BINDING PROPERTIES OF MEAT BLENDS AS AFFECTED BY NaCl TYPE, BLENDING TIME AND POST-BLENDING STORAGE

By

JOHN FRANKLIN CAMPBELL

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1980

ABSTRACT

BINDING PROPERTIES OF MEAT BLENDS

AS AFFECTED BY NaCl TYPE,

BLENDING TIME AND POST-BLENDING STORAGE

By

John Franklin Campbell

A study was designed and conducted to evaluate the binding properties of red meat sausage blends as they are influenced by NaCl type, blending time and post-blending storage time. Three commercially available NaCl types were evaluated. These NaCl types were flake, dendritic and evaporated/granulated. Blending times of 0, 2, 4, 6, 8 and 10 minutes and post-blending storage times of 0, 1, 2, 3, 4 and 5 days were also evaluated. In order to evaluate these treatments, model systems measurements such as soluble phase volume, soluble phase protein concentration, blend pH and blend stability were collected and evaluated. A second part of this study was conducted to evaluate the more promising blending and post-blending storage times. Blending and post-blending storage times utilized in this portion were 6, 8 and 10 minutes and 0, 1 and 5 days, respectively.

Blends manufactured utilizing flake NaCl exhibited superior binding properties, as indicated by higher blend pH's, lower soluble phase volumes and greater soluble phase protein concentrations compared to blends prepared with dendritic or evaporated/granulated NaCl's ($P<0.05$). These observations also carried over to blend stability parameters, with the flake NaCl blends exhibiting significantly less loss due to thermal treatment than the other two NaCl types ($P<0.05$). Dendritic NaCl produced blends which were consistently superior to the evaporated/granulated NaCl type for all parameters evaluated ($P<0.05$).

Blending time exhibited no influence on blend pH value. As blending time increased from zero to eight minutes, soluble phase volume and soluble phase protein concentration responded accordingly reaching their optimal values following a blending time of eight minutes ($P<0.05$). The blend stability parameters of water loss and total loss responded accordingly to blending time ($P<0.05$). However fat loss reached a minimum following six minutes of blending and no additional improvement was noted ($P<0.05$) with additional blending time.

Post-blending storage time had no apparent influence on blend pH. However, soluble phase volume decreased to a minimum following four days of post-blending storage and then exhibited an increase after one additional day

of storage ($P<0.05$). Soluble phase protein concentration increased as blending time increased, with a maximum value following five days of storage ($P<0.05$). Blend stability parameters of total and water loss exhibited initial decreases ($P<0.01$) following one day of storage and then remained relatively constant throughout the five day storage period.

There was a significant interaction between blending time and post-blending storage time on the soluble phase protein concentration ($P<0.05$). This interaction suggested that blending times of four, six and ten minutes resulted in essentially the same concentrations of soluble protein from two throughout five days of storage. The second part of this study showed that no differences existed in yield or product composition due to blending time. Yields were maximum ($P<0.01$) following one day of post-blending storage as compared to zero and five days of storage.

Sensory evaluation of the experimental bologna products showed no differences due to either blending time or post-blending storage time.

These results indicate that a blending time of six to eight minutes coupled with a post-blending storage time of one day in a preblending system utilizing ribbon blenders will result in a product with improved yield, increased protein functionality and no perceptible alterations in the sensory quality of bologna type products.

To the Mag

No blend correction required

ACKNOWLEDGMENTS

To those people who served on my committee, Drs. Price, Steffe, Wishnetsky, Dawson, Nicholas and Reynolds a thank you is extended. In addition, a special note of appreciation is extended to Roger Mandigo of the University of Nebraska for it was Roger who initially took a chance on me and has taught me virtually everything I know about the expedition of bureaucratic processes and the manufacture of meat products.

To Bea Eichelberger, who taught me how to play bridge, and to Dora Spooner who kept me in register and put up with my occasional audacity, thank you. To those fellow graduate students who are still enduring the trials and tribulations of their programs a note of encouragement is in order.

A thank you is extended to Tom Koppel for being my sole source of physical assistance throughout this project. For all of the equipment he cleaned and all the sausage he stuffed a mere thank you seems inadequate.

For the financial assistance and loan of equipment used in this study I would like to recognize Diamond Crystal Salt, Co. and Boldt Industries for their awareness of the urgent need for this type of work.

For my parents, who have encouraged me to ardently pursue my education for the past 21 years, words are totally inadequate. Without these two people I would have never had the desire necessary to pursue advanced degrees.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Egyptian hieroglyphics dating to the year 1000 B.C. constitute the earliest recorded history of meat processing and preservation through the use of crude salt preparations. During this infancy of meat processing technology very little, if anything, was known concerning the factors involved in meat product manufacture. These early meat processors only knew what techniques worked well in order to preserve meat. Today, almost 3000 years later, most of the processes used by our early Egyptian counterparts are understood at least to some degree. However, new developments and process alterations have staged entirely new areas of interest and concern for modern meat scientists.

Like the early Egyptians we are still concerned with delaying the spoilage and degradation of meats. However, we are also involved with attempting to attain a fuller understanding of the changes and alterations which animal tissues undergo during meat processing. Among these changes which meats experience during processing is the solubilization of proteins. In order for sausages and sectioned and formed meat products to be of acceptable texture a certain amount of the muscle proteins must be solubilized in order to bind the fats or other pieces of meat together.

This is accomplished in the same way as the Egyptians did it 3000 years ago, through the addition of salt.

Today's meat processing industry is a multi-faceted business whose ultimate goal is to show a reasonable profit in a very competitive industry which operates on a high volume-low profit basis. In order to achieve and maximize profits it is imperative that the processor utilize the meat and non-meat ingredients to their fullest potential.

As one might expect the most costly ingredient in a sausage product is the protein which is derived primarily from the lean meat fraction. Among the ways in which one can promote the solubilization and subsequently the utilization of these proteins is the process of preblending. Preblending involves blending some or all of the meat with part of the non-meat ingredients, primarily salt and sodium nitrite, at some time prior to actual product manufacture. The practice of preblending, although probably as old as meat processing itself, has only recently become the subject of scientific investigations.

The objectives of this study were:

(1) to determine changes in protein solubilization as influenced by blending time and post-blending storage.

(2) to characterize the nature of the soluble proteins

in preblended meat systems.

(3) to determine differences in soluble protein composition and solubilization pattern as influenced by type of NaCl.

(4) to evaluate the effects of preblending on the fat and water binding properties of a meat blend.

REVIEW OF LITERATURE

Optimization of protein solubilization and functionality in sausage products is a dynamic area of research. This review of literature will focus on previous advances and current knowledge concerning protein solubilization as it is influenced by blending time, post-blending storage and non-meat ingredients, these being the primary considerations of this study.

Meat Emulsions

In order to more fully understand the influence of protein solubilization on sausage products a review of the theory of and concepts involved in emulsion sausage manufacture is in order at this point.

Although scientists and laymen alike have frequently referred to products such as bologna and frankfurters as meat emulsions, this term is not totally appropriate for these products. Becher (1965) defined an emulsion as consisting of two immiscible substances one of which is dispersed in the other. Particle size of the discontinuous or dispersed phase was also restricted to a maximum of 100 μm by this definition.

In a meat system the actual size of fat globules (the dispersed or discontinuous phase) have been reported to be as small as 0.1 μm in diameter (Borchert et al., 1967). Likewise, Ackerman et al. (1971) reported numerous fat globules of less than 5 μm in diameter. These results indicate that true meat emulsions do exist. However, work reported by Theno and Schmidt (1978) showed quite clearly that although some frankfurter type products are true emulsions, as indicated by fat globule size, many commercial products lack the degree of comminution necessary to be classified as true emulsions.

Numerous researchers have studied the morphology of finely comminuted meat products. Hansen (1960), Swift et al. (1961), Helmer and Saffle (1963), Carpenter and Saffle (1964) and Meyer et al. (1964) have all studied the properties of these products using light microscopy and have reported that although these products appear to resemble classical oil in water emulsions as previously defined, the continuous phase of the product, consisting of water and salts, is permeated by a protein matrix hence immobilizing the discontinuous phase. Therefore it is apparent that highly comminuted meat products are probably best described as pseudo-emulsions which is the terminology that will be used in the remainder of this study.

Pseudo-Emulsion Formation

In order to form a pseudo-emulsion in a meat system there are two fundamental requirements; an input of energy and the presence of an emulsifying agent to stabilize the dispersion of fat within the matrix (Saffle, 1968). The energy requirement may be fulfilled in a number of different ways all of which result in applying a shearing force to the system. These methods include blending, chopping, high speed emulsification and various combinations of these three methods (Hamm, 1960). The role of the emulsifying agent is fulfilled by muscle proteins which are solubilized by the addition of ionic compounds, primarily NaCl, simultaneously with the application of a shearing force (Saffle, 1968).

It is extremely important to recognize that there is both a minimum and a maximum level of energy which can be introduced into the meat system in order to obtain a stable matrix (Saffle, 1968). The energy level must be great enough to finely comminute the meats and to render the fat into a semi-liquid state whereas it must not be so great as to denature the muscle proteins therefore rendering them useless for matrix formation. Hamm (1960) has reported that the energy applied to a meat system is more efficiently utilized when the lean component of the sausage is chopped briefly with NaCl in the absence

of added water prior to the addition of the remaining sausage ingredients. This technique results in increased solubilization of muscle proteins since the muscle fibers exert more resistance to the knives of the chopper causing them to be cut more sharply and finely than in systems where water is added. The addition of NaCl at this point causes the hydration of the muscle proteins to increase and also aids in solubilization for reasons which will be discussed later.

Not only is the presence of soluble proteins necessary for pseudo-emulsion formation, but also the ionic properties of these soluble proteins are of the utmost importance (van Eerd, 1971). The primary property of these soluble proteins which dictates their functionality in matrix formation, is their relative proportions of hydrophilic and lipophilic side chains. This characteristic of proteins and other surfactants can be quantitated and assigned a value known as the hydrophilic-lipophilic balance (HLB). This HLB is one-fifth of the percentage of hydrophilic groups on the protein molecule, therefore an HLB in excess of 10 indicates a predominately hydrophilic molecule. In this capacity the HLB indicates the degree of affinity for both fat and water which are the two substances of interest in sausage systems.

