key constituent and the resulting benefits associated therewith have been addressed by many individuals in both muscle and nonmuscle protein foods (Saffle and Galbreath, 1964; Trautman, 1964; Acton and Saffle, 1969; Wolf, 1970; Acton, 1972; Kinsella,1979; Chou and Morr, 1979). Inherent properties of post-mortem muscle such as rigor state and the ionic environment insitu in combination with exogenous forces such as temperature, solvent environment, and mechanical treatment are first and foremost when considerations are given to the functional properties of muscle proteins.

Myofibrillar Proteins

Solvent environment is altered by the addition of either NaCl or a combination of NaCl and polyphosphates and this alteration, either by a direct or an indirect mechanism, changes the solute properties of primarily the myofibrillar proteins by increasing their hydration. This action is important because the changes that occur at this level are reflected as changes in solubility (Chou and Morr, 1979). Earlier supportive work by Sayer and Briskey (1963),

on the influence of physiological condition of muscle on protein solubility, revealed a significant negative correlation between protein solubility and juice retention by muscle (-.71) when rigor mortis was complete. The role of phosphates in improving water holding capacity (WHC) and water binding in intact muscle foods along with comminuted products has received considerable attention in the past and continues to be of interest since inconsistencies do exist with respect to their mode of action.

Trout and Schmidt (1983) reviewed the possible mechanisms that explain the function of phosphates in processed meats. The proposed mechanisms are; 1.) increased pH, 2.) increased ionic strength, 3.) dissociation of the actomyosin complex, and 4.) chelation of divalent cations.

Offer and Trinick (1983) observed changes in myofibrillar hydration under various solvent conditions and found that increasing the pH from 7 to 9 produced slight swelling in irrigated myofibrils while reducing the pH from 7 to 5 resulted in shrinkage. Phosphates produce slight increases in pH, 0.1 to 0.3 pH units, depending on phosphate type. However, this small increase does not seem to produce any significant effect on water uptake in cooked products (Trout and Schmidt, 1984). Increased values of pH associated with polyphosphates are greater for uncooked than cooked products and only small changes in pH are required to significantly affect water binding capacity (Trout and Schmidt, 1983). The addition of different polyphosphates

results in different pH responses and this variability would partially explain why certain phosphates are superior to others in enhancing meat protein functionality. Shults, Russell, and Wierbicki (1972) studied the influence of pyrophosphate (PP), tripolyphosphate (TPP), and hexametaphosphate (HMP) on pH increases with and without NaCl. An increase of approximately 0.2 pH units occurred in cooked muscle in the presence of 1% NaCl and .5% PP. The pH increased by 0.1 units for TPP at the same salt and phosphate concentrations while HMP produced no increase in pH.

The influence of ionic strength on the solubility of protein can be described best in terms of the "salting in" and "salting out" phenomena (von Hippel and Schleich, 1969). By increasing the ionic strength of a particular solution a protein of a higher structural order is converted to a state of randomness and greater thermodynamic stability. As a result the proteins become more soluble. On the other hand, solubility is reduced as salt concentration is increased to a point where protein-protein interaction increases and results in protein precipitation (von Hippel and Schleich, 1969). These authors attribute this effect of salts on conformational stability to an indirect action of salts in their ionized state on alterations in the water structure. Direct association of either anions or cations to charged groups on the molecule can also occur. These associations change protein stability by a shift to either a net positive

or a net negative charge. This shift results in internal repulsive forces that "open" up the molecular conformation of the protein. The effect of ionic strength is strongly dependent on the valency and concentration of the particular ions (Moore, 1962) and in processed meats those ions of importance are Na, Cl, and polyphosphates. Ionic strength exerts its effect because the degree of swelling or hydration in the protein network depends on the concentration of the ions in solution (Sherman, 1962).

Muscle proteins under various solvent conditions have been discussed by several authors with respect to meat processing (Wierbicki, Cahill, and Deatherage, 1957a; Wierbicki, Cahill, and Deatherage, 1957b.; Sherman,1962; Shults, Russell, and Wierbicki, 1972; Ishioroshi, Samejima, and Yasui, 1979; Turner, Jones, and MacFarlane, 1979; and Offer and Trinick; 1983). As a result, the importance of solvent environment and specifically ionic strength in protein performance in muscle foods is clearly evident. Trout and Schmidt (1984) point out that pH 6.00 and ionic strength (IS) 0.63 produce maximum tensile strength while pH 5.90 and IS .57 resulted in the greatest cook yield for restructured beef rolls. Australian workers reported maximal yields of crude myosin at 1M NaCl and .25% sodium tripolyphosphate (STPP)(Turner et al., 1979). These workers also demonstrated that their was maximum protein solubilized at pH 6.5, .16% STPP, and 1.0M NaCl. However, bind strength did not differ significantly between .125,

.25, and .5% STPP in the presence of 1M NaCl. Trout and Schmidt (1984) stated that increased extraction of myofibrillar proteins is only effective in increasing the tensile strength up to a point, beyond that there is no benifit realized.

Questions have been raised concerning a possible synergism between polyphosphates and NaCl relative to protein solubility. Sherman (1962) indicated that polyphosphates appear to bind water to a greater extent than an equivalent amount of chloride ions. Yasui, Masako, and Hashimoto (1964), suggested that the affinity of pyrophosphate and tripolyphosphate is greatly improved by the formation of a sodium salt of myosin (Na-myosinate). Therefore, added NaCl may form Na-myosinate that in turn binds phosphate anions. Subsequently the superior water binding capabilities of phosphate anions result in greater water binding verses NaCl alone. Work by Offer and Trinick (1983) on specific mechanisms of swelling and shrinking in myofibrils illustrates the structural changes that accompany hydration in the presence NaCl and a combination of NaCl plus polyphosphates. In terms of synergistic effects, pyrophosphate reduced the amount of NaCl needed to produce maximum swelling of myofibrils by a large degree. The location on the sarcomere where extraction occurred varied for the two conditions. In the presence of pyrophosphate, the A-band was completely removed beginning from its ends, however with NaCl alone solubilization occurred at the

center of the A-band. The respective salt concentrations for NaCl and the NaCl plus pyrophosphate combination were 1.0M and 0.4M.

Improved meat functionality associated with the use of pyrophosphates may also be due to a dissociation of the actomyosin complex (Young, 1974), providing myosin ATPase or myosin tripolyphosphatase (TPase) is present. Work by Ishioroshi et al. (1979) demonstrated little change with ATPase over storage time, therefore, STPP, in addition to it's effect on pH and ionic strength may result in the dissociation of actomyosin. The resulting hydrolysis product, pyrophosphate, because of its greater effect on pH (Shults et al., 1972) and consequently configurational changes in the myosin molecule, offers another explanation for the enhanced binding observed in the presence of phosphates. However, Trout and Schmidt (1984) refuted this possible contribution of actomyosin dissociation to improved binding. Their work showed that in the presence of 2% NaCl there was not a significant difference in bind strength when either pyrophosphate, tripolyphosphate, or hexametaphosphate were used, as might be expected if tripolyphosphate is capable of causing actomyosin dissociation.

Hellendoorn (1962) observed that upon addition of a strong Ca^{+2} chelating agent, such as ammonium oxalate or EDTA, water binding did not change. Inklaar (1967) found that phosphate added meat possessed no ability to bind or

chelate Ca^{+2} or Mg^{+2} ions. Dialysable Ca^{+2} in the standard was not different from that where phosphate was added to the meat. Thus the chelation of divalent cations does not appear to play a major role in the effect of phosphates on meat (Trout and Schmidt, 1983).

Collagen

Most of the early work on collagen solubility in various alkaline solutions was facilitated by leather manufacturers. Bowes and Kenten (1948) observed that after exposure to alkaline or acid solutions, alkali treated collagen (pH 13.0) had an appreciably greater water uptake than the untreated or natural collagen. Their results showed that alkaline treatment resulted in solubilization of approximately 5% of the collagen and the hydrolysis of amide groups. In the context of hydration, the increased swelling of collagen in alkaline solutions was attributed to a decrease in cohesion between collagen fibrils as a result of a high concentration of hydroxyl anions (Bowes and Kenten, 1950).

Other researchers (Goll, Hoekstra, and Bray, 1964; Goll, Bray, and Hoekstra, 1964; Herring, Cassens, and Briskey, 1965; Miller, Karmas, and Fu Lu, 1983), have discussed the association between age and changes in acid, alkali, and neutral salt soluble collagen from both corium collagen and connective tissue collagen. As the animal ages, there is an increased number of cross-links formed

thus making the collagen fibrils more resistant to the solubilizing effect of various solutions and any collagen soluble in NaCl is attributed to newly synthesized tropocollagen (Dutson, 1976).

The possible use of bovine hide collagen as a food ingredient has warranted research regarding its solute properties and the implications relative to protein functionality. Ranganayaki, Ashgar, and Henrickson (1982) investigated the influence of various cations and anions on hydration of bovine hide collagen. These workers found a pH effect on hydration in the presence of 6% NaCl. Salt concentration of 6% increased hydration in the pH range 6-8, but at pH values lower than 5 and greater than 8, added NaCl depressed hydration. The authors explained this in terms of a shielding effect of Na and Cl ions on the negative and positive charges at these extreme pH values. They also studied the influence of various phosphate salts (0.5% level) on the hydration of freeze dried collagen at 22 C and 70 C. The authors found that polyphosphate salts depressed the hydration of freeze-dried collagen at 22 C in the pH range of 6-8. At pH values lower than 5 all phosphates except hexametaphosphate increased hydration. In the presence of 2% NaCl and .5% phosphate salts, heating at 70 C for 10 minutes caused an increase in hydration to occur.

CHAPTER III

MATERIALS AND METHODS

Materials

The Eastern Regional Research Center at Philadelphia, PA provided comminuted native wet collagen for this study. This comminuted collagen had been frozen in No. 10 cans and classified as product No. 1 based on pH, particle size, protein denaturation, and viscosity (Elias, Komanowsky, Sinnamon, and Aceto, 1970). All collagen was removed from frozen storage (-13 C) and thawed at 4 C. It was strained with the aid of a Buchner funnel to separate the solid and liquid portions of the material and subsequently, a ratio of 2.18:1 (solids:liquid) was obtained. This ratio was determined so a constant proportion of solids to liquid could be maintained if more collagen were needed for conducting further experiments. Following filtration, the liquid and solids were recombined and mixed thoroughly. This standardized material was packaged in 250 gm portions into 16oz freezer containers for -13 C storage.

A beef inside round from a choice grade carcass was removed from frozen storage and thawed at 4 C. Following thawing, it was manually freed of separable fat and

superficial connective tissue, ground once through a 1/2 inch plate and ground twice through a 3/16 inch plate. Fifty gram portions of the minced muscle were packaged in whirlpak bags for storage at -13 C. The individually packaged frozen meat and collagen were thawed as necessary for experimentation. Food grade NaCl, granular food grade sodium tripolyphosphate (STPP), and distilled water were used to formulate the extracting solutions.

Methods

Three percent NaCl and a combination of three percent NaCl and 0.44%STPP were used as the extracting solutions (Figure 1). The meat block consisted of either 100% meat or meat replacement by collagen at 10, 20, 30, or 100% levels. These collagen levels (0, 10, 20, 30 and 100%) denote the meat blocks used in this experiment. Fifteen and nine-tenths grams of the meat block were weighed into a 400 ml blender cup and blended with 159.1 gms of extractant at 6400 rpm for one minute using a Sorvall Omni-mixer. The resulting slurry was blended for an additional minute following a three minute rest period (Turgut and Sink, 1983). This preparation of 175 gms of slurry was repeated four times to obtain sufficient sample for the heat treatment. The four blends were combined and the pH of the composite blend was adjusted to 6.00 with either 1N NaOH or 1N HCl. This concentration of base and acid was used in order to reach the designated pH without significantly

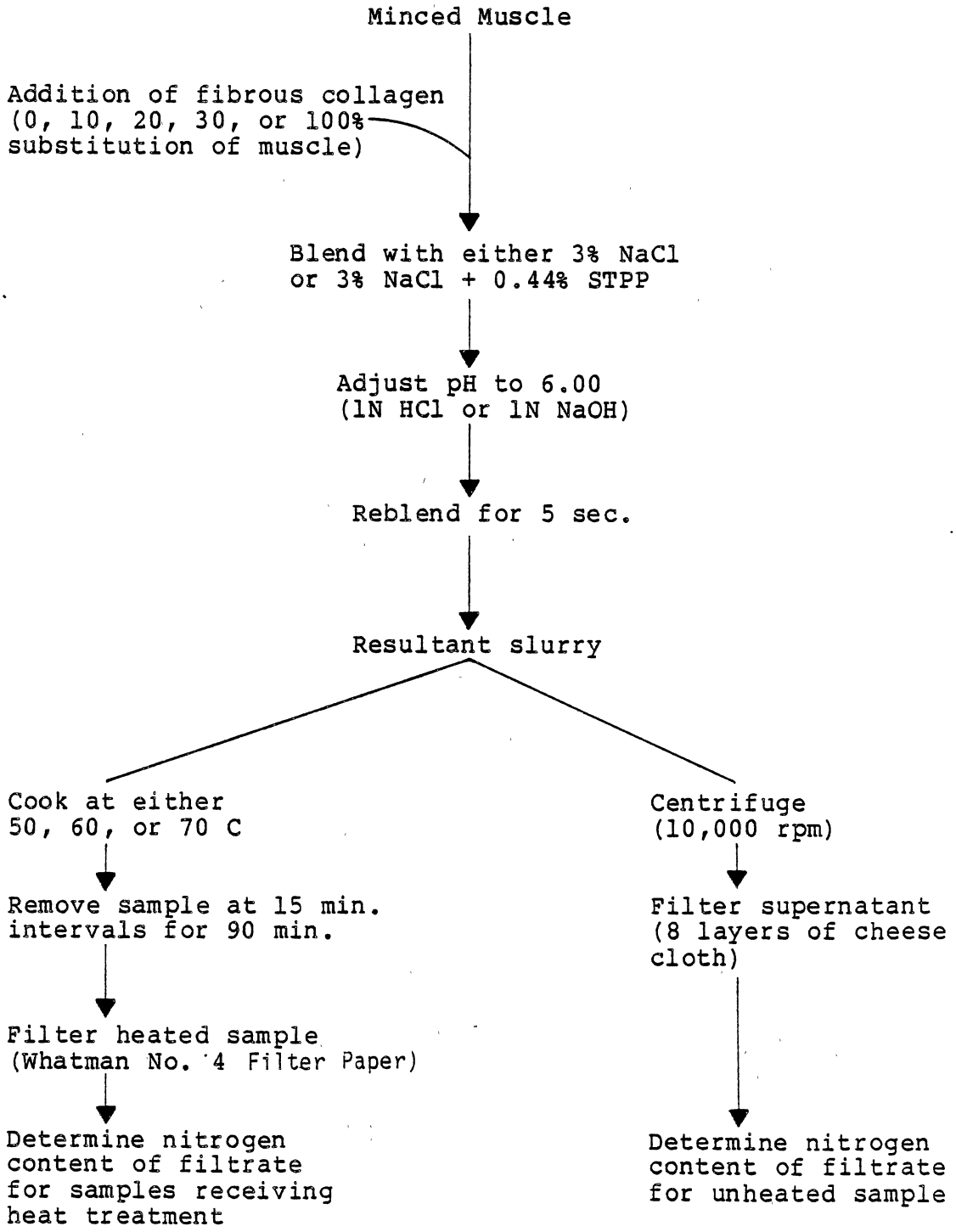

Figure 1. Flow Diagram of Experimental Procedure for Monitoring Changes in Nitrogen Solubility under Different Hydrothermal Conditions.

affecting the total volume of the composite slurry. Following pH adjustment, it was necessary to reblend the slurry because both added collagen and inherent muscle collagen had adhered to the stirring bar and thus were no longer uniformly distributed throughout the slurry. Redistribution of the collagen was accomplished using a Waring Commercial Blendor.

Subsequent to the redistribution step, fifty grams of the resulting slurry were weighed into each of 12 test tubes for heat treatment in an oscillating water bath at either 50, 60, or 70 C depending on the treatment conditions. Two test tubes were removed at 15 minute intervals until 90 minutes had elapsed. Upon removal from the water bath, the contents of each tube were immediately filtered through Whatman No. 4 filter paper and the filtrate was placed in an ice bath (Figure 2).

The original slurry, in excess of that needed for heat treatment, was used for determination of the total nitrogen content of the slurry and the nitrogen content of the time-temperature control (0 min heating time). For determining the soluble nitrogen content of the control, a portion of the aforementioned meat slurry was weighed into a 50 ml teflon tube and placed in an RC2-B refrigerated centrifuge for centrifugation at 10,000 rpm for 10 min. The resulting slurry supernatant was filtered through eight layers of cheese cloth and the filtrate was used in the nitrogen analyses.

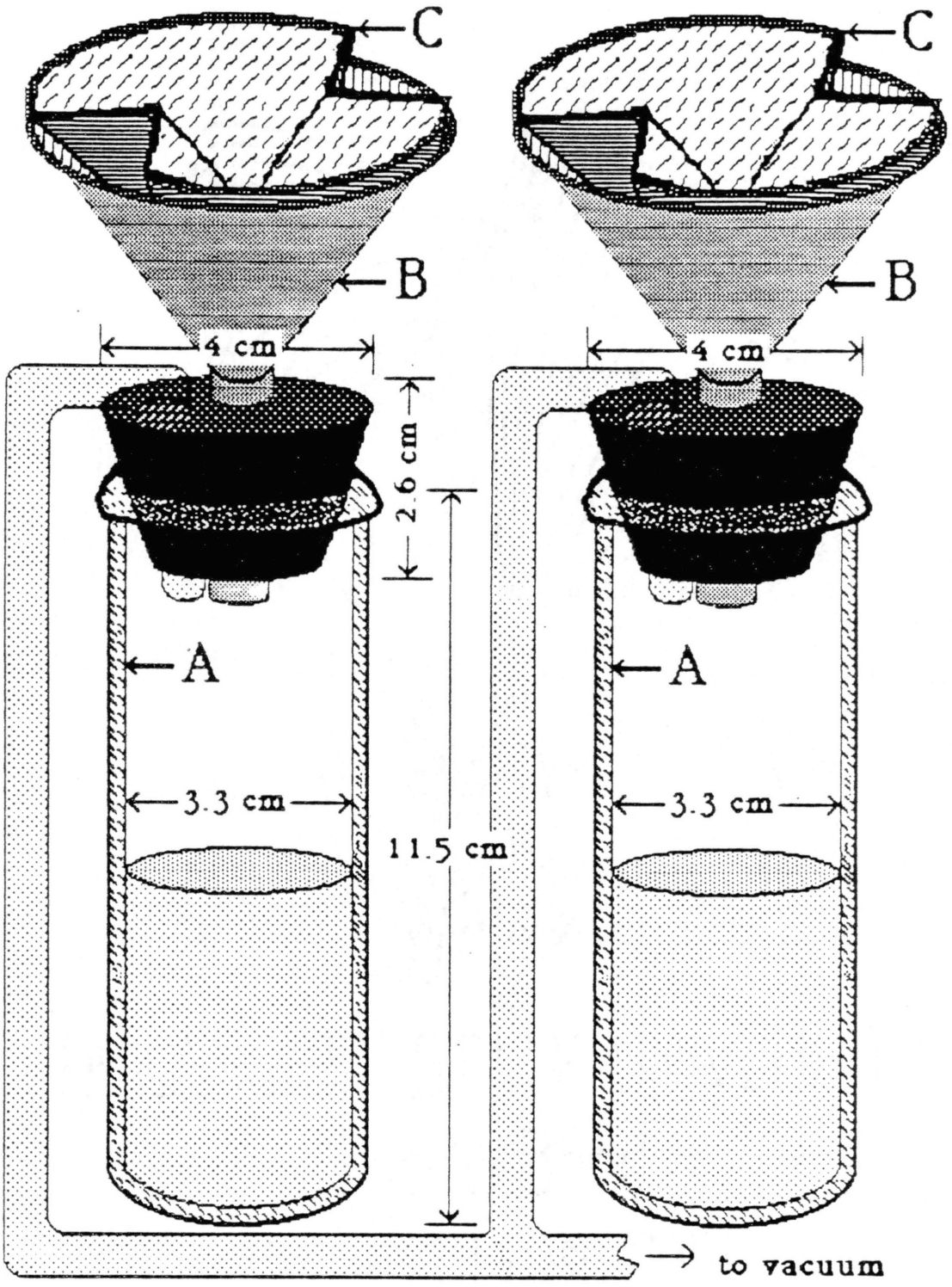

Figure 2. Illustration of Filtration Apparatus Used to Separate Liquid and Solids of Heated Sample (A, Test Tube; B, Funnel; C, Filter Paper).

In summary, nitrogen analyses and pH measurements were conducted on each pair of filtrates at 15, 30, 45, 60, 75, and 90 min intervals. Kjeldahl N was obtained following AOAC methods using the Tecator 1013 digestion unit and the Kjeltec 1030 autoanalyzer and pH was measured with a Corning Model 130 pH meter. In addition to the 6 fifteen minute intervals, nitrogen analysis was conducted in duplicate on the unheated filtrate obtained from the previously described centrifugation step.

Experimental Design and Data Analysis

The experiment was conducted using a Randomized Complete Block Design with a split-plot arrangement of treatments. In each block, 5 collagen levels, 3 temperature levels, and 2 phosphate levels denote the main-unit treatment factors for a total of thirty possible treatments (5 X 3 X 2) and within each treatment, seven time intervals represent the subunit treatment factors (Figure 3). Each treatment was replicated three times for a total of three blocks.

The data were analyzed using Statistical Analysis System (SAS) (Barr and Goodnight, 1972). In the first block of treatments, the 0 minute time interval was not measured for all treatments and in addition there was missing data due to some sample loss in each block of the thirty treatments. Therefore, in order to have balanced data,

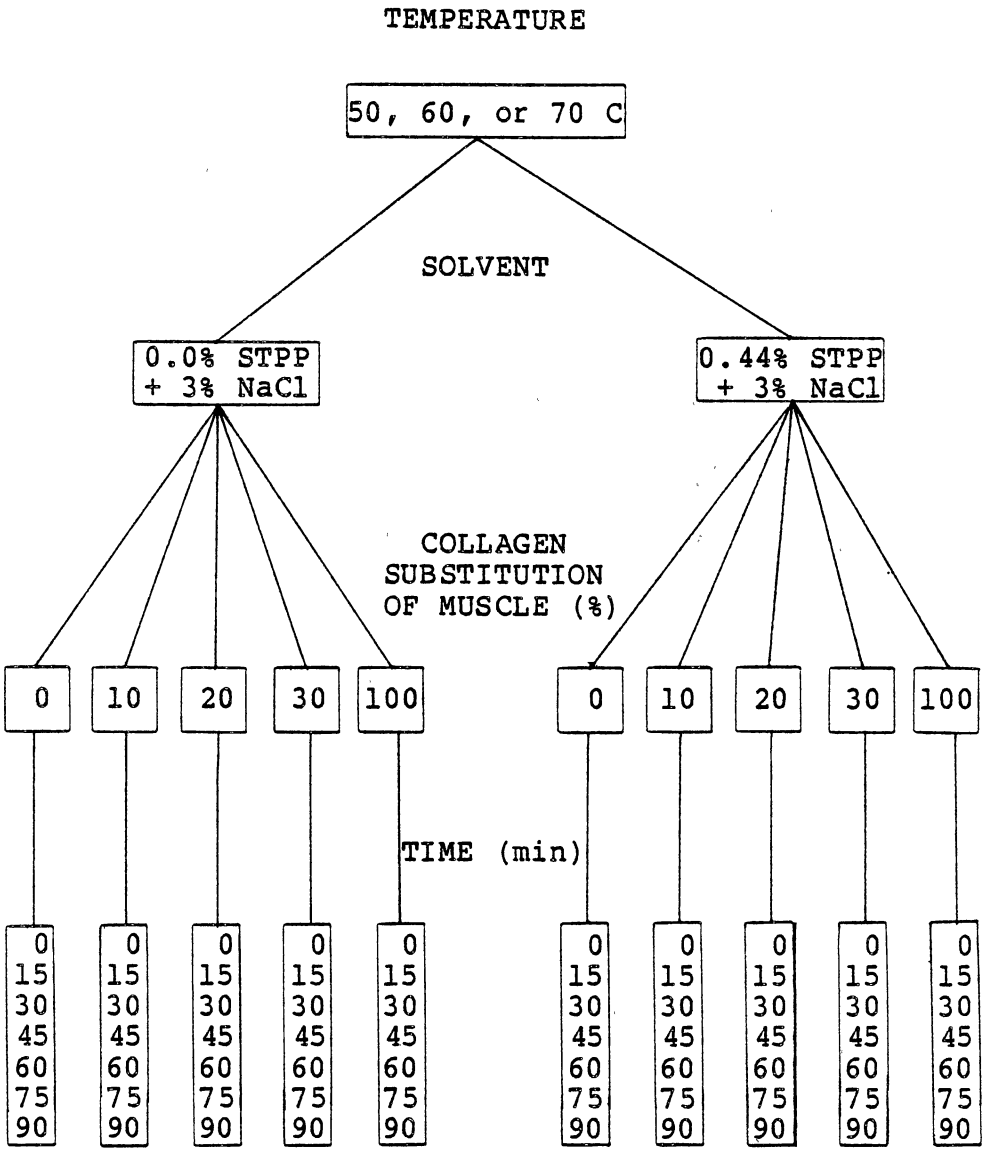

Figure 3. Diagram of Experimental Design that Includes Three Temperature Levels, Two STPP Levels, Five Collagen Levels, and Seven Time Intervals.

missing observations were predicted and these values were included in the data for subsequent analysis. F-tests from the analysis of variance were performed to determine if there were significant differences between levels of each treatment factor and also the significance of any two-way interactions among wholeunit treatment factors and between wholeunit treatment factors and subunit treatment factors. The presence of quadratic trends in the data associated with time of heating was verified using the general linear models (GLM) procedure of SAS.