Fats and oils can be similarly assigned required HLB's,

these being indicative of the HLB of the surfactant which would be most effective in stabilizing a dispersion of the particular fat in question. Van Eerd (1971) reported that the required HLB of animal fats was approximately 14 in-cating that they require an extremely hydrophilic surfactant in order to achieve emulsion stability. He also reported in the same paper that the HLB of salt soluble muscle proteins was very close to this value. It is important to note that since proteins are composed of amino acids, which are ampholytes, proteins themselves display ampholytic properties. Because of this, as the pH of the environment in which the proteins are present changes, likewise the HLB of the proteins will also change due to a change in the overall charge of the molecule.

Role of Protein Type and Source

Type of protein is also of extreme importance when a finely comminuted sausage is being manufactured. Numerous researchers have studied the emulsification and binding properties of various muscle proteins. Maurer and Baker (1966) studied the influence of collagen content on the emulsifying capacity of poultry meats in a model system. These workers reported that as the collagen content of the meat increased the subsequent ability of the system to emulsify fat decreased. A correlation between collagen content and emulsification capacity of light fowl meat of

-0.895 was reported by these researchers. Other research conducted on the effect of protein type on emulsification properties in poultry meat was reported by Maurer et al. (1969a). These researchers reported that proteins extracted in the presence of 3% NaCl had a greater emulsifying capacity than water soluble proteins. Carcass part had no effect on emulsification as long as the protein contents of the fractions were adjusted to a constant level. This work also focused on the effect of the presence of NaCl in the emulsification system by studying salt soluble proteins which had had the NaCl removed by dialysis. They reported that emulsification properties were detrimentally influenced by the removal of NaCl from the system. The emulsification properties of the fractions studied in descending order were: salt soluble proteins and NaCl, salt soluble proteins dialyzed, water soluble proteins and NaCl, and water soluble proteins. These results indicate that NaCl exerts a positive influence on emulsification through its mere presence regardless of the type of protein involved, however type of protein still appears to be of extreme importance.

Further work on the emulsifying capacity of salt-soluble proteins was conducted by Maurer et al. (1969b). These researchers studied the yields of salt soluble proteins from different poultry sources and reported that as yield increased the emulsification capacity of the

protein, when expressed on a per unit protein basis, decreased, indicating an inverse relationship between protein concentration and emulsification capacity. However, these researchers emphasized that although the efficiency of emulsification was appreciably less at higher protein concentrations the total oil emulsified per unit muscle was considerably greater.

Hegarty et al. (1963) focused their research efforts on the emulsification capacities of purified beef muscle proteins. These workers ranked the emulsification capacities of the proteins from greatest to least as follows: actin in the absence of KCl, myosin, actomyosin, sarcoplasmic proteins and actin in 0.3 M KCl. It was suggested that actin without salt was present in the filamentous form and that actin in 0.3 M KCl was present as globular actin therefore explaining the vast differences in the emulsification properties of these two preparations. Regarding stability, these workers reported that all emulsions prepared with actin had a low level of stability whereas those emulsions prepared with myosin and synthetic actomyosin possessed superior stability. Also of extreme interest here was the reported observation that at normal meat pH's, 5.6 to 5.8, that the sarcoplasmic (water soluble) proteins produced the most stable emulsions. This observation regarding the stabilizing properties of

sarcoplasmic proteins is the first published report of this nature.

More extensive work on the emulsification properties of purified chicken muscle proteins has also been reported (Galluzzo and Regenstein, 1978a,b,c). Galluzzo and Regenstein (1978b) studied the emulsification capacities of several purified muscle proteins in a 0.6 M NaCl, 20 mM citrate-phosphate buffer. Protein solutions studied by these workers included myosin, actin and a synthetic actomyosin. The synthetic actomyosin samples were studied in the absence of and in the presence of 5 mM ATP in order to observe the effects of the actomyosin linkage as opposed to a combination of actin and myosin. The emulsification capacities of the proteins reported were from highest to lowest: myosin ≈ synthetic actomyosin + ATP > synthetic actomyosin - ATP > actin. These results concerning the emulsification capacities of myosin, actomyosin without ATP and actin are in agreement with those previously reported by Hegarty et al. (1963). It was also noted that myosin was rapidly removed from solution during emulsification whereas actin was less readily removed, indicating an increased affinity of the oil for myosin as opposed to actin. The emulsions resulting from myosin were thick and creamy whereas actin stabilized emulsions were described as thin and coarse. Synthetic actomysin in

the absence of ATP behaved in the same manner as myosin while the use of synthetic actomyosin + 5 mM ATP resulted in the preferential incorporation of myosin into the emulsion while actin remained in solution.

Further studies on chicken muscle proteins reported by Galluzzo and Regenstein (1978c) focused on the emulsification properties of native actomyosin. This work showed that the emulsification capacity of native actomyosin was less than that previously reported by Galluzzo and Regenstein (1978b) for myosin and synthetic actomyosin + 5 mM ATP, however when 5 mM ATP or 5 mM pyrophosphate were added the emulsification capacities were found to be essentially equal. Again this was attributed to the disassociation of the actin-myosin linkage in actomyosin. During timed emulsification of native actomyosin disassociated with 5 mM ATP, myosin was again preferentially removed from solution as in the case of synthetic actomyosin + 5 mM ATP which was previously reported by these same workers.

Several researchers have studied the emulsification properties of intact and semi-extracted myofibrils from beef semitendinosus (Fukazawa et al., 1961a,b,c) and from chicken breast muscle (Galluzzo and Regenstein, 1978c). Fukazawa et al. (1961b) studied the binding properties of intact fibrils, actin, tropomyosin poor fibrils and

actin-myosin poor fibrils in an experimental sausage. They reported that sausages manufactured using intact fibrils were quite similar to those sausages manufactured using pre-rigor beef muscle whereas sausages made from synthetic myofibrils had binding properties which resembled those of sausages made from 7 day post-mortem muscle.

Galluzzo and Regenstein (1978c), in studies involving the timed emulsification of chicken myofibrils, reported that tropomyosin was not involved to any large extent in emulsion formation and that the troponins were only marginally involved. These conclusions are in agreement with those of Fukazawa et al. (1961c) who reported these findings in addition to the conclusion that actin was not a major stabilizer as has also been stated by Galluzzo and Regenstein (1978b).

Protein Solubilization

Research concerning the solubilization of muscle proteins has recently been conducted by several laboratories. There are several factors which exert influences on the solubilization of proteins and therefore the literature relating to each of these factors will be reviewed separately.

The major factors involved in meat protein solubilization are:

1. Mechanical Action
2. Ionic Strength
3. pH
4. Temperature
5. Vacuum
6. Preblending

Effects of Mechanical Action

Several types of mechanical action are routinely applied to meats during their transformation from raw materials to finished products. Among these are blending, chopping, grinding and massaging and/or tumbling. Blending, chopping and grinding are confined primarily to finely comminuted sausage products whereas massaging and tumbling are most widely used in the production of sectioned and formed meat products.

Theno et al. (1977) reviewed the past advances and the current state of the art in meat processing systems with a focus on the effects of mechanical action on protein solubilization, in particular meat massaging. These workers defined meat massaging as the process of imparting mechanical work to meat pieces or chunks by mixing or churning them in such a manner that they become soft and pliable with a creamy, tacky exudate on the

surface. This exudate is composed of water, salt and solubilized protein and as such serves as an adhesive in the binding of these meat pieces.

Protein solubilization is promoted through the introduction of mechanical energy into the meat massaging system (Weiss, 1974). Theno et al. (1978a) studied the effects of massaging on the microstructural composition of muscle exudate using conventional light microscopy techniques. This work showed that as massaging time increased, up to 24 hours, that muscle fibers were progressively disrupted and extensive quantities of fiber fragments began to appear in the meat exudate. As massaging progressed definite clouds of solubilized protein were also observed. These authors found that overall increased mechanical action due to massaging resulted in improved binding ability of the product. This was attributed to the increased disruption of the muscle fibers which was observed. Cassidy et al. (1978) used the same basic approach in the study of tumbled meat and reported similar results.

More in depth studies concerning the nature of the protein exudate, in mechanically worked meat pieces have also been reported (Siegel et al., 1978a). These researchers reported that although other factors, such as NaCl and phosphate concentration, greatly influenced the composition

of the protein exudate the singular effect of massaging only facilitated the availability of these proteins for solubilization rather than functioning directly in the solubilization process itself.

Maesso et al.(1970a,b) studied, among other factors, the influence of mechanical mixing on cubes of poultry meat. They reported that as the extent of mechanical mixing increased that the intracellular content of broken muscle cells was released resulting in an increase in bind. Other work concerning the effects of mechanical mixing was conducted by Belohlavy (1975). This work utilized pork muscle flaked using an Urschel "Comitrol" and although no measures of protein solubilization or availability for solubilization were made it was reported that binding ability increased to a maximum at 8 minutes of mixing time and then decreased to a minimum at 14 minutes.

Gorbatov et al. (1972) and Gorbatov and Gorbatov (1974) have extensively studied the effects of chopping on the rheological properties of sausage meats. These workers characterized the changes in consistency and viscosity which meats experience during chopping and reported that the time of chopping required to achieve a specific degree of viscosity was largely dependent on the water content of the batter as this greatly influences protein solubilization. Likewise, Hamm (1960) stated that

protein solubilization during chopping is greatly enhanced by chopping for several bowl revolutions prior to the addition of water. This practice theoretically causes the muscle fibers to resist the knives more and thus experience a greater degree of comminution therefore exposing more proteins for solubilization due to the resulting increase in surface area of the meat particles.

Although grinding of meat effectively increases the surface area the relative benefits of grinding in comparison to chopping regarding protein solubilization are rather small. This is due to the decreased magnitude of the surface area increase as compared to that which is accomplished by chopping (Hamm, 1960).

Emulsification as accomplished by high speed mills, such as the Griffith Mincemaster is responsible for a great deal of the mechanical energy which is contributed to meat batters during processing (Saffle, 1968). These machines operate on the same basic principle as meat grinders, however, the perforations in the emulsitator plates are considerably smaller in diameter than those in grinder plates resulting in a greater degree of comminution. The basic contribution of emulsitators in the processing of a pseudo-emulsion type product is not one of solubilization but rather one of particle size reduction hence resulting in a more stable emulsion.

Recently, machines have been introduced into the United States from Europe which are capable of performing the combined functions of blenders and choppers (Johnston, 1979). These machines are reported to produce increased protein solubilization while saving valuable time as compared to the use of separate machines. At present, this type of machinery is not enjoying wide usage in the meat industry due primarily to poor durability and reliability of the prototype equipment.

Effects of Ionic Strength

Most of the proteins which are of interest in the stabilization and binding of finely comminuted sausages and other meat products are soluble only in salt solutions (Hamm, 1973). Thus the effect of ionic strength on meat protein solubilization plays a major role in the production of these products.

Hamm (1960) discussed the influence of salts on meat protein hydration. In living tissue the effect of ions is primarily due to osmotic effects, whereas in post-rigor tissue the salts freely migrate into the muscle fibers due to the destruction of the semipermeability of the cell membranes. Once inside the muscle fibers these salts exert lyotropic effects which result in the swelling of the fiber.

Hamm (1957) studied the effects of various metal chlorides and of the sodium salts of different strong acids on the hydration of muscle homogenates at various ionic strengths. At ionic strength of less than 0.1 no differences were observed related to the particular salt employed, however, when the ionic strength of 0.1 was exceeded, significant differences between the salt types were observed. It was reported that at the pH of sausage systems the influence of the various anions on hydration followed the lyotropic series quite closely. The effects of the anions on hydration were F^-< Cl^-< Br^-< CNS^-, I^- with all of these anions resulting in increased hydration. Regarding the metal chlorides studied it was reported that the effects of the different metals were K^+< Na^+, Mg^{++}< Ca^{++}< Li^+< Ba^{++} again with all metals improving hydration. It was also reported by Hamm (1957) that the pH of the environment greatly influenced the relative effects of the different salts on hydration.

The manner in which salt ions bind to proteins is primarily electrostatic due to the attraction of the salt ions by the positively or negatively charged groups of the proteins (Schellman, 1953). It is also suspected that Van der Waal's forces may play a role with anions of high molecular weight, however, this is merely a hypothesis.

Cann and Phelps (1955) stated that in the acidic range of the isoelectric point of muscle the electrostatic repulsion between positively charged groups of the protein is reduced by the binding of anions therefore decreasing the net charge of the protein in the following manner:

$$\underset{\text{(repulsion)}}{R\text{-}NH_3^+} \quad {}^+H_3N\text{-}R \xrightarrow{+A^-} R\text{-}NH_3^+A^- \quad A^{-+}NH_3\text{-}R' \quad \underset{\text{(no repulsion)}}{}$$

Due to this reaction the protein structure therefore tightens resulting in a decrease in hydration. The stronger the ion is bound, therefore the stronger is the effect of dehydration.

In the basic range of isoelectric point, however, an opposite effect is exerted by the salt since more negatively charged groups are available for the formation of salt bridges with the positive charges in the following manner:

$$\underset{\text{(no repulsion)}}{R\text{-}NH_3^+} \quad {}^-OOC\text{-}R' \xrightarrow{+A^-} R\text{-}NH_3^+A^- \quad {}^-OOC\text{-}R' \quad \underset{\text{(repulsion)}}{}$$

The result in this case is a loosening of the protein structure and therefore an increase in hydration. The more tightly the anion is bound the stronger the hydrating value of that anion will be. The binding of anions

therefore also lowers the isoelectric point of the protein due to increased negative charges. This effect of lowered isoelectric point due to bound ions is primarily responsible for the increased solubilization due to the addition of ions at the pH of processed meat systems.

The binding of cations is in the same manner as for anions. However, while it is best to have a strongly bound anion it is preferable to have a weakly bound cation. This is due to the fact that the anions result in a decreased isoelectric point while the binding of cations does not (Weber and Portzehl, 1952).

Weber and Portzehl (1952) suggested that at ionic strengths in excess of 1.0 and at pH values greater than 7.0 salts were capable of partially dissociating actomyosin into its respective components. However, this effect does not influence meat systems due to the unusual conditions at which it occurs.

Most hydration is maximum at NaCl concentrations of μ=0.8 to 1.0 (Hamm, 1957). These figures correspond to 5% NaCl with no water added and approximately 6.5% NaCl with 30% added water. Hamm and Grau (1958) also reported that systems with ionic strengths of 0.8 to 1.0 also exhibited maximum hydration after thermal processing.

Numerous reports have recently been published regarding the effects of various NaCl levels on massaged hams

(Siegel et al., 1978a,b; Theno et al., 1978a,b). These workers reported that as NaCl content increased that the degree of protein solubilization also increased with a NaCl content of 2% producing optimal binding properties in the finished product and no noticeable difference between the binding properties of the 2 and 3% NaCl hams. Theno et al. (1978b) clearly showed through the use of scanning electrom microscopy that the presence of NaCl in these hams aided in the disintegration and overall disruption of muscle fiber integrity. These results concur with the statements made by Hamm (1960) regarding the loss of membrane semipermeability due to the presence of NaCl. Theno et al. (1978a) reported that the exudate formed on massaged hams showed evidence of higher levels of soluble protein as NaCl level increased and these results were substantiated by the reports of Siegel et al. (1978a).

The use of phosphates in meat products has also been extensively studied. Reports on intact muscle products have shown that phosphate greatly increases protein solubilization and ultimately product bind (Krause et al., 1978a,b; Siegel et al., 1978a,b; Theno et al., 1978a,b; Ockerman et al., 1978; Cassidy et al., 1978). Although phosphates are highly ionized it is also suspected

that the cleavage of the actomyosin linkage is in part responsible for this increase in solubilization as has been demonstrated by Hamm (1973).

Pepper and Schmidt (1975) and Neer (1975) have studied the effects of NaCl and phosphates on comminuted and cooked red meat products. Neer reported that 2% NaCl and 0.25% phosphate produced a product with optimal binding characteristics while products containing more than these levels did not show any significant improvements in bind.

Effects of pH

Due to the fact that proteins are composed of amino acids which are ampholytic molecules, in that they display properties of both acids and bases, proteins are also ampholytes (Lehninger, 1975). As the pH of the environment in which a specific protein is present changes the charge of that particular protein also changes until the pH of the solution reaches a point where the net charge on the protein is zero. At this pH, also known as the isoelectric point of the protein, the protein is no longer soluble and therefore precipitates resulting in a total destruction of protein functionality.

A great deal of work has been done concerning the effect of pH on the properties of muscle proteins (Froning and Janky, 1971; Froning and Neelakantan, 1971; Hegarty et al., 1963; Hwang and Carpenter, 1975; McCready and

Cunningham, 1971; Swift and Sulzbacher, 1963). These workers have all reported that as the pH of the system is moved away from the isoelectric point of muscle proteins (≈5.2)the emulsification properties of the protein are increased. This is probably due to improved solubility of the proteins at these pH's.

Hamm (1973) reported that the effect of pH on the protein solubility of a meat system is dependent on the presence of other factors in the system. The most common instance of this is exemplified by the effects of the presence of NaCl. As was discussed previously the presence of NaCl results in a lowering of the isoelectric point of the proteins thus significantly increasing the solubilization of proteins at pH's where without NaCl only a small portion of the protein is solubilized.

This theory supports the findings of Swift and Sulzbacher (1963) who reported that the addition of NaCl to sytems containing water soluble proteins resulted in improved emulsification properties.

Without exception all reports on the binding properties of meat systems as influenced by pH have shown that as the pH is raised away from the isoelectric point that bind improves indicating increased protein functionality and solubilization.

Effects of Temperature

Saffle (1968) reported that protein solubilization was maximized at a temperature of 4.4°C. This temperature is higher than those which are normally used for the laboratory preparation of protein fractions (0-2°C) and no doubt results in the denaturation of some proteins. However, the benefits of increased solubilization at these temperatures more than offsets the slight degree of denaturation which occurs.

Final temperatures which are realized during the production of finely comminuted sausages normally fall in the range of 15-22°C (Hansen, 1960; Helmer and Saffle, 1963). Although these temperatures are greatly in excess of those reported by Saffle (1968) as resulting in maximum protein solubilization they are necessary in order to maximize the fat binding properties of the proteins.

Brown and Toledo (1975) studied the relationships between chopping temperature and the fat and water binding properties of finely comminuted sausage batters. These researchers reported that as temperatures increased in excess of 22°C the fat and water binding properties of the batter decreased. However, these workers also found that when the temperature of the batter was lowered by the addition of dry ice a subsequent increase in binding properties was evident. Although this increase was not

sufficient to restore the binding properties to a state equal to that of batters which had not been subjected to adverse treatments it does indicate that some of the thermally abused protein in these batters was capable of recovering at least part of its original functionality.

Bard (1965) reported that raising meat temperature from -5 to 3°C resulted in a four-fold increase in protein solubility with approximately 5% of the toal protein being soluble at 3°C. These results coincide with those reported by Saffle (1968).

Saffle (1965) stated that the usuage of frozen and thawed meat should be limited in finely comminuted meat products due to poor binding properties. However, work reported by Buttkus (1970) and Johnson (1975) indicated that protein extractability is essentially unchanged by freezing and thawing. This indicates that it is the functionality rather than the solubility of protein which is affected by freezing. Further confirmatory reports of instability due to the excessive use of frozen and thawed meats have also been made (Hargus et al., 1970; Froning, 1970; Morrison et al., 1971).

Effects of Vacuum

The application of vacuum to a meat system during blending and/or comminution has been shown to improve the final product in several ways (Starr, 1979). Increased solubilization of proteins due to vacuum blending has been reported by Schmidt (1979). This work involved the application of vacuum blending to a sausage system and revealed increased solubilization of myofibrillar proteins as a direct effect of vacuum.