CHAPTER IV

RESULTS AND DISCUSSION

Effects of Temperature and Time

As shown in Figure 4, the greatest reduction in nitrogen solubility for the 0% collagen substitution level at 50 C occurred from 0 to 15 minutes ($p < .05$) for both solvent conditions with a subsequently smaller reduction from 15 to 30 minutes. However, from 30 to 90 minutes, nitogen solubility did not exhibit a noticeable change ($p > .05$) for either solvent. Changes in nitrogen solubility followed a similar pattern at 60 C with values that were proportionally lower than those values obtained at 50 C from 15 to 90 minutes. At 70 C there was one significant reduction ($p < .05$) from 0 to 15 minutes, after which time the values remained stationary. Regression analysis across time revealed a significant quadratic effect for each temperature, thus supporting the previously described trends in the data.

In summary, there was a time-dependent reduction in solubility either from 0 to 15 minutes at 70 C or from 0 to 30 minutes at 50 and 60 C. When comparisons were made between temperatures at times greater than 30 minutes, there

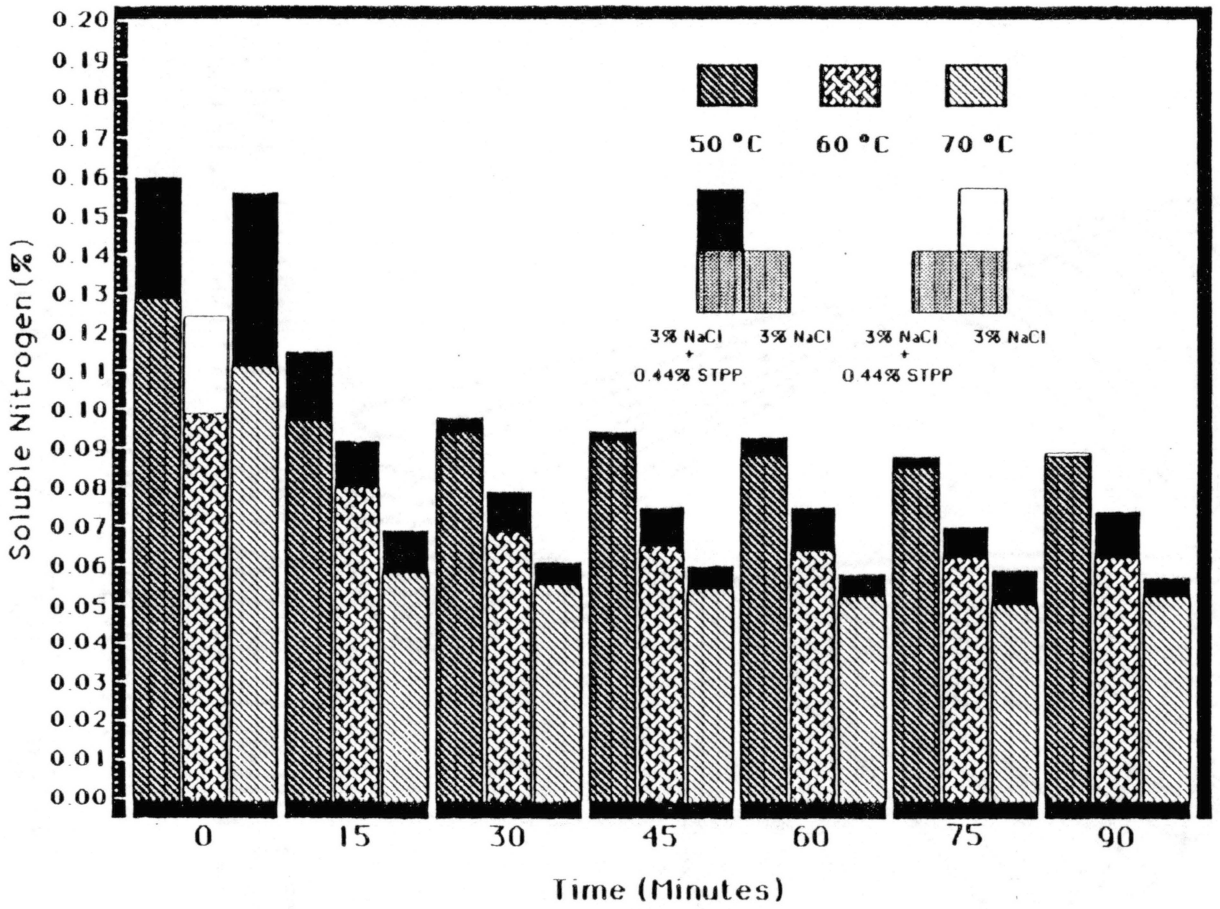

Figure 4. Effect of Temperature, Time, and Solvent on Nitrogen Solubility for 0% Meat Replacement with Fibrous Collagen. Values for both solvents are superimposed at each bar to illustrate STPP effect.

was a temperature dependent reduction in solubility as shown by proportionally lower values at progressively higher temperatures. Data was not available at temperatures greater than 70 C to indicate if this temperature dependent response continued to occur. However, some preliminary nonprotein nitrogen data at 70 C suggested that nonprotein nitrogen accounted for the majority of the soluble nitrogen at this temperature thus it is unlikely that a significant reduction will occur at higher temperatures as a result of futher protein denaturation.

Using gel electrophoresis, Cheng and Parrish (1979) reported that alpha-actinin became insoluble at 50 C with myosin, actin, and the regulatory proteins (troponin and tropomyosin) becoming insoluble at 55 C, 70-80 C, and 80 C respectively. In addition, Wright et al. (1977) observed similar changes using differential scanning calorimetry (DSC) when they assigned Tmax's (temperature of maximum rate of heat input as a thermal denaturation criterion) of 60, 70, and 80 C to myosin, sarcoplasmic protein, and actin denaturation respectively. These workers also indicate that the individual proteins, actin and myosin, will not respond to heat in a manner similar to the actomyosin complex. Also, proteins in situ are likely to be more heat resistant than dilute solutions of proteins (Hamm, 1966; Paul et al., 1966) thus these factors must also be considered to accurately interpret thermal denaturation studies.

Therefore, an interpretation of the current

experimental results in terms of the aforementioned DSC and electrophoretic studies may explain the temperature dependent changes in nitrogen solubility for heating periods longer than 30 minutes. The different levels at which soluble nitrogen stabilizes may represent the differential denaturation of the individual components of muscle tissue. Heating at 50 and 60 C should precipitate some myosin without affecting the other more heat resistant components (actin and the sarcoplasmic proteins) but at 70 degrees there should be coagulation of some if not all of these three proteins, thus giving rise to the stepwise reduction in solubility between 30 and 90 minutes as temperature increases from 50 to 70 C (Figure 4). A comparison of the initial reduction in solubility from 0 to 15 minutes between 50 and 70 C reveals that at the higher temperature, further reduction in solubility associated with increasing temperature by 20 C was not as great as the initial reduction observed at 50 C. This sharp reduction at 50 C may represent the denaturation of myosin since it is the most abundant muscle protein (34% of the total protein) and also one of the more heat sensitive components.

The dissimilar response of collagen to heat denaturation as compared to the previously discussed meat proteins is shown in Figure 5. This graph illustrates that at all hydrothermal conditions studied for 100% collagen replacement of the minced muscle the amount of nitrogen in the filtrate increased with increasing temperature and with

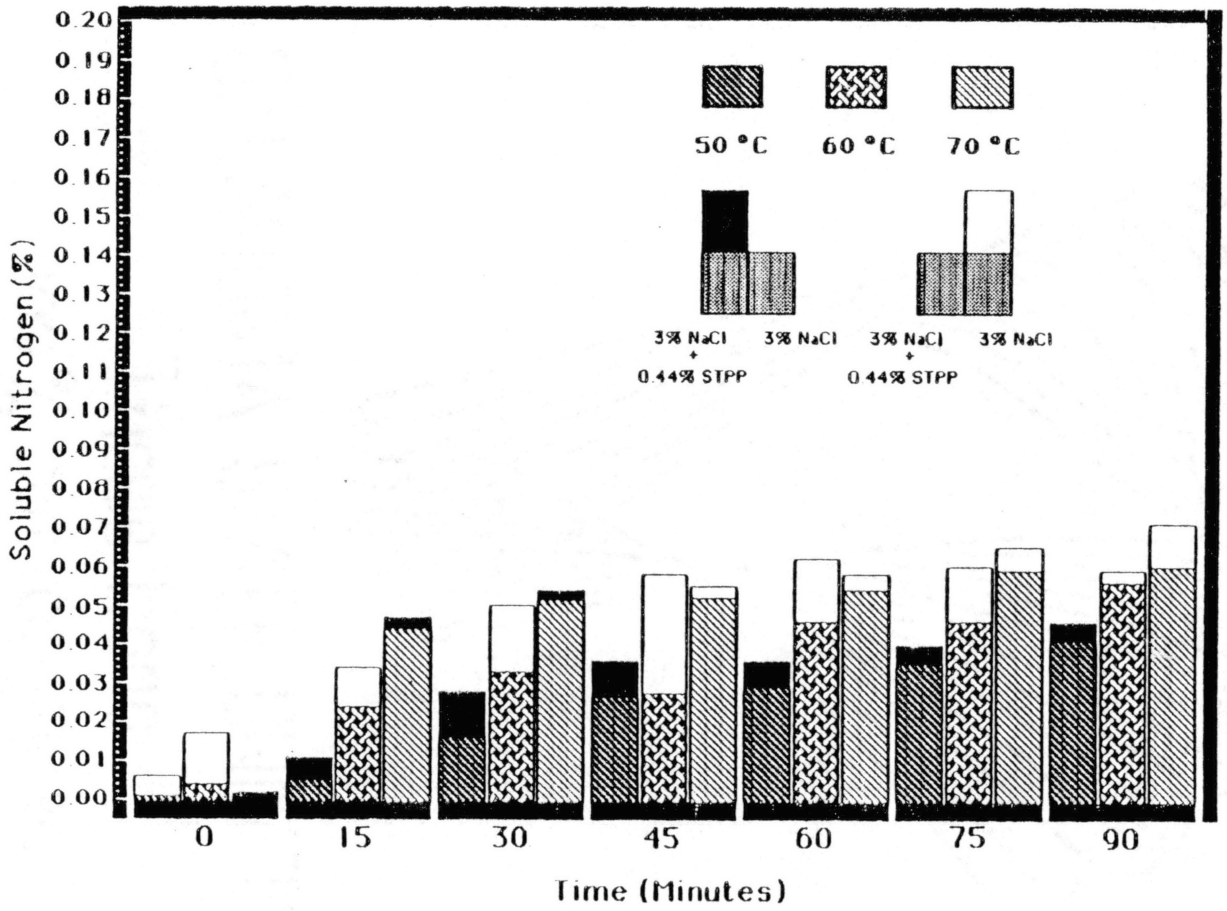

Figure 5. Effect of Temperature, Time, and Solvent on Nitrogen Solubility for 100% Meat Replacement with Fibrous Collagen. Values for both solvents are superimposed at each bar to illustrate STPP effect.

increasing time at a given temperature. In the abscence of STPP at 50 C there was no significant increase in solubility from 0 to 15 minutes whereas there was a significant increase ($p < .05$) from 15 to 30 minutes, after which time the values remained relatively constant. At 70 C with 3% NaCl as the solvent, there was a marked increase ($p < .05$) from 0 to 15 minutes of heating followed by a stationary response to increasing time from 15 to 90 minutes. A similar response was observed at 60 C when 3% NaCl, however, the initial increase at 15 min. was not as great as seen at 70 C and 3% NaCl although it was significantly different from the nitrogen extracted from the unheated sample ($p < .05$), illustrating again that the thermal hydrolysis of collagen appears to be responsible for the increase in soluble nitrogen.

The initial increase in solubility at 50 C may have been due to a release of the soluble tropocollagen molecule as a consequence of breaking the weak noncovalent forces associated with the secondary structure of collagen (Goll, 1964a) since the primary force responsible for stabilizing the collagen triple helix is the hydrogen bonding associated with structural water and hydroxyproline (Aberle and Mills, 1983). This release of soluble material is accompanied by shrinkage of the fibrous protein (Ts=39 C). At 70 C, the increased solubilization of collagen above that observed at 50 C may be the result of breaking the continuity of the interchain water bridges, the hydroxyproline related

stability, and the aldol type crosslinkages associated with hydroxylysine. Burjanadze (1979) reported a correlation coeffecient of 0.91 between Tm and total hydroxyproline content and he further stated that the hydroxyproline content at the third position of the triplet is more closely related to thermal stability for vertebrate collagens. Thus, the energy present at 60 and 70 C may have been sufficient to overcome the covalent related stability thus resulting in the greater release of soluble nitrogen as compared to 50 C.

Effect of Collagen Replacement

Prior to heating, 0 minutes, the substitution of meat with collagen produced a significant decrease ($p < .0001$) in soluble nitrogen (Figure 6). A comparison of the solubilities for 0% collagen substitution (100% minced muscle) and 100% collagen substitution (0% minced muscle) at 0 minutes reveals that the former is rather soluble while the latter is practically insoluble. Thus, replacing portions of the minced muscle with the insoluble collagen diluted the amount of muscle that was available for extraction with either 3% NaCl or 3%NaCl + 0.44% STPP and therefore resulted in less soluble nitrogen. This dilution effect is evident at each time interval for both solvents at 50 C (Figures 6 and 7) and for 3% NaCl + 0.44% STPP at 60 C (Figure 8). These observations are consistent with data from Asghar and Henrickson (1982). They reported that the

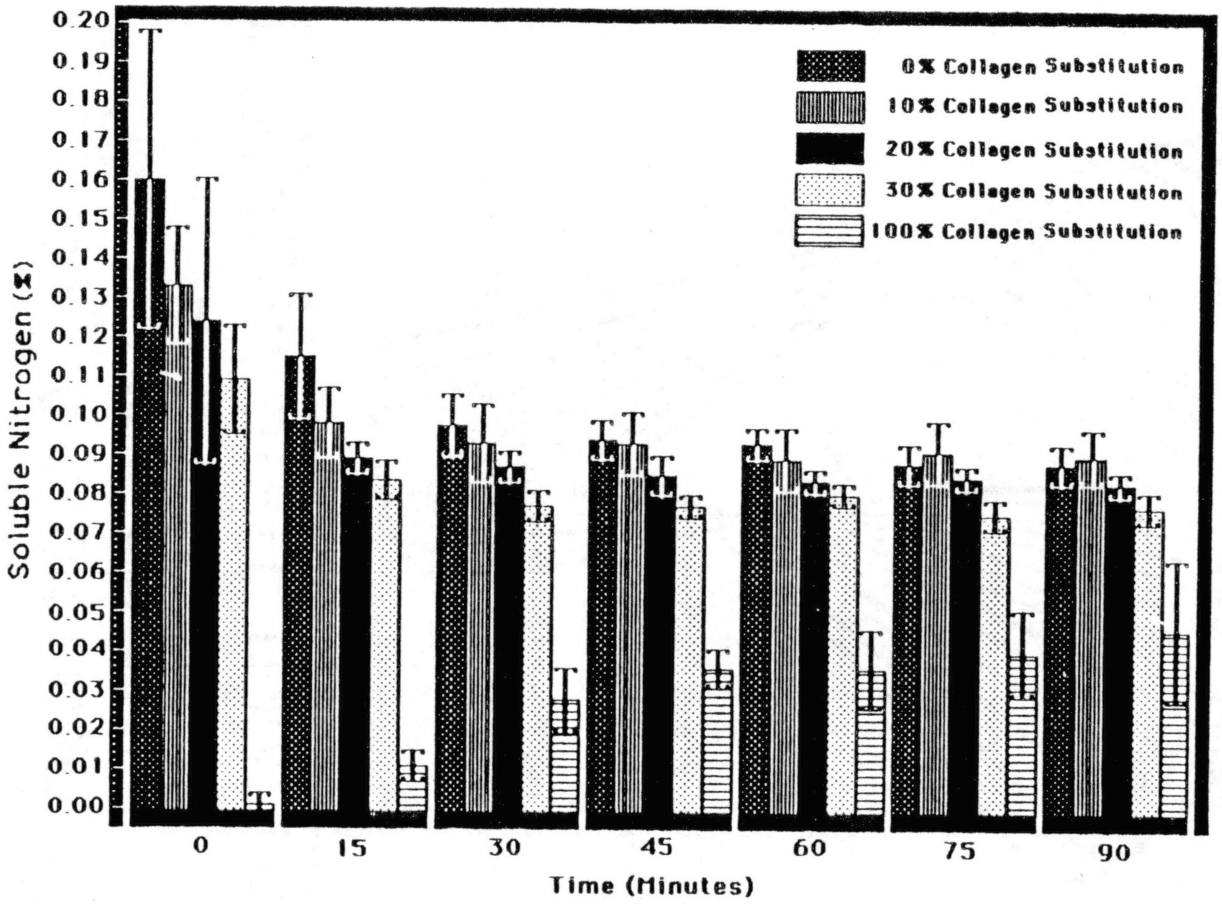

Figure 6. Effect of Collagen Substitution and Heating Time on Soluble Nitrogen at 50 C in the Presence of NaCl and STPP.

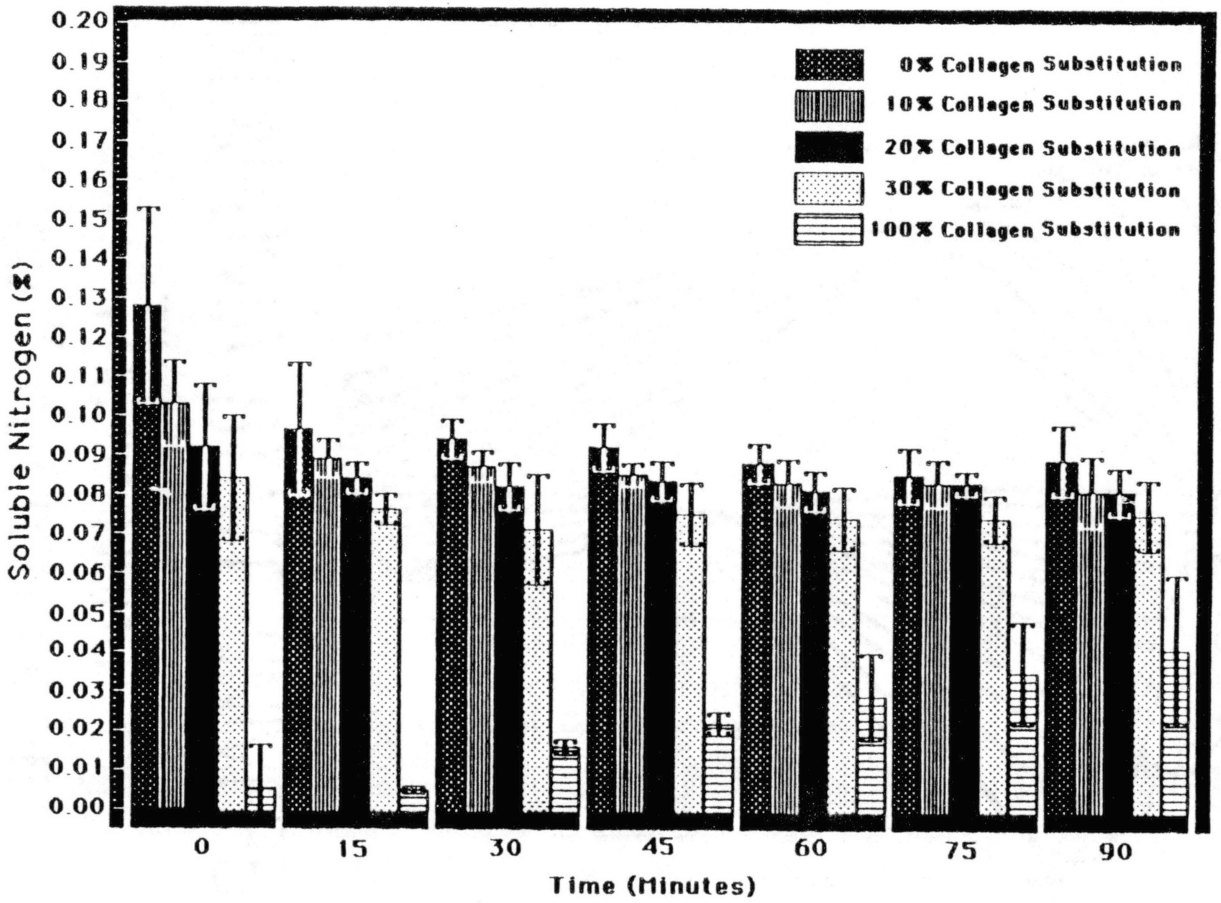

Figure 7. Effect of Collagen Substitution and Heating Time on Soluble Nitrogen at 50 C in the Presence of NaCl.

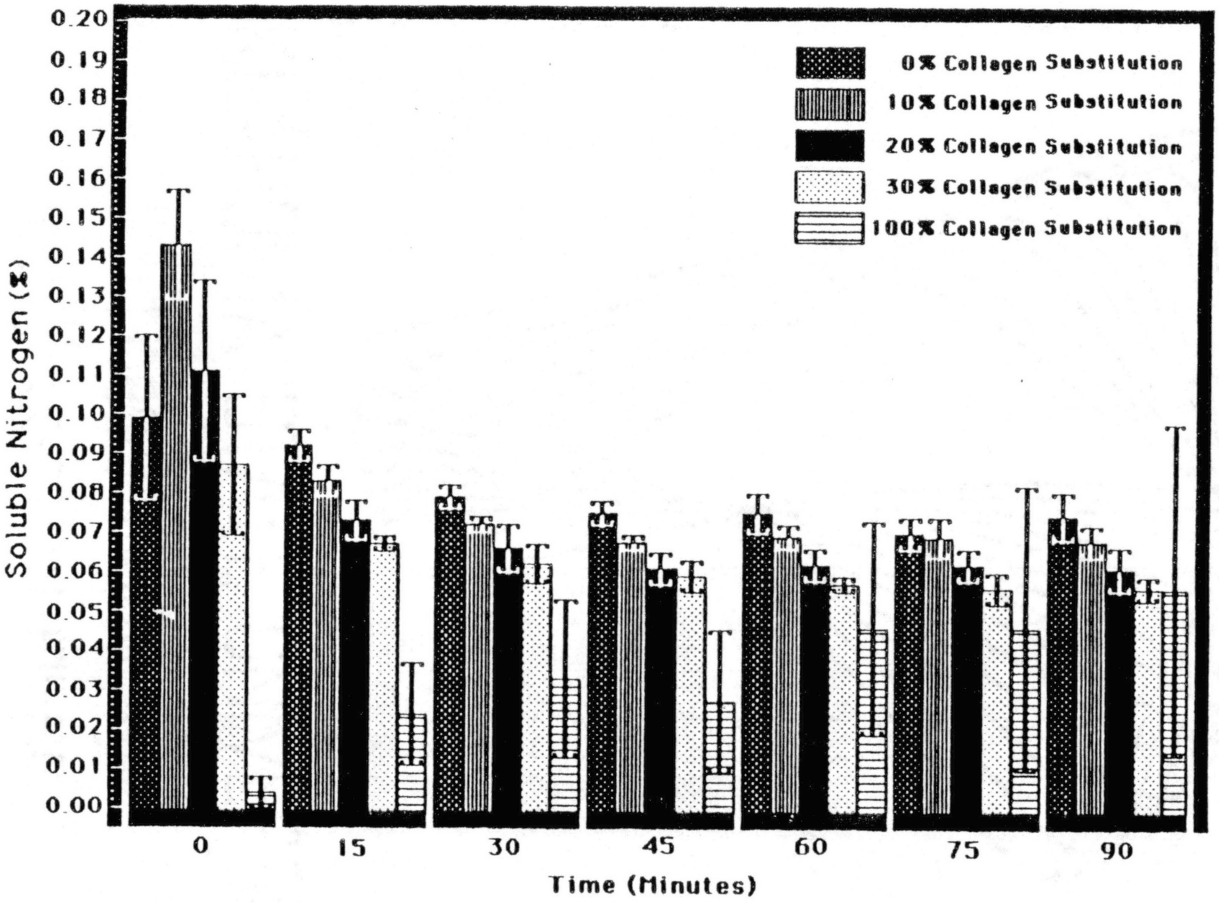

Figure 8. Effect of Collagen Substitution and Heating Time on Soluble Nitrogen at 60 C in the Presence of NaCl and STPP.

decrease in the quantity of sarcoplasmic and myofibrillar protein was due to a collagen dilution effect.

There are two opposing responses affecting the quantity of soluble nitrogen as a result of heat denaturation; a reduction in soluble nitrogen due to the heat coagulation of soluble meat proteins and an increase in solubility associated with the thermal hydrolysis of collagen. At 50 C for both solvents (Figures 6 and 7), the former appeared to exert a greater influence on the quantity of nitrogen in the filtrate than the latter for 10, 20, and 30% replacement levels as shown by the subsequent reduction in soluble nitrogen with increasing collagen substitution of minced muscle. However, at 60 C with 3% NaCl as the solvent (Figure 9) and at 70 C for both solvents (Figures 10 and 11), the difference associated with collagen replacement was not as distinct for heating periods longer than 30 minutes. The majority of the reduction in nitrogen solubility associated with the coagulation of salt-extractable meat proteins had already occurred at these prolonged heating times, thus the primary factor causing changes in solubility was the hydrolysis of fibrous collagen.