The underlying theory responsible for this increase in solubilization has been presented by Starr (1979). This report stated that when a vacuum is applied to a meat system the meat particles experience a swelling phenomenon which results in increased surface area available for interaction with the extraction solution and/or knives of the chopper. Although this appears to be a very viable explanation for the effects of vacuum on protein solubilization there is also an enhancement of the functionality of the soluble proteins due to the application of vacuum. This may be partially explained by the fact that when a vacuum is applied to the system free air and air which is entrapped within the blend is partially removed. This air, if not removed, would require soluble proteins to stabilize its position in the sausage matrix therefore decreasing the

amount of soluble protein which would be available for the stabilization of fat particles (Saffle, 1968).

Effects of Preblending

Very little research has been conducted on the influence of preblending on protein solubilization in meat systems. Webb (1968) defined preblending as the process of presalting some or all of the meat ingredients and allowing the resulting blend to undergo a passive extraction at cooler temperatures for some time prior to final product manufacture.

Increased efficiency of fat emulsification properties has been reported for proteins which were extracted from preblended beef check muscles (Borton et al., 1968). The results of studies which were reported by Johnson et al. (1977) also indicated that preblending for 24 hours significantly improved the water binding properties of beef and pork blends as compared to samples which were not preblended. Froning and Janky (1971) and Acton (1973) have studied the effects of preblending on poultry muscle and have reported noticeable increases in emulsion stability and binding properties in products made from these tissues due to preblending.

Although preblending is widely employed in industry the mechanisms involved in product improvement through preblending are poorly understood and more research is needed in this area (Webb, 1968).

METHODS AND MATERIALS

This study was conducted in two parts, the first of which was a model systems experiment designed to ascertain the effects of NaCl type, blending time and post-blending storage time on protein solubility and binding properties of sausage blends. The second part of this study was then designed to study the applications of the results of part I on a pilot plant sausage system.

Part I

Source of Meat

All meats utilized in this study were obtained in the fresh, unfrozen state from local meat packers. The pork was obtained as boneless picnics and the beef as boneless cow fronts. Meats were delivered to the meat laboratory three days prior to processing and immediately stored at $4^{o}C$. Samples were taken for protein, moisture and fat analysis upon arrival (AOAC, 1970). The mean proximate composition of these meat sources is shown in Table 1. Meat ingredients were always obtained from the same sources in order to minimize variations in composition.

Table 1. Means and standard deviations for the proximate composition of raw materials (Part I).

	MEAT SOURCE	
	Boneless Cow Fronts[1]	Boneless Picnics[1]
Moisture (%)	67.06 ± 0.34	58.51 ± 1.19
Fat (%)	12.94 ± 0.56	24.39 ± 1.56
Protein (%)	18.96 ± 0.23	15.95 ± 0.32

[1] N = 6

Source of NaCl

Three types of commercially available NaCl were used in this study. The three types were:

(1) Flake

(2) Dendritic

(3) Evaporated and granulated

Samples of these were supplied by the Diamond Crystal Salt Co1, St. Clair, MI. Information regarding typical analyses is given in Appendix A.

Processing

A flow diagram of the processing sequence is shown in Figure 1. The boneless picnics and boneless cow fronts were ground through 9.5 and 4.8 mm plates, respectively. This difference in grind size was utilized in order to maximize the surface area of the lean portion of the blend as is normally done commercially. Appropriate amounts of the two meats were then weighed and placed in a Reitz blender (capacity ≈ 80 kg) equipped with twin ribbon shafts, so that the final meat block represented 68 kg of meat containing 17% protein.

At this time the blender was started and 1700g of one of the three experimental salts and 8.2 g of $NaNO_2$, dissolved in 25 ml of 17°C tap water, were added. This resulted in a blend containing 2.5% NaCl and 120 ppm $NaNO_2$,

The mixture was blended for 30 seconds in order to distribute the NaCl and $NaNO_2$. At this time the blender

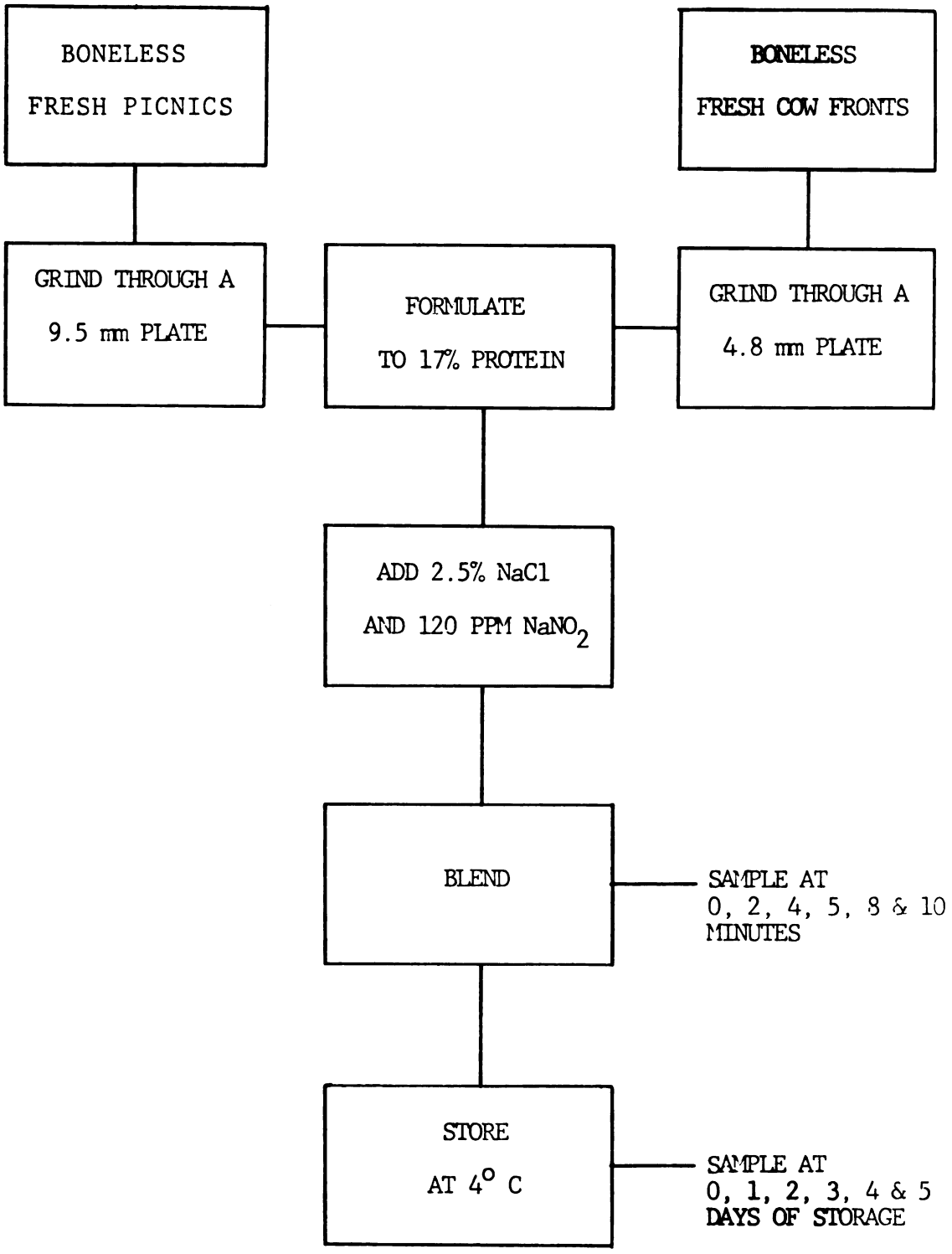


Figure 1. Processing flow chart (Part I).

was stopped and a 1.8 kg sample was removed and placed in a polyethylene bag. Blending was then resumed and subsequent samples were collected, in the same manner as the initial sample, at 2, 4, 6, 8 and 10 additional minutes of blending. All blending was accomplished at room temperature utilizing a ribbon speed of 45 rpm.

Following the completion of blending the samples were placed in a 4°C cooler prior to analysis. All samples were analyzed for protein solubility, pH, blend stability and soluble protein composition via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing in polyacrylamide gels (IFPA). These analyses were performed initially and following 1, 2, 3, 4 and 5 days of post-blending storage at 4°C.

Determination of Soluble Phase

In order to extract the soluble phase from the blend samples 25 g of blend were homogenized for five seconds in a Waring blendor containing 25 ml of 4°C 4% NaCl solution (w/v) (Reagent Grade). The resulting slurry was decanted into a 75 ml polypropylene centrifuge tube and subjected to centrifugation at 20,000 x g for 30 minutes in a Sorvall RC-2B automatic refrigerated centrifuge equipped with a Sorvall SS-34 fixed angle rotor and operated at a rotor temperature of 4°C.

Following centrifugation the soluble phase (Figure 2) was decanted by loosening the fat cap with a micro-spatula and inverting the centrifuge tube into a Nalgene funnel lined with two layers of cheesecloth. The resulting filtrate was collected in a graduated 15 ml conical centrifuge tube. Following 30 minutes of filtration the volume of filtrate was noted as the volume of the soluble phase. This filtrate was then subjected to protein determination and 1 ml aliquots were frozen with an equal volume of glycerol for later use in SDS-PAGE and IFPA.

Determination of pH

10 g of blend sample were homogenized for 30 seconds with 50 ml of distilled water in a Waring blendor. The resulting slurry was subjected to pH determination using a Beckman model 3560 pH meter equipped with a combination electrode, which had previously been standardized with pH 7.00 and 4.01 standard buffers. pH determinations were performed in duplicate for each blend with the average of the two determinations being recorded as the blend pH.