In the context of collagen hydrolysis, the quantity of nitrogen in the filtrate is also dependent on the amount of fibrous collagen that replaced the minced muscle component of the meat block. At 0% (Figure 4) and 10% (Figure 12) substitution levels a disparity existed between 60 and 70 degrees at all periods greater than 0 minutes whereas for

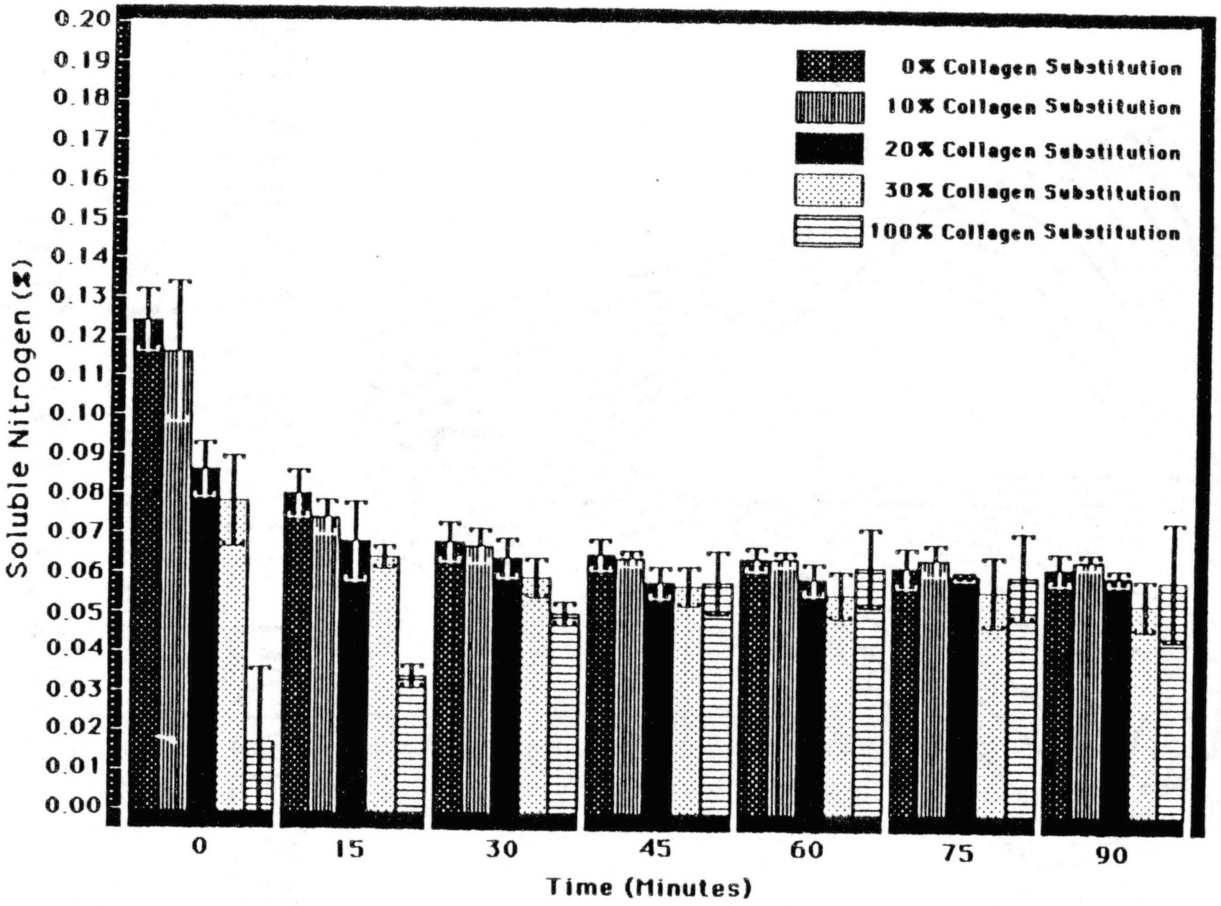

Figure 9. Effect of Collagen Substitution and Heating Time on Soluble Nitrogen at 60 C in the Presence of NaCl.

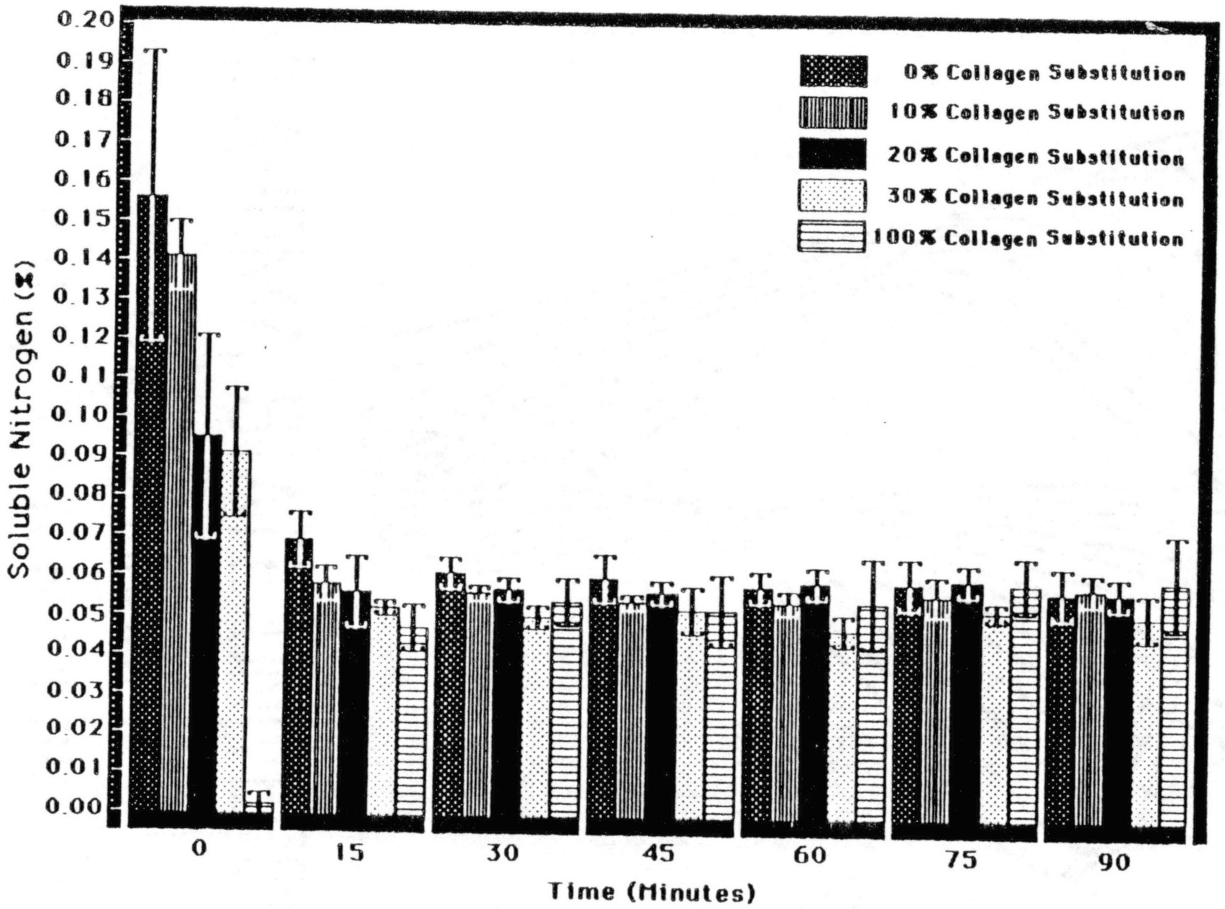

Figure 10. Effect of Collagen Substitution and Heating Time on Soluble Nitrogen at 70 C in the Presence of NaCl and STPP.

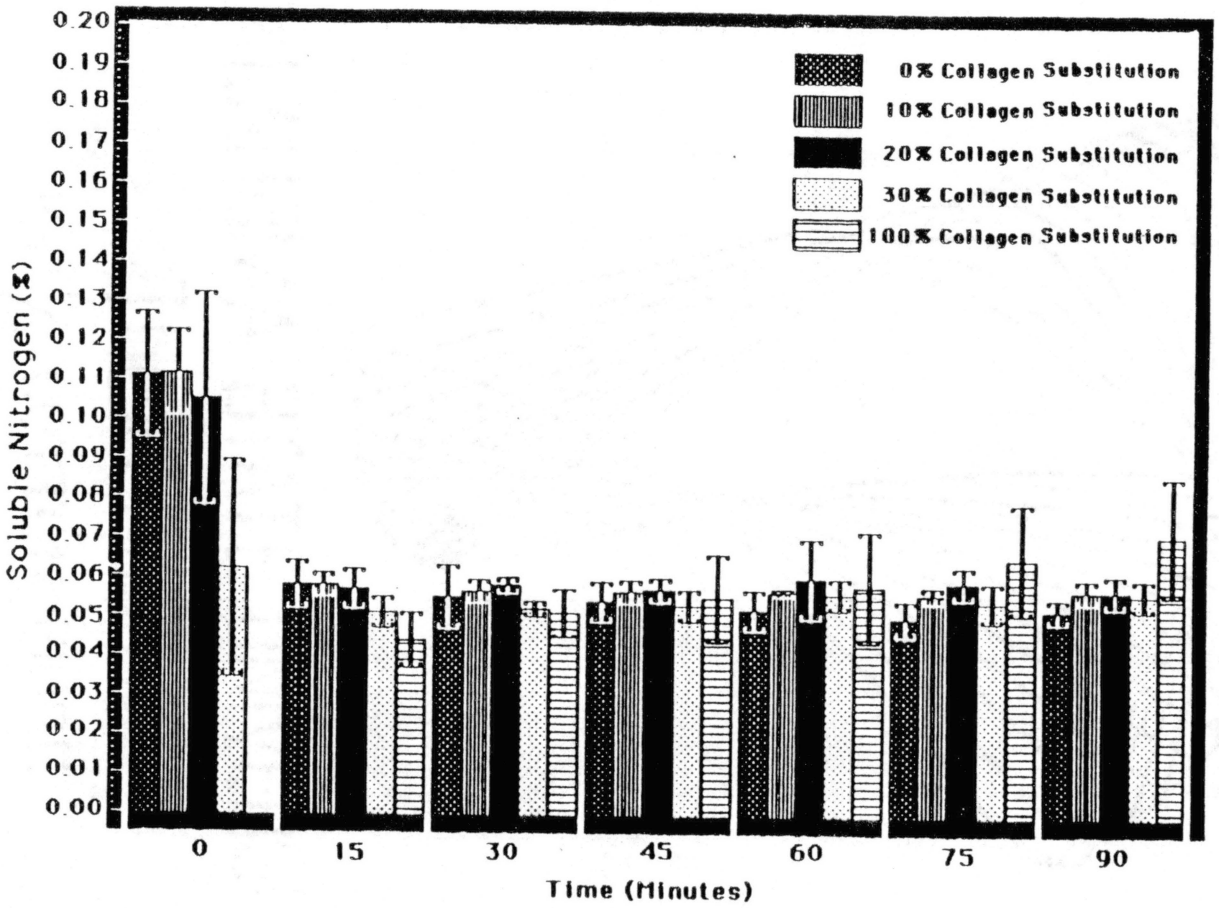

Figure 11. Effect of Collagen Substitution and Heating Time on Soluble Nitrogen at 70 C in the Presence of NaCl.

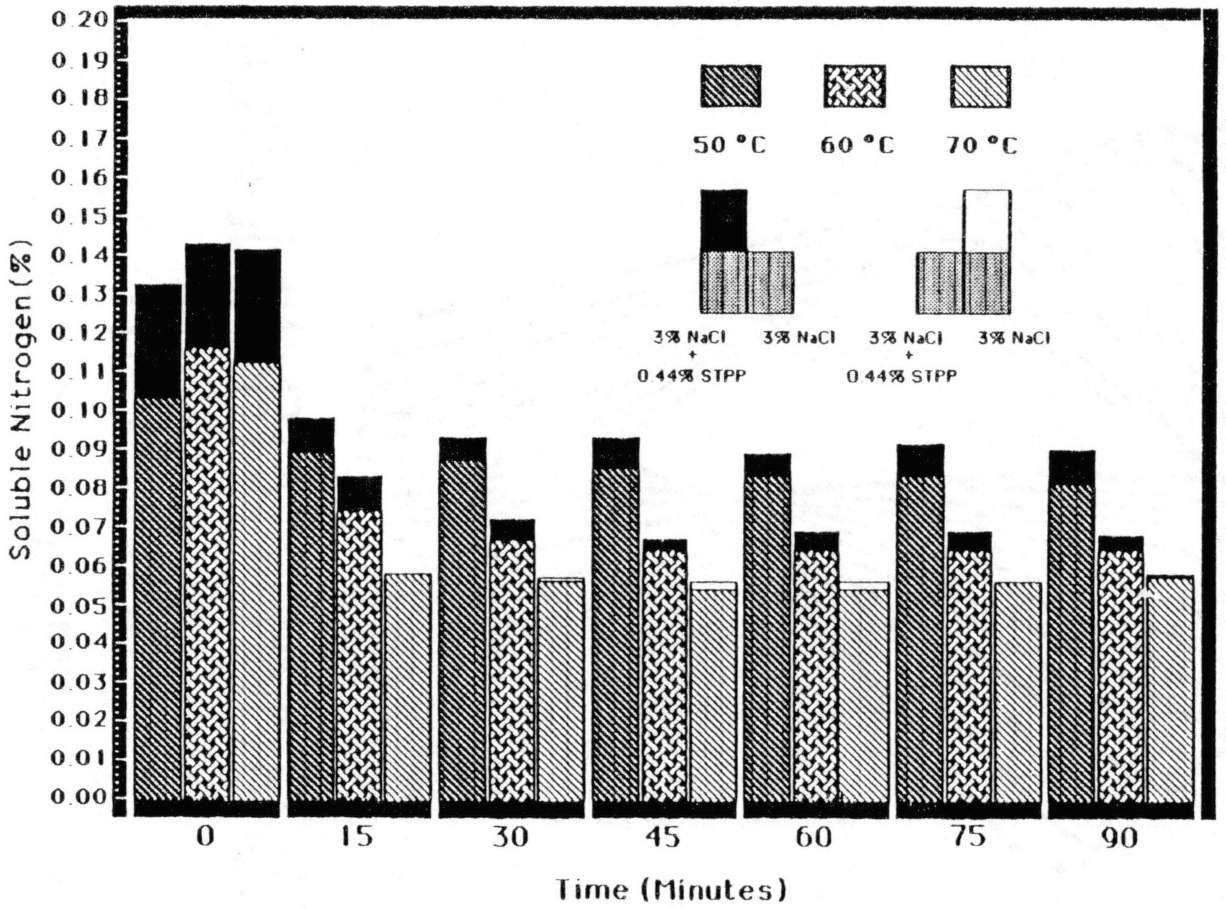

Figure 12. Effect of Temperature, Time, and Solvent on Nitrogen Solubility for 10% Meat Replacement with Fibrous Collagen. Values for both solvents are superimposed at each bar to illustrate STPP effect.

the 20% (Figure 13) and 30% (Figure 14) substitution levels this difference had been negated at prolonged periods of heating. Thus illustrating that more collagen hydrolysis was occurring at 20 and 30 percent substitution levels because more fibrous collagen was present.

Effect of STPP

Sodium tripolyphosphate (STPP) produced an increase ($p < .005$) in the amount of soluble nitrogen when averaged across all levels of temperature, time, and collagen (Table XII of Appendix B). This effect was evident at each combination of temperature and time for the 0 % collagen substitution level as shown by the darkened portion of each bar (Figure 4). The increase between samples extracted with and without phosphate was less for 60 and 70 C than for 50 C at heating times greater than 15 minutes. This difference indicated that the protein components most susceptible to precipitation by heat were most affected by the presence of STPP. This inequality was probably due to the fact that there was a greater quantity of these proteins to be acted upon.

Offer and Trinick (1983) observed that in the presence of 0.4M NaCl and 1mM pyrophosphate the extraction of A and I bands was more extensive than when NaCl was used alone. Therefore, the response difference observed with and without STPP between 50 C and both 60 and 70 C, respectively, may be

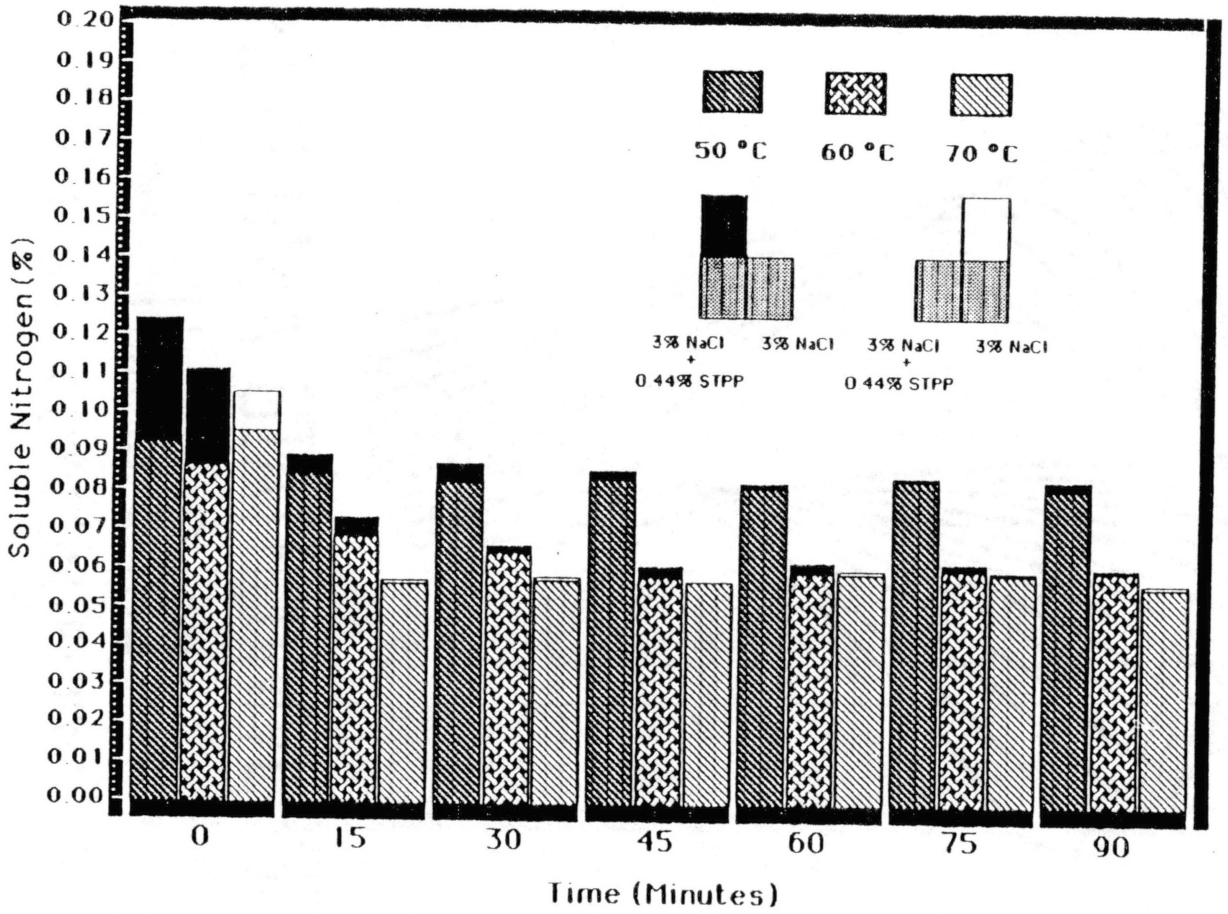

Figure 13. Effect of Temperature, Time, and Solvent on Nitrogen Solubility for 20% Meat Replacement with Fibrous Collagen. Values for both solvents are superimposed at each bar to illustrate STPP effect.

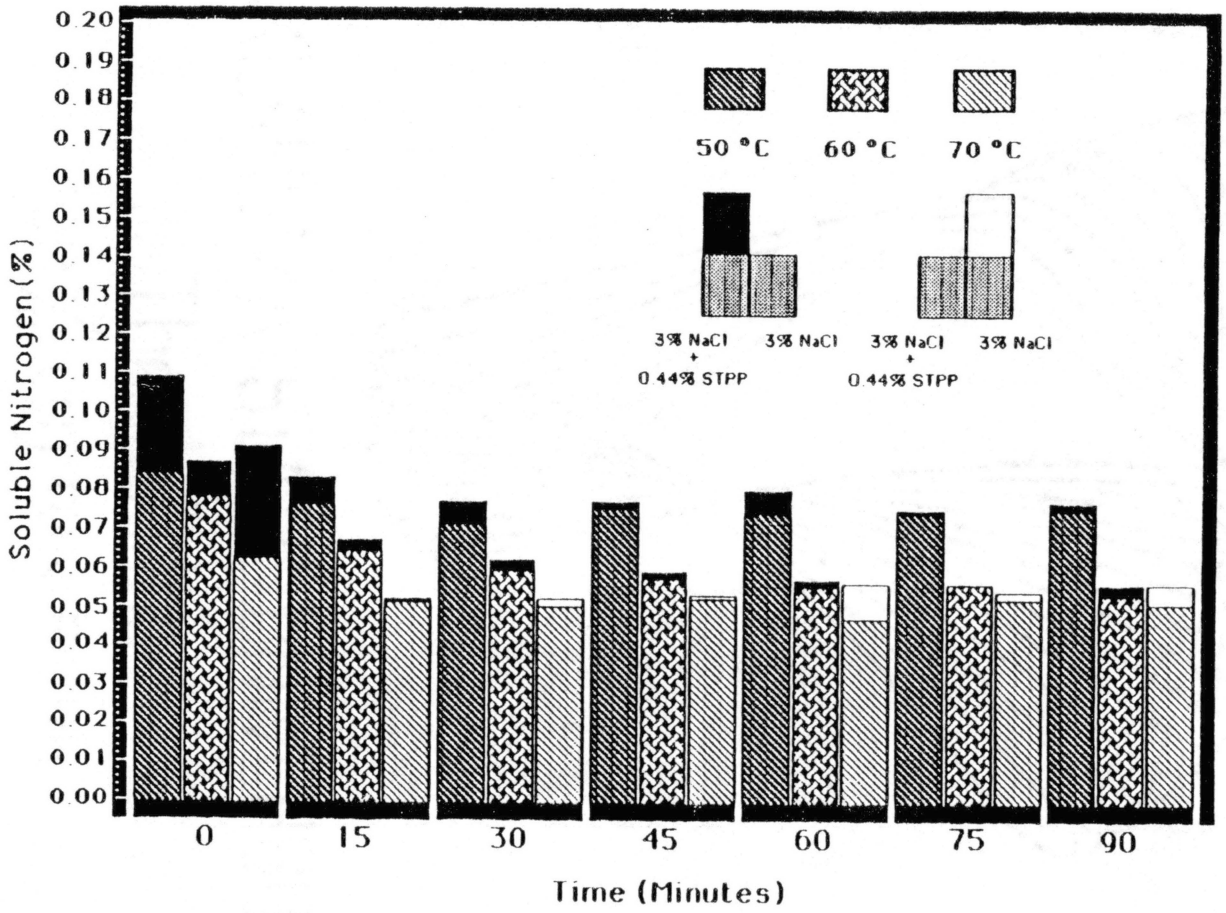

Figure 14. Effect of Temperature, Time, and Solvent on Nitrogen Solubility for 30% Meat Replacement with Fibrous Collagen. Values for both solvents are superimposed at each bar to illustrate STPP effect.

associated with a significant increase in the release of actomyosin. Based on the previously mentioned temperatures at which myosin is denatured (Tm = 55 C) it is plausible to make this assumption (Wright et al., 1977; Cheng and Parrish, 1979).

The increased solubility associated with the use of STPP for 0% collagen substitution and 50, 60, and 70 C was not observed for 10, 20, and 30% collagen substitution at 70 C and heating times greater than 15 minutes as may be seen when comparing results portrayed in Figure 4 and Figures 11, 12, and 13, respectively. As the collagen level increased, soluble nitrogen values approached equality for the two solvent conditions studied. Under the protocol of this experiment, this response illustrated the diverse behavior of collagen and myofibrillar proteins in the presence of heat and was not due to STPP. Three factors that might explain these observations are; 1.) soluble nitrogen attributable to meat proteins was diluted as a result of collagen substitution, 2.) myofibrillar and sarcoplasmic proteins precipitated when heated, thus decreasing the quantity of nitrogen in the filtrate and 3.) collagen solubility increased due to thermal hydrolysis as exhibited by greater soluble nitrogen values. Meat protein dilution and coagulation were no longer contributing to further changes in soluble nitrogen as a result of heat inactivation, whereas, the thermal hydrolysis of collagen was increasing soluble nitrogen for prolonged heating times

at 60 and 70 C and thus resulted in the small differences between solvents that were observed under the conditions employed. The latter response appeared to offset the protein coagulation that had occurred from 60 to 70 C.

The mechanisms whereby STPP increased protein solubility are explained best in terms of its effect on pH and ionic strength. Table I illustrates that pH was significantly greater in the presence of STPP and it is also accurate to assume that ionic concentration was greater when STPP was combined with NaCl than when NaCl was used alone.

TABLE VI

EFFECT OF SOLVENT AND COLLAGEN SUBSTITUTION ON MEAT SLURRY pH PRIOR TO ADJUSTMENT TO pH 6.00[a]

Collagen level	3% NaCl	3% NaCl + 0.44% STPP
0	5.46 (0.15)	6.57 (0.07)
10	5.45 (0.06)	6.62 (0.15)
20	5.52 (0.13)	6.81 (0.11)
30	5.67 (0.08)	6.87 (0.13)
100	7.38 (0.17)	8.38 (0.22)

[a]values in parentheses represent the standard deviation

Meat particle size had been significantly reduced due to the mechanical action of the Omni-mixer and the solvent was present as ten times the amount of the meat block. The combined effects of blending, pH, and the high ratio of the extractant to the meat block produced optimum conditions for

the diffusion and action of sodium tripolyphosphate.