Blend Stability

A modification of the emulsion stability procedure described by Townsend et al. (1971) was used. 35 g of blend was tightly packed into a 50 ml polypropylene disposable syringe which was then stoppered using the neoprene piston supplied with the syringe. This neoprene piston

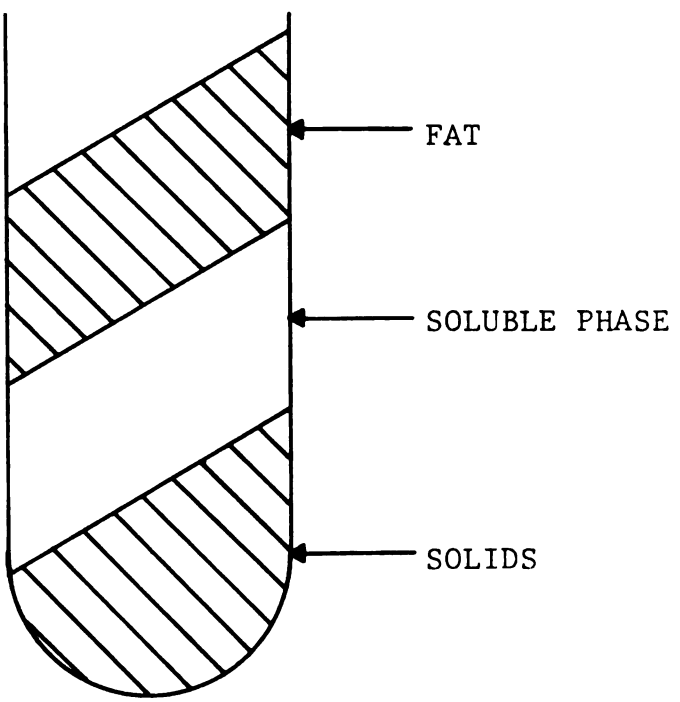


Figure 2. Cross-sectional view of a soluble phase preparation following centrifugation at 20,000 X g for 30 minutes.

had previously had small sections of the rim removed in order to accomodate venting during heating.

Duplicate samples of each blend were transferred to a preheated 70°C water bath and heated to an internal temperature of 69°C as indicated by a thermometer inserted into the geometric center of an identical blend sample which had been prepared specifically for this purpose. The cooked blend samples were immediately removed and decanted into Pyrex funnels, the free fluid resulting from this step was collected in 15 ml graduated conical centrifuge tubes. Following 15 minutes of draining the total fluid volume, fat volume and water volume present in the released fluid were recorded.

Determination of Protein in the Soluble Phase

The micro-biuret procedure described by Goa (1953) was used to determine protein concentration in the soluble phase (Appendix B).

One ml of soluble phase was diluted with 49 ml of 4°C 4% NaCl (w/v) (Reagent Grade). Two ml of this dilution were placed in each of two test tubes containing 2 ml of 6% NaOH (w/v) and 0.2 ml of biuret reagent. Following incubation at room temperature for 15 minutes the absorbance of the solutions at λ = 540 nm was determined using a Beckman model 24 spectrophotometer utilizing distilled water as a blank. Protein concentration was determined using a

standard curve prepared with bovine myofibrils (Figure 3). Myofibrils were prepared according to Goll et al. (1964) (Appendix C).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed using a modification of the procedure outlined by Porzio and Pearson (1977) (Appendix D).

Samples of the soluble phase were thawed at 4°C and aliquots sufficient to yield a final protein concentration of 0.8 µg/µl were diluted with a pH 7.2 SDS tracking dye composed of 1% SDS (w/v), 1 mM EDTA, 0.5 M Tris:HCl, 0.5% 2-mercaptoethanol (v/v), 20% glycerol (w/v) and 0.01% pyronin Y (w/v). The ratio of tracking dye to protein was always maintained in excess of 4:1 in order to insure proper denaturation. The diluted protein samples were heated at 100°C for five minutes and allowed to equilibrate to room temperature. 25 µl of this solution containing 20 µg of protein was applied to the gel and overlayed with chamber buffer (0.2 M Tris:glycine, 0.1% SDS (w/v), 0.1 mM EDTA).

The acrylamide solution utilized in the preparation of the gels consisted of the following: 10 ml stock acrylamide, 5 ml of 2.0 M Tris:glycine, 2.5 ml glycerol (50% w/v), 1 ml of 2.5% SDS:2.5 mM EDTA, 10 µl of TEMED (N,N,N',N' tetramethylenediamine), 5.5 ml of deionized distilled water and 1.0 ml of 1% ammonium persulfate (w/v). The ammonium persulfate was added to the solution immediately prior to casting.

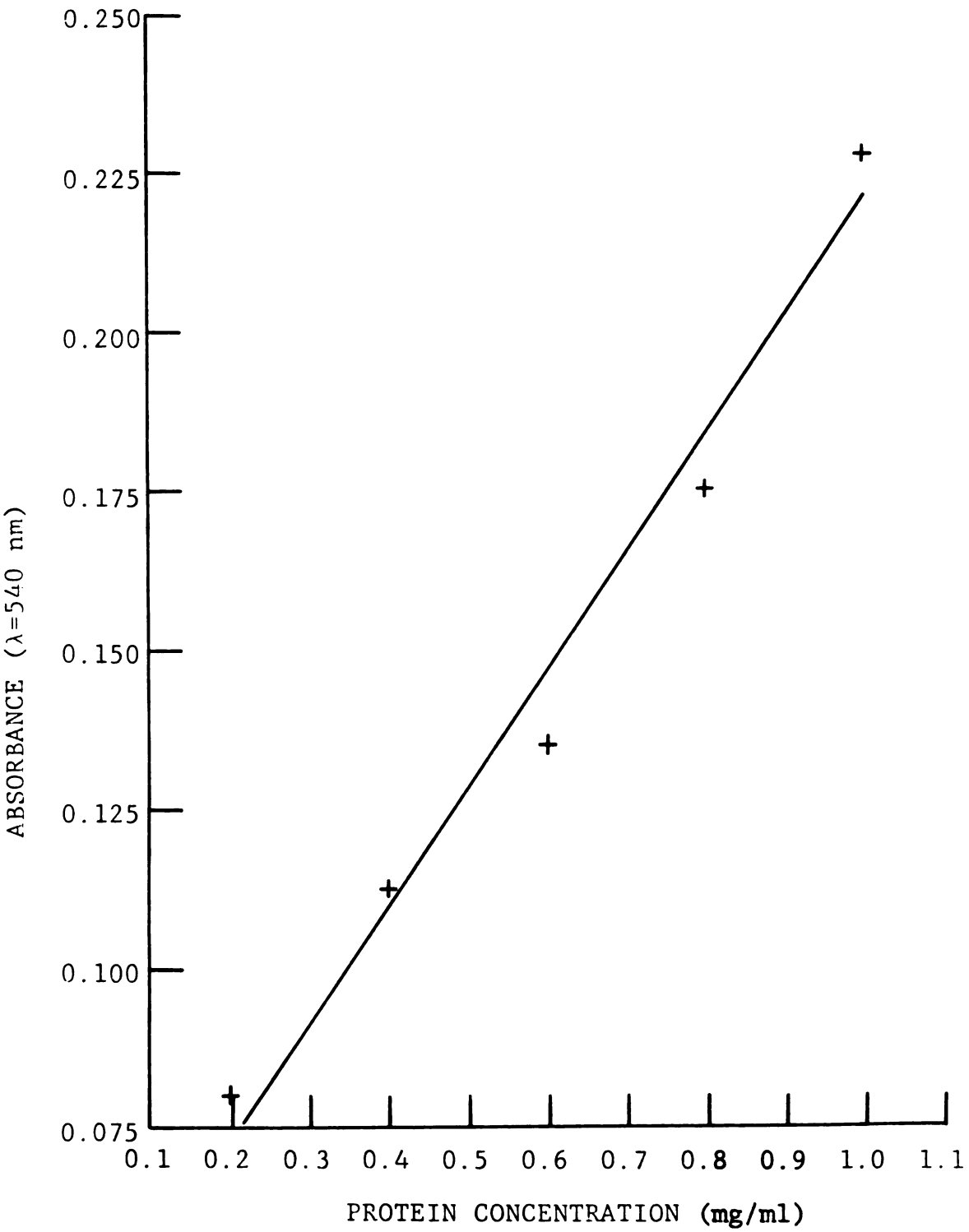
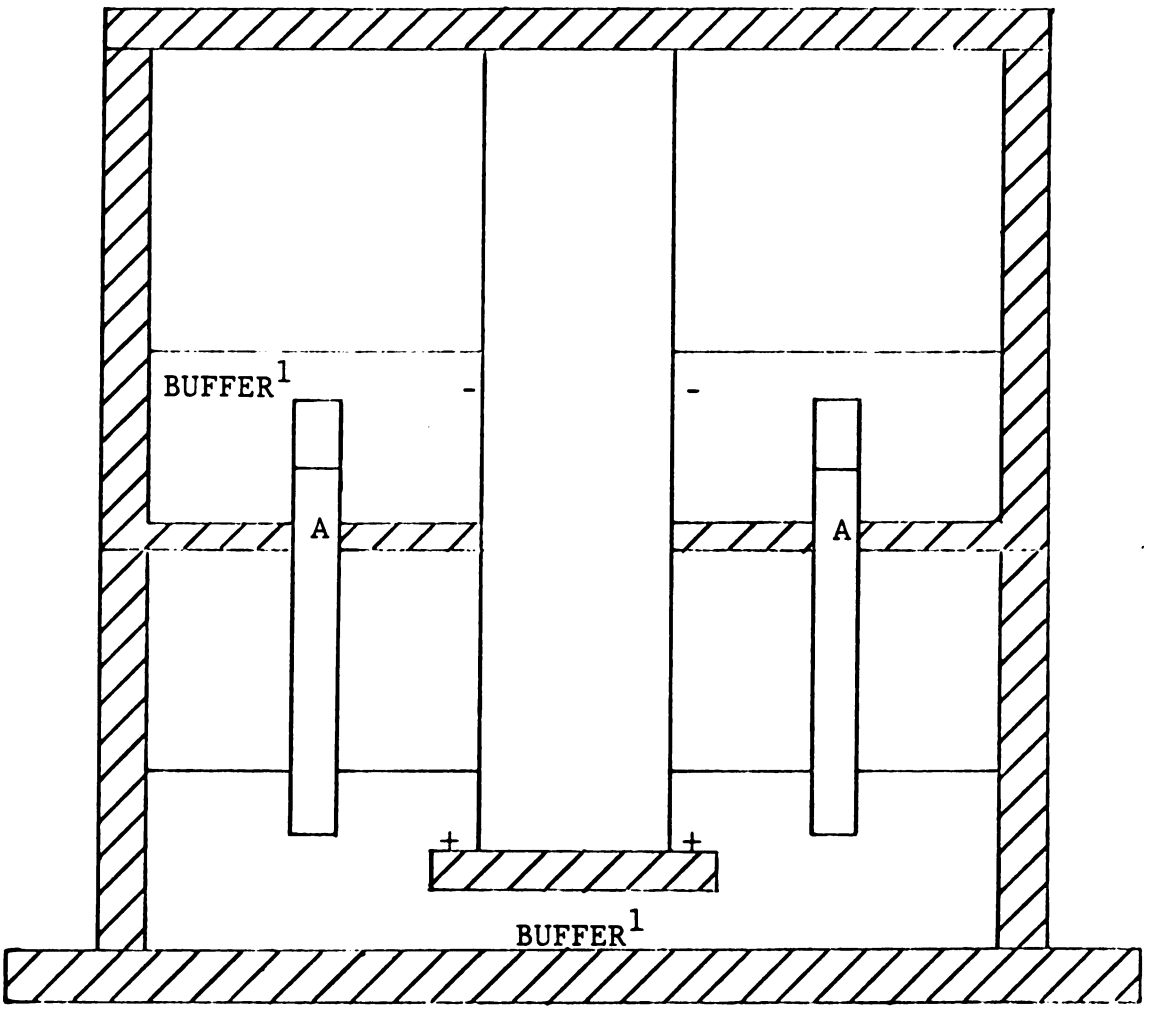