Data in figure 6 revealed that at 50 C, 3% NaCl + 0.44% STPP increased soluble nitrogen for the 100% collagen replacement level, above that found when 3% NaCl was used as the extractant. The collagen solubilized at 50 C may represent newly synthesized collagen since this collagen is stabilized by weak hydrogen bonds, van Der Waals forces, and some electrostatic bonding. Therefore, the addition of STPP, and thus ionic strength and pH effects, may destabilize these bonds to a point that results in more complete release of this constituent of collagen at the 15 minute heating period.

At 60 C and 70 C, heat had less effect on the release of soluble nitrogen in the presence of STPP than when 3% NaCl was used as the extractant. Phosphate could possibly bind to collagen forming bonds that reduce the susceptibility of collagen to thermal hydrolysis. However, this direct mechanism is unlikely, since there appears to be a clearer relationship between increasing pH and greater thermal stability as suggested by Dick and Nordwig (1966) and Stabursvik and Martens (1980).

Dick and Nordwig (1966) studied the effect of pH on the stability of the collagen fold and found that the Ts for a calfskin citrate extract was highly dependent on pH with Tm increasing from 32.1 at pH 1.90 to 37.9 at pH 4.5. These results were interpreted in light of the repulsive forces associated with surplus positive charges. The pH conditions

of this experiment were not that extreme but it could be postulated that, as in meat, the initial increase in pH from 7.38 to 8.38 produced by STPP (Table I) would change the balance of negative charges on the protein molecule and thus affect the solubility of tropocollagen by increasing inter and intramolecular spacing in the protein and subsequently improving hydration.

The increased solubility of collagen at 50 C in the presence of phosphate may represent an effect on the soluble tropocollagen whereas at higher temperatues the suppressing effect seems to be associated with the action of STPP on the more heat resistant component (i. e. intact mature collagen fibrils). Stabursvik and Martens (1980) reported that the Tmax attributable to intramuscular collagen was shifted from 67 to 70 C as pH increased from the region of 5.4-6.0 to 8.00. Ranganyaki et al. (1982) found that at 6% NaCl, hydration was reduced at pH values greater than 8.00 and less than 5.00. This was attributed to a shielding effect of ions on the negative and postive charges at these extreme pH values. This work may indicate that the degree of hydration affects the degree of thermal stability for fibrous collagen.

In this experiment, however, the effect of temperature was preeminent since with both solvent conditions more collagen was solubilized at 70 C, followed by 60 C and 50 C, respectively (Figure 5). A consideration of what portion of soluble nitrogen was due to newly synthesized collagen and

what portion was due to mature collagen may clarify why 3% NaCl + 0.44% STPP produced an increase in solubility at 50 C but suppressed solubility at 60 and 70 C when compared to those values obtained for 3% NaCl.

Statistical Analysis

F-tests from the analysis of variance revealed highly significant collagen by treatment temperature and collagen by STPP interactions for the whole units when 100% collagen was included in the analysis (Table XII of Appendix B). There were significant differences in soluble nitrogen associated with Temperature ($p < .0001$), Collagen ($p < .0001$), and STPP ($p < .005$). When the analysis was conducted excluding data from the 100% collagen level (Table XIII of Appendix B) the collagen by treatment temperature and collagen by STPP interactions for the whole units were not significant ($p < .05$).

The diverse physicochemical properties of each protein source that is evident by the manner each protein manifests heat denaturation, could have contributed to these interactions. For example 0% substituted collagen (100% meat) (Figure 4) and 100% collagen (Figure 5) did not respond in a similar manner at either 50, 60, or 70 C. The initial decline in soluble nitrogen for 0% collagen substitution was matched by an almost equal and opposite response for 100% Collagen during the first 15 minutes of heating. Muscle proteins coagulated and became insoluble

whereas collagen solubility increased on exposure to heat.

Temperature by time, collagen by time, and STPP by time interactions were highly significant with or without the inclusion of 100% collagen in the analysis. The quantity of nitrogen present in the filtrate prior to heating decreased as collagen increased therefore the initial drop was more drastic during the first 15 min. interval (Figure 6) for 0% collagen substitution than for 30% collagen substitution, denoting a dissimilar response for 0% and 30% collagen at the 15 minute heating time. Exclusion of the data from the 0 minute time interval produced a nonsignificant temperature by time and STPP by time interaction in addition to a nonsignificant collagen by STPP and temperature by STPP interactions. Thus, based on this analysis, the majority of the changes for both the temperature and collagen treatment factors occurred during the initial 15 minute heating period.

Regression analysis revealed significant quadratic effects across time for each collagen and temperature level when 100% collagen was included in the analysis ($p < .0001$). This trend indicated that the major changes in protein solubility occured initially, followed by a stationary response for all levels of meat replacement. Quadratic trends at each collagen and STPP level were highly significant ($p < .0001$) as well. There was also a highly significant quadratic effect observed at 60 and 70 C ($p < .0001$) across all levels of collagen and STPP; whereas the

data at 50 C did not follow with as significant a quadratic effect ($p < .005$).

Duncan's multiple range test was used to compare means at each level of time for each of the thirty treatment combinations under study (Tables II-VI of Appendix A). In general, this procedure revealed significant differences among means between 0 and 15 minutes or 0 and 30 minutes after which periods there was virtually no difference ($p < .05$) among means. This analysis is supportive of the regression analysis results that revealed the significant quadratic trends among the treatment factors.

Experimental Problems

Those data shown at the 0 minute interval for 0, 10, 20, 30, and 100% collagen substitution (Figures 4, 12, 13, 14, and 5, respectively) exhibited considerable variation. These values should have been similar for a given collagen level regardless of the temperature specified (50, 60, or 70 C) because each represented the unheated sample and thus, had received the same treatment prior to heating.

As previously mentioned, missing values were predicted and used in subsequent statistical analysis. There were missing values at the 0 minute time interval because this level was not considered for experimentation initially in the first block of treatment combinations. Also, there was some missing data due to sample loss. The value obtained at the 0 minute time level represented the upper limit for 0,

10, 20, and 30% collagen substitution and the lower limit for 100% collagen substitution. Therefore, it is expected that regression analysis will underestimate the extremely high values and overestimate the extremely low values. This limitation may partially account for the inconsistencies observed at the 0 minute time level for a given level of collagen substitution.

Some variability may be accounted for by the filtration technique used for the unheated sample. Eight layers of cheese cloth were used, thus if suspended insoluble protein from the bottom of the centrifuge tube were allowed to pass through the cheese cloth the values may be erroneously high. Filter paper, as was used for the cooked sample, may have alleviated this problem by preventing this suspended insoluble material from passing into the filtrate.

Future Research

Future research should include studies that would detect which components of collagen are least resistant to heat, aside from those components released initially at the thermal shrinkage temperature (39 C). It may also lend to a clearer understanding of the results found in this study if collagen had been subjected to heat in the abscence of both NaCl and STPP, since ionic environment has such a profound effect on protein behavior. Research might also be conducted to detect if there is any association between heat labile collagen and actin, myosin, and actomyosin and if so,

elucidate the mechanism for the association. If there are any interactions between these proteins, this knowledge might contribute to optimizing the use of hide collagen in such meat systems as frankfurters and bologna.

CHAPTER V

SUMMARY AND CONCLUSIONS

The objectives of this study were to determine how the substitution of collagen affected soluble protein content under various hydrothermal and solvent conditions and to ascertain if any interaction between collagen and soluble muscle proteins existed.

Changes in nitrogen solubility were monitored as an indication of protein solubility to determine the effect of different heating schedules, increased collagen substitution levels, and sodium tripolyphosphate (STPP) on protein solubility. Temperatures of 50, 60, and 70 C and heating times of 0, 15, 30, 45, 60, 75, and 90 minutes constituted the heating schedule. Minced meat with no collagen substitution and collagen substitutions of 10, 20, 30, and 100% were designated as the meat block. The meat block was blended with ten parts of either 3% NaCl or 3% NaCl + 0.44% STPP and the resulting slurry was adjusted to pH 6.00. Following pH adjustment, the sample was heated at the specifed temperature and time. The cooked sample was immediately filtered and the filtrate was subsequently analyzed for nitrogen.

The quantity of nitrogen in the filtrate was

significantly reduced after 15 minutes at either 50, 60, or 70 C. This reduction appeared to be due primarily to the denaturation of the more heat sensitive protein (myosin) at 50 and 60 C and a comibination of both myosin and the less heat sensitive proteins at 70 C.

The replacement of meat with increasing amounts of collagen produced a proportional decrease in the quantity of soluble nitrogen observed at the 0 minute time period. This decrease appeared to be due to a reduction in the amount of minced muscle available in the meat block for solvent extraction. This trend was apparent at 50 C for both solvents and at 60 C in the presence of STPP for all heating times. However, at 60 C without STPP present in the solvent and at 70 C with both solvents, the difference associated with collagen substitution was not as distinct for heating periods longer than 30 minutes. The merging of the results under these conditions was apparently due to the thermal solubilization of collagen at the higher temperatures, prolonged heating times, and increased collagen levels. For the 100% collagen level, a large increase in the amount of nitrogen in the filtrate at 70 C occurred after 15 minutes of heating. A similar trend was observed for 60 C at the 100% collagen level, but it was smaller in magnitude than at 70 C. Sodium tripolyphosphate inhibited the release of soluble nitrogen at 60 C and 70 C, however at 50 C, STPP facilitated the heat extraction of collagen. Sodium tripolyphosphate significantly increased the amount of

soluble nitrogen in the filtrate for the 0, 10, 20, and 30% collagen levels as a result of its action on the minced muscle component of the meat block.

The presence of quadratic trends and the interactions among main unit and subunit treatment factors were dependent on the changes in solubility for each collagen level during the initial 15 minute heating period.

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APPENDIXES

APPENDIX A

TABLES OF MEANS

TABLE II

EFFECT OF TEMPERATURE, TIME, AND SOLVENT ON SOLUBLE NITROGEN (%) FOR 0% MEAT REPLACEMENT WITH COLLAGEN[a,b]

Time (min)	50 C		60 C		70 C	
	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP
0	0.128a	0.160a	0.124a	0.099a	0.111a	0.156a
15	0.097b	0.115b	0.080b	0.092a	0.058b	0.069b
30	0.094bc	0.098c	0.068c	0.079b	0.055b	0.061bc
45	0.092bc	0.094cd	0.065c	0.075b	0.054b	0.060bc
60	0.088bc	0.093cd	0.064c	0.075b	0.052b	0.058c
75	0.085c	0.088d	0.062c	0.070b	0.050b	0.059c
90	0.089bc	0.088d	0.062c	0.074b	0.052b	0.057c

[a]Means in columns not followed by the same letter are significantly different ($p < 0.05$).
[b]Each value is a mean of six observations with predicted values replacing missing observations.

TABLE III

EFFECT OF TEMPERATURE, TIME, AND SOLVENT ON SOLUBLE NITROGEN (%) FOR 10% MEAT REPLACEMENT WITH COLLAGEN[a,b]

Time (min)	50 C		60 C		70 C	
	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP
0	0.103a	0.133a	0.116a	0.143a	0.112a	0.141a
15	0.089b	0.098b	0.074b	0.083b	0.058b	0.058b
30	0.087b	0.093b	0.067bc	0.072c	0.057b	0.056b
45	0.085b	0.093b	0.064c	0.067c	0.056b	0.054b
60	0.083b	0.089b	0.064c	0.069c	0.056b	0.054b
75	0.083b	0.091b	0.064c	0.069c	0.056b	0.056b
90	0.081b	0.090b	0.064c	0.068c	0.057b	0.058b

[a]Means in columns not followed by the same letter are significantly different ($p < 0.05$).
[b]Each value is a mean of six observations with predicted values replacing missing observations.

TABLE IV

EFFECT OF TEMPERATURE, TIME, AND SOLVENT ON SOLUBLE NITROGEN (%) FOR 20% MEAT REPLACEMENT WITH COLLAGEN[a,b]

Time (min)	50 C		60 C		70 C	
	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP
0	0.092a	0.124a	0.086a	0.111a	0.104a	0.095a
15	0.084ab	0.089b	0.068b	0.073b	0.057b	0.056b
30	0.082ab	0.087b	0.064c	0.066bc	0.058b	0.057b
45	0.083ab	0.085b	0.058bc	0.061c	0.057b	0.057b
60	0.081b	0.082b	0.059bc	0.062c	0.060b	0.059b
75	0.083ab	0.084b	0.060bc	0.062c	0.059b	0.060b
90	0.081b	0.083b	0.060bc	0.061c	0.057b	0.056b

[a]Means in columns not followed by the same letter are significantly different ($p < 0.05$).
[b]Each value is a mean of six observations with predicted values replacing missing observations.