Figure 3. Standard curve used in the determination of protein by the micro-biuret method.

Casting was accomplished by pouring the gel solution into 5 x 90 mm glass tubes to a level of 80 mm. The solution was overlayed with distilled deionized water and allowed to polymerize. The resulting gels were 10.1% acrylamide as a percentage of gel volume and 0.01% bis as a percentage of total acrylamide (10.1% T:0.01% C).

The loaded gels were placed in an electrophoresis chamber and both the anodic and cathodic reservoirs of the chamber were filled with a chamber buffer composed of: 0.05 M Tris, 0.15 M glycine, 0.1% SDS (w/v) and 0.1 mM EDTA (Figure 4). Subsequent electrophoresis was conducted by applying a current of 0.2 mA/gel for a period of 16 hours. At this time the amperage was increased to 0.5 mA/gel until the dye front was approximately 0.5 cm from the end of the gel. Identical samples of a bovine myofibril preparation (Appendix C) were co-electrophoresed in order to serve as molecular weight standards for use in the identification of specific proteins in the experimental samples (Figure 5).

The electrophoresed gels were removed from the tubes by rimming with water and were immediately stained in a solution containing: 0.03% Coomassie Blue R250 (w/v) (Sigma), 50% methanol (v/v) and 7% glacial acetic acid (v/v). Following 24 hours of staining the gels were destained by diffusion for 24 hours in a 30% methanol (v/v), 10% glacial acetic acid (v/v) solution and subsequently stored in a 7.5% glacial acetic acid solution prior to densitometry.



A - SDS Polyacrylamide Gels

[1]Buffer = 0.2 M Tris:Glycine; 0.01% SDS; 0.01 mM EDTA; pH 8.8.

Figure 4. Diagram of an electrophoretic chamber prepared for SDS-PAGE.

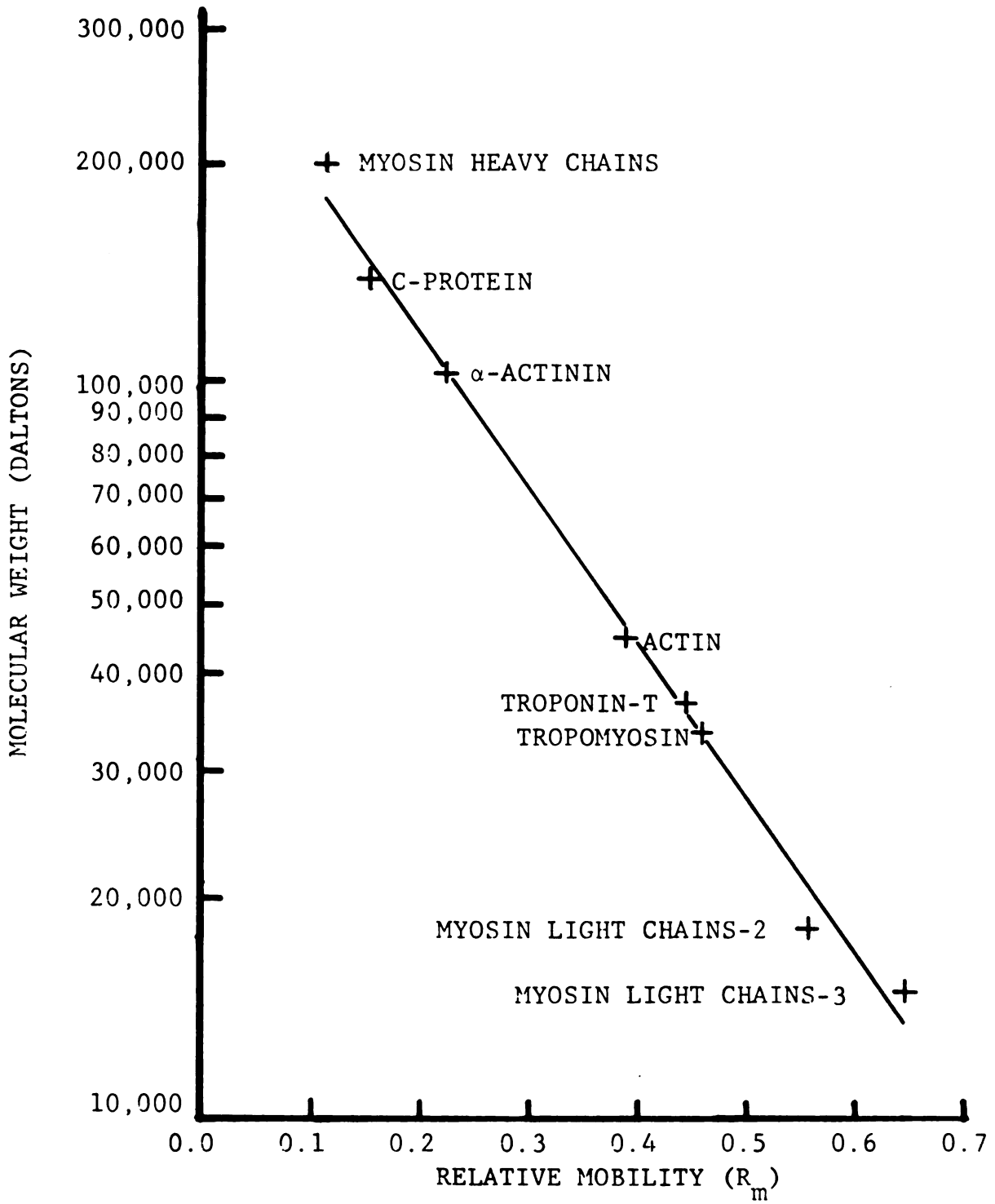


Figure 5. Standard curve used in the determination of molecular weight by SDS-PAGE.

Isoelectric Focusing in Polyacrylamide Gels (IFPA)

IFPA was accomplished using the primary procedure outlined by O'Farrell (1975) with additional modifications as reported by Baumann and Chrambach (1976) and Florini et al. (1971) (Appendix E).

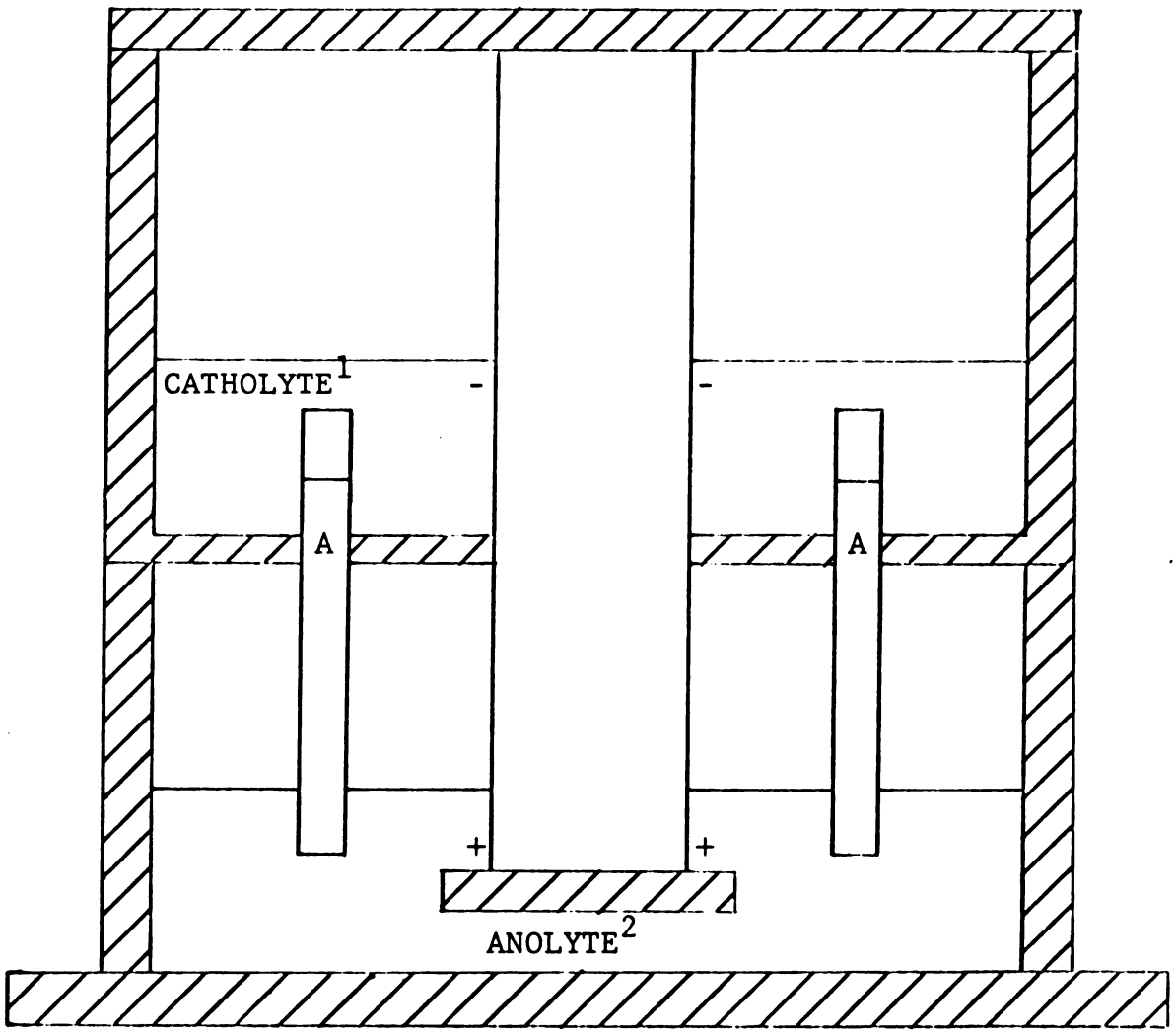
Samples were prepared by thawing the frozen soluble phase samples at 4°C and diluting an aliquot containing a known quantity of protein to a protein concentration of 0.8 µg/µl with a solution consisting of: 9.5 M urea, 2% Triton X-100 (Packard Scintillation Grade) (w/v), 2% carrier ampholytes (Biorad, pH range 3-10) (w/v) and 5% 2-mercaptoethanol (v/v). The resulting solution was briefly vortexed and 25 µl containing 20 µg of protein was applied to gels which had been prepared in the following manner.