TABLE V

EFFECT OF TEMPERATURE, TIME, AND SOLVENT ON SOLUBLE NITROGEN (%) FOR 30% MEAT REPLACEMENT WITH COLLAGEN[a,b]

Time (min)	50 C		60 C		70 C	
	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP
0	0.084a	0.109a	0.078a	0.087a	0.062a	0.091a
15	0.076b	0.083b	0.064b	0.067b	0.051b	0.052b
30	0.071b	0.077b	0.059bc	0.062bc	0.052b	0.050b
45	0.075b	0.077b	0.057bc	0.059bc	0.053ab	0.052b
60	0.074b	0.080b	0.055bc	0.057c	0.056ab	0.048b
75	0.074b	0.075b	0.056bc	0.056c	0.054ab	0.052b
90	0.075b	0.077b	0.053c	0.056c	0.056ab	0.051b

[a]Means in columns not followed by the same letter are significantly different ($p < 0.05$).
[b]Each value is a mean of six observations with predicted values replacing missing observations.

TABLE VI

EFFECT OF TEMPERATURE, TIME, AND SOLVENT ON SOLUBLE NITROGEN (%) FOR 100% MEAT REPLACEMENT WITH COLLAGEN[a,b]

Time (min)	50 C		60 C		70 C	
	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP
0	0.006e	0.001e	0.017d	0.004e	0.000e	0.002c
15	0.005e	0.011d	0.034c	0.024d	0.044d	0.047b
30	0.016d	0.028c	0.050b	0.033c	0.051cd	0.054ab
45	0.022cd	0.036bc	0.058a	0.027cd	0.055c	0.052ab
60	0.029bc	0.036bc	0.062a	0.046b	0.058bc	0.054ab
75	0.035ab	0.040ab	0.060a	0.046b	0.065ab	0.059a
90	0.041a	0.046a	0.059a	0.056a	0.071a	0.060a

[a]Means in columns not followed by the same letter are significantly different ($p < 0.05$).
[b]Each value is a mean of six observations with predicted values replacing missing observations.

TABLE VII

EFFECT OF TEMPERATURE, TIME, AND SOLVENT ON pH FOR 0% MEAT REPLACEMENT WITH COLLAGEN[a,b,c]

Time (min)	50 C		60 C		70 C	
	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44STPP	3%NaCl	3%NaCl + 0.44%STPP
0	5.94c	6.00a	5.96b	6.00a	5.99a	6.00a
15	5.89d	5.96b	6.01a	5.98a	6.01a	5.98a
30	6.02b	5.98ab	6.02a	5.98a	6.01a	5.97a
45	6.05ab	5.98ab	6.03a	5.97a	6.01a	5.97a
60	6.06ab	5.99ab	6.02a	5.99a	6.01a	5.99a
75	6.06a	6.00a	6.03a	5.97a	6.01a	5.99a
90	6.04ab	6.00a	6.03a	5.98a	6.00a	5.98a

[a]Means in columns not followed by the same letter are significantly different ($p < .05$).
[b]Each value is a mean of six observations with predicted values replacing missing observations.
[c]The values from 15 to 90 minutes were obtained from the filtrate of the cooked slurry that had been adjusted to pH 6.00 prior to heating.

TABLE VIII

EFFECT OF TEMPERATURE, TIME, AND SOLVENT ON pH FOR 10% MEAT REPLACEMENT WITH COLLAGEN[a,b,c]

Time (min)	50 C		60 C		70 C	
	3%NaCl	3%NaCl + 0.44STPP	3%NaCl	3%NaCl + 0.44STPP	3%NaCl	3%NaCl + 0.44STPP
0	5.98a	6.01b	6.00a	6.01a	6.02ab	6.00a
15	5.94b	6.02b	5.92b	6.01a	6.01b	5.97b
30	5.97ab	6.07a	5.91b	6.01a	6.02ab	5.97b
45	5.96ab	6.06a	5.92b	6.01a	6.04ab	5.97b
60	5.96ab	6.06a	5.92b	6.01a	6.04ab	5.97b
75	5.97ab	6.07a	5.91b	6.01b	6.05a	5.97b
90	5.97ab	6.07a	5.90b	6.02a	6.05a	5.97b

[a]Means in columns not followed by the same letter are significantly different ($p < 0.05$).
[b]Each value is a mean of six observations with predicted values replacing missing observations.
[c]The values from 15 to 90 minutes were obtained from the filtrate of the cooked slurry that had been adjusted to pH 6.00 prior to heating.

TABLE IX

EFFECT OF TEMPERATURE, TIME, AND SOLVENT ON pH FOR 20% MEAT REPLACEMENT WITH COLLAGEN[a,b,c]

Time (min)	50 C		60 C		70 C	
	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP
0	6.03a	6.02a	6.04a	6.01a	6.06b	6.00b
15	5.93c	6.03a	5.97b	5.99a	6.10a	6.02ab
30	5.96cb	6.03a	5.98b	6.00a	6.09a	6.03ab
45	5.97b	6.02a	5.98b	5.99a	6.11a	6.03ab
60	5.97b	6.05a	5.99b	6.00a	6.11a	6.04a
75	5.96b	6.04a	5.99b	5.99a	6.12a	6.04ab
90	5.97b	6.04a	5.99b	5.99a	6.12a	6.04ab

[a]Means in columns not followed by the same letter are significantly different ($p < .05$).
[b]Each value is a mean of six observations with predicted values replacing missing observations.
[c]The values from 15 to 90 minutes were obtained from the filtrate of the cooked slurry that had been adjusted to pH 6.00 prior to heating.

TABLE X

EFFECT OF TEMPERATURE, TIME, AND SOLVENT ON pH FOR 30% MEAT REPLACEMENT WITH COLLAGEN[a,b,c]

Time (min)	50 C		60 C		70 C	
	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP
0	6.07d	6.03a	6.08a	6.01a	6.09a	6.00b
15	6.03e	5.95c	6.00b	6.03a	6.09a	6.08a
30	6.11c	5.96bc	5.99b	6.03a	6.08a	6.07a
45	6.24a	5.96bc	6.00b	6.03a	6.07a	6.07a
60	6.21a	5.98bc	6.01b	6.04a	6.07a	6.07a
75	6.15b	5.98b	6.01b	6.04a	6.07a	6.07a
90	6.18b	5.99b	6.02b	6.04a	6.08a	6.07a

[a]Means in columns not followed by the same letter are significantly different ($p < .05$).
[b]Each value is a mean of six observations with predicted values replacing missing observations.
[c]The values from 15 to 90 minutes were obtained from the filtrate of the cooked slurry that had been adjusted to pH 6.00 prior to heating.

TABLE XI

EFFECT OF TEMPERATURE, TIME, AND SOLVENT ON pH FOR 100% MEAT REPLACEMENT WITH COLLAGEN[a,b,c]

Time (min)	50 C		60 C		70 C	
	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP	3%NaCl	3%NaCl + 0.44%STPP
0	6.39c	6.09b	6.37c	6.05a	6.34e	6.00a
15	6.51a	6.13a	6.25c	6.00b	6.39d	5.97a
30	6.50ab	6.13a	6.21b	6.01b	6.39d	5.98a
45	6.41c	6.13a	6.24a	6.01b	6.42c	5.99a
60	6.50ab	6.13a	6.26a	6.02b	6.47b	6.00a
75	6.31d	6.14a	6.26a	6.01b	6.41cd	5.99a
90	6.47b	6.13a	6.29a	6.01b	6.50a	5.99a

[a]Means in columns not followed by the same letter are significantly different ($p < .05$).
[b]Each value is a mean of six observations with predicted values replacing missing observations.
[c]The values from 15 to 90 minutes were obtained from the filtrate of the cooked slurry that had been adjusted to pH 6.00 prior to heating.

APPENDIX B

TABLES OF ANALYSIS OF VARIATION

TABLE XII

ANALYSIS OF VARIATION FOR SOLUBLE NITROGEN INCLUDING ALL DATA

Source	DF	Mean Square	F Value	PR>F
Total	1259	0.95986662		
Block (B)[a]	2	0.00184338	33.87	0.0001
Collagen (C)	4	0.07805242	130.89	0.0001
Temp (Te)	2	0.03047339	51.10	0.0001
STPP (P)	1	0.00530130	8.89	0.0042
C X Te	8	0.01117309	18.74	0.0001
C X P	4	0.00153660	2.58	0.0468
Te X P	2	0.00121803	2.04	0.1389
C X Te X P	8	0.00058842	0.99	0.4558
Error (a)	58	0.00059633		
Time (Ti)	6	0.01686052	77.20	0.0001
Error (b)	12	0.00021841		
C X Ti	24	0.00865005	46.27	0.0001
Error (c)	56	0.00018694		
Te X Ti	12	0.00041139	2.85	0.0110
Error (d)	28	0.00014441		
P X Ti	6	0.00121189	10.44	0.0002
Error (e)	14	0.00011612		
C X Te X P X Ti	132	0.00030296	2.86	0.0001
Error (f)	250	0.00010578		

[a]Each effect is tested using the first error term appearing below it.

TABLE XIII

ANALYSIS OF VARIATION FOR SOLUBLE NITROGEN EXCLUDING THE 100% COLLAGEN LEVEL

Source	DF	Mean Square	F Value	PR>F
Total	1007	0.54436140		
Block (B)[a]	2	0.00413779	92.92	0.0001
Collagen (C)	3	0.01330625	38.81	0.0001
Temp (Te)	2	0.06195322	180.70	0.0001
STPP (P)	1	0.00924629	26.97	0.0001
C X Te	6	0.00055422	1.62	0.1641
C X P	3	0.00044358	1.29	0.2878
Te X P	2	0.00039783	1.16	0.3224
C X Te X P	6	0.00023892	0.70	0.6534
Error (a)	46	0.00034285		
Time (Ti)	6	0.03656591	147.12	0.0001
Error (b)	12	0.00024855		
C X Ti	18	0.00141059	26.68	0.0001
Error (c)	42	0.00004919		
Te X Ti	12	0.00104178	9.48	0.0001
Error (d)	28	0.00010989		
P X Ti	6	0.00155003	48.48	0.0001
Error (e)	14	0.00003197		
C X Te X P X Ti	102	0.00021558	4.84	0.0001
Error (f)	192	0.00010815		

[a]Each effect is tested using the first error term appearing below it.

TABLE XIV

ANALYSIS OF VARIATION FOR SOLUBLE NITROGEN EXCLUDING THE 0 MINUTE TIME INTERVAL

Source	DF	Mean Square	F Value	PR>F
Total	1079	0.39614305		
Block (B)[a]	2	0.00081948	27.41	0.0001
Collagen (C)	4	0.03184669	68.88	0.0001
Temp (Te)	2	0.03123610	67.56	0.0001
STPP (P)	1	0.00132820	2.87	0.0954
C X Te	8	0.01248797	27.01	0.0001
C X P	4	0.00090400	1.96	0.1134
Te X P	2	0.00073950	1.60	0.2108
C X Te X P	8	0.00062298	1.35	0.2391
Error (a)	58	0.00046233		
Time (Ti)	5	0.00014707	6.45	0.0063
Error (b)	10	0.00002280		
C X Ti	20	0.00130953	10.00	0.0001
Error (c)	48	0.00013094		
Te X Ti	10	0.00014007	0.99	0.4776
Error (d)	24	0.00014141		
P X Ti	5	0.00011195	0.88	0.5215
Error (e)	12	0.00012678		
C X Te X P X Ti	110	0.00004890	2.15	0.0001
Error (f)	206	0.00002271		

[a]Each effect is tested using the first error term appearing below it.

TABLE XV

ANALYSIS OF VARIATION FOR SOLUBLE NITROGEN EXCLUDING THE 0 MINUTE TIME INTERVAL AND 100% COLLAGEN LEVEL

Source	DF	Mean Square	F Value	PR>F
Total	863	0.19203092		
Block (B)[a]	2	0.00215721	202.28	0.0001
Collagen (C)	3	0.00556098	33.30	0.0001
Temp (Te)	2	0.06547494	392.05	0.0001
STPP (P)	1	0.00284513	17.04	0.0002
C X Te	6	0.00070259	4.21	0.0019
C X P	3	0.00048823	2.92	0.0437
Te X P	2	0.00041426	2.48	0.0948
C X Te X P	6	0.00010667	0.64	0.6986
Error (a)	46	0.00016701		
Time (Ti)	5	0.00130387	67.07	0.0001
Error (b)	10	0.00001944		
C X Ti	15	0.00015263	6.09	0.0001
Error (c)	36	0.00002506		
Te X Ti	10	0.00023592	2.66	0.0242
Error (d)	24	0.00008879		
P X Ti	5	0.00007726	2.08	0.1390
Error (e)	12	0.00003719		
C X Te X P X Ti	110	0.00001595	2.07	0.0001
Error (f)	206	0.00000770		

[a]Each effect is tested using the first error term appearing below it.

VITA 2

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Candidate for the Degree of

Master of Science

Thesis: INFLUENCE OF TEMPERATURE, TIME, AND SODIUM TRIPOLYPHOSPHATE ON THE SOLUBLITY OF COLLAGEN AND SALT-EXTRACTABLE MEAT PROTEINS

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