Gels utilized for IFPA were cast from a solution composed of: 9 M urea, acrylamide (5% T), N,N' Diallyltartaramide (Aldrich) (15% C), 2% Triton X-100, 2% carrier ampholytes (pH range 3-10), 0.07% TEMED and 0.01% ammonium persulfate. The gel solution was degassed by water aspiration prior to casting and the ammonium persulfate was added immediately following this step. Gels were cast in 5 x 90 mm glass tubes to a length of 80 mm, overlayed with distilled deionized water and allowed to polymerize for three hours prior to electrophoresis.

Following polymerization the water was removed from the gels and they were placed in an electrophoresis chamber containing 0.02 M H_3PO_4 in the anodic chamber (Figure 6). The gels were overlayed with 20 µl of the solution used in sample dilution and the tubes were filled with 0.04 M NaOH. Additional 0.04 M NaOH was added to the cathodic chamber until the cathode was covered. These gels were pre-run using the following schedule: 200 V for 30 minutes, 300 V for 15 minutes and 400 V for 15 minutes. This pre-run schedule served to establish the desired pH gradient and to eliminate the ammonium persulfate from the gel.

Following pre-running the catholyte was removed and 25 µl of the prepared protein samples containing 20 µg of total protein were applied to the gels and overlayed with a solution containing: 9 M urea and 1% carrier ampholytes. The gel tubes were then refilled with 0.04 M NaOH and the level of catholyte was again raised in order to cover the cathode. A voltage of 400 V was then applied to this system for a total of 16 hours.

Upon the completion of focusing the gels were removed from the tubes by rimming with water and two randomly selected gels were profiled using a Biorad gel "propHiler" in order to determine the pH gradient (Figure 7). All gels were then stained for 2 hours using a solution containing 0.25% FCF Fast Green (Sigma) (w/v) and 10% glacial acetic



A - Polyacrylamide Isoelectric Focusing Gels

[1]CATHOLYTE = 0.04 M NaOH

[2]ANOLYTE = 0.02 M H_3PO_4

Figure 6. Diagram of an electrophoretic chamber prepard for IFPA.

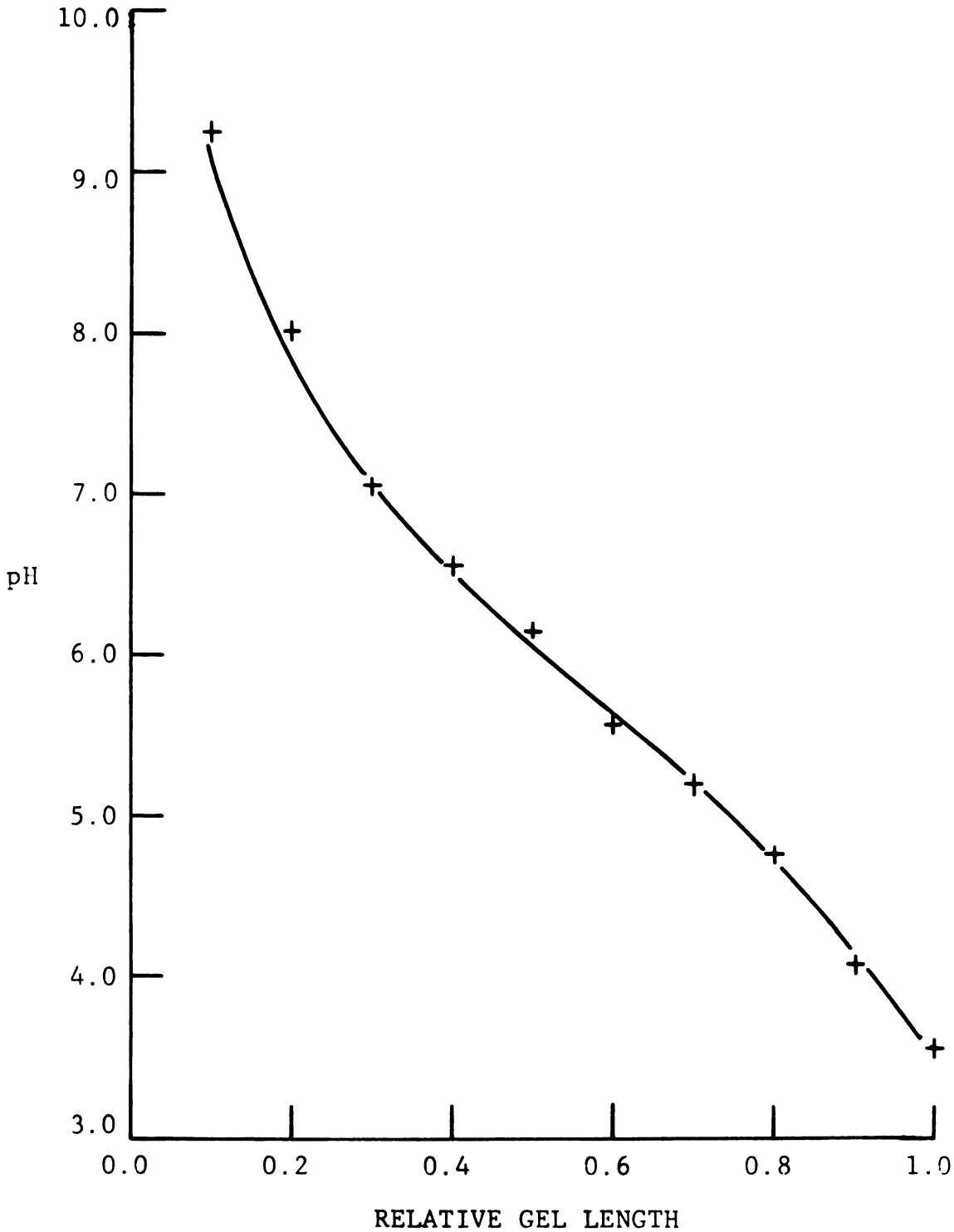


Figure 7. Typical pH gradient achieved in IFPA.

(v/v). Subsequent destaining was accomplished by diffusion in a 30% methanol (v/v), 10% glacial acetic acid (v/v) solution for a period of 48 hours. These gels were stored in 7.5% glacial acetic acid (v/v) prior to densitometry (Gorovsky et al., 1970).

Gel Densitometry

Gels were scanned using a Beckman DU spectrophotometer equipped with a model 2520 Gilford gel scanner linked to a model 3380 Hewlett Packard integrator. The gels were scanned at a scan rate of 0.5 cm/minute and a chart speed of 2 cm/minute. Start delay and slope sensitivity settings were 0.25 minutes and 3.00 mV/minute, respectively. SDS-PAGE gels were scanned at a wavelength of 550 nm and IFPA gels were scanned using a wavelength of 639 nm according to the procedures outlined by Potter (1974). The relative areas of the individual protein peaks were recorded.

Statistical Design

Phase I was designed as a split plot arrangement of treatments with the main plot treatment, NaCl type, completely randomized and the sub plot treatments, blending time and post-blending storage time, in a randomized complete block design with two replications (Figure 8). The analysis of variance table and the partitioning of the degrees of freedom are shown in Table 2.

	FLAKE NaCl						DENDRITIC NaCl						EVAPORATED-GRANULATED NaCl					
	B_1	B_2	B_3	B_4	B_5	B_6	B_1	B_2	B_3	B_4	B_5	B_6	B_1	B_2	B_3	B_4	B_5	B_6
S_1	1_N																	
S_2																		
S_3																		
S_4																		
S_5																		
S_6																		

^{1}N=2 B_1=0 min. blending B_4=6 min. blending S_1=0 days storage S_4=3 days storage
B_2=2 min. blending B_5=8 min. blending S_2=1 day storage S_5=4 days storage
B_3=4 min. blending B_6=10 min. blending S_3=3 days storage S_6=5 days storage

Figure 8. Experimental design (Part I).

Table 2. Analysis of variance (Part I).

SOURCE OF VARIATION	DEGREES OF FREEDOM	
TOTAL	215	
Between Main Plot Units	5	
NaCl Type		2
Error A		3
Within Main Plot Units	210	
Blend Time		5
Post-Blend Storage		5
Blend Time x Post-Blend Storage		25
Blend Time x NaCl Type		10
Post-Blend Storage x NaCl Type		10
Blend Time x Post-Blend Storage x NaCl Type		50
Error B		105

Data Analysis

Data were analyzed according to least-squares analysis of variance procedures and significant differences were determined using the Neuman-Keuls multiple range test (Snedecor and Cochran, 1967).

Phase II

Source of Meat

Meat utilized in phase II consisted of fresh boneless cow fronts, fresh boneless picnics and frozen regular pork trimmings. The proximate analysis data for these materials are shown in Table 3. All analyses were performed according to AOAC (1970).

Processing

Figure 9 shows the processing sequence utilized in phase II for the production of bologna. The boneless cow fronts were ground once through a 4.8 mm plate and the boneless picnics and regular pork trimmings were ground once through a 9.5 mm plate.

The initial lean blends were formulated to 17% protein using boneless cow fronts and boneless picnics so that the final blend weight was 32 kg. These meat ingredients were added to a Buffalo paddle blender with NaCl (2.5% of the meat block) (Hardy's Institutional Salt) and 120 ppm of $NaNO_2$. Identical blends were made using blending times of 6, 8 and 10 minutes. Immediately following the completion

Table 3. Proximate composition of raw materials (Part II).

		MEAT SOURCE		
		Boneless Cow Fronts	Boneless Picnics	Regular Pork Trimmings
Moisture	(%)	65.04	59.31	34.30
Fat	(%)	15.01	24.98	54.95
Protein	(%)	18.97	15.62	9.73

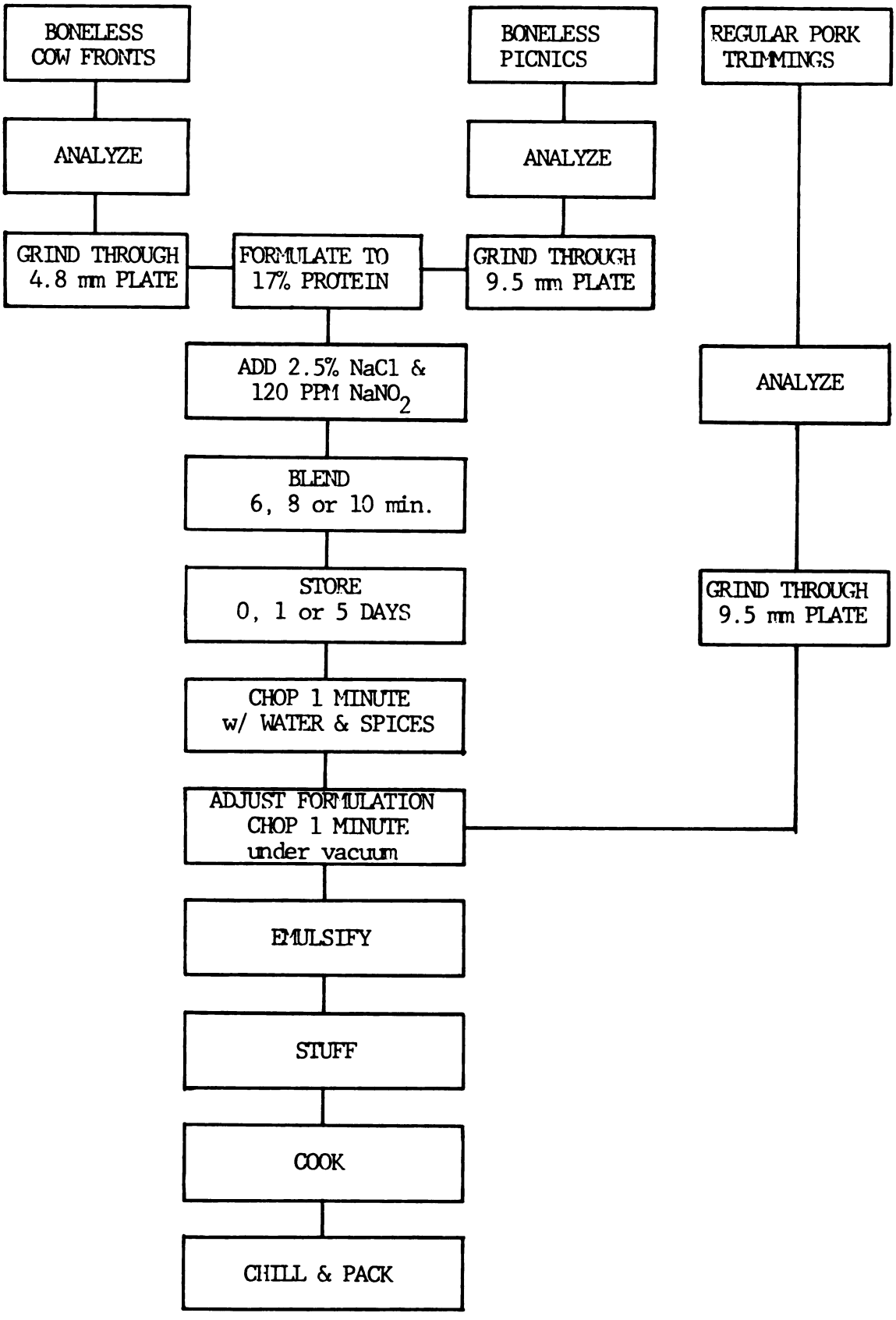


Figure 9. Processing flow chart (Part II).

of blending aliquots of the blends were placed in a Hobart Vertical Cutter Mixer (VCM) with spices (Table 4), the remaining $NaNO_2$ and NaCl and an amount of moisture consisting of 50% ice and 50% 17°C water sufficient to produce a finished product containing 30% fat and a moisture content equivalent to a 4 X finished protein + 10% assuming a 95% smokehouse yield. These ingredients were chopped for one minute at high speed. At this time the amount of regular pork trimmings necessary to establish the final fat content of 30% were added and chopping was resumed under a vacuum equivalent to 635 mm of Hg for one additional minute.

Following chopping, the batter was passed through the 2.0 mm plate of a one plate Griffith "Mincemaster" with the knives set at a torque of 100 ft-lbs. The temperature of the resulting batter was immediately measured and recorded. The batter was then stuffed into number 5½ U.C. E-Z PEEL casings (Union Carbide Corp.). The first, second and third sticks of bologna were labeled, rinsed with 17°C water, dried and weighed. These sticks were hung on a standard smokehouse tree for thermal processing. All products were cooked in an air-conditioned two cage smokehouse (Drying Systems Co., Inc.) using the following schedule of temperatures and relative humidities (R.H.):

Table 4. Non-meat ingredients in bologna formulation.

INGREDIENT	g/100kg meat
NaCl	2934.3
Sugar	1934.5
Ground White Pepper	423.7
Mustard	241.7
Mace	121.2
Nutmeg	121.2
Paprika	121.2
Onion Powder	60.6
Sodium Ascorbate	54.9
$NaNO_2$	12.0

1 hr at 57°C dry bulb and 38°C wet bulb; R.H. = 30%
1 hr at 63°C dry bulb and 48°C wet bulb; R.H. = 44%
1 hr at 69°C dry bulb and 57°C wet bulb; R.H. = 56%
to internal 68°C at 79°C dry bulb and 70° wet bulb;
R.H. = 65%.

Following the completion of this schedule the products were showered with cold water for 10 minutes and placed in a 2°C cooler. Upon the completion of 12 hours of chilling the bolognas were weighed and yields were calculated as a percentage of stuffed weight. Immediately after weighing the bolognas were peeled and 3.2 mm thick slices were removed using a meat slicer. Six of these slices from each treatment were vacuum packaged using a "Multivac" (Koch, Inc.) vacuum packaging machine and designated as samples for proximate analysis. Ten identical six slice packages were also made for use in sensory testing. The samples were stored at 2°C until analysis and evaluation. Products were also manufactured in the same manner following one and five days of post-blending storage at 4°C.

Proximate Analysis

AOAC (1970) approved procedures were utilized for the determination of fat, moisture and protein in the finished product. The procedures used were as follows:

fat - Goldfisch method using anhydrous ether
moisture - drying at 105°C for 24 hours
protein - from total nitrogen by Micro-Kjeldahl

Sensory Evaluation

Triangle tests were conducted using the comparisons listed in Table 5. Twenty judges evaluated each comparison using the form shown in Figure 10. All samples were assigned three digit random identification numbers prior to testing. Results were analyzed using a table comprising the binomial expansion with $p = 0.33$.

Statistical Design

Phase II was designed as a split plot arrangement of treatments with the main plot treatment, blending time, and the sub plot treatment, post-blending storage time completely randomized. Two complete replications were performed (Figure 11). The analysis of variance table and the partitioning of the degrees of freedom are shown in Table 6.

Data Analysis

Data were analyzed in the same manner as those collected in phase I of this project.

Table 5. Treatment comparisons used in the triangle difference test.

[1]BLEND TIME	[2]POST-BLEND STORAGE TIME
6 & 8 minutes	0 & 1 day
6 & 10 minutes	0 & 5 days
8 & 10 minutes	1 & 5 days

[1]Blend time comparisons were made using 0 days post-blending storage samples.

[2]Post-blend storage time comparisons were made using 6 minute blending time samples.

TRIANGLE TEST

Circle the number of the different sample.

____

____

____

Figure 10. Triangle test ballot.

SIX MINUTE BLEND TIME			EIGHT MINUTE BLEND TIME			TEN MINUTE BLEND TIME		
l_N S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3

$l_N = 2$

S_1 = 0 days storage

S_2 = 1 day storage

S_3 = 5 days storage

Figure 11. Experimental design (Part II).

Table 6. Analysis of variance (Part II).

SOURCE OF VARIATION	DEGREES OF FREEDOM	
TOTAL	17	
Between Main Plot Units	5	
Blend Time		2
Error A		3
Within Main Plot Units	12	
Post-blending storage		2
Post-blending storage X Blend Time		4
Error B		6

RESULTS AND DISCUSSION

Part I

Effects of NaCl Type

The effects of NaCl type on protein solubilization and pH of the meat blends are shown in Table 7.

The pH of the blends which were made using flake NaCl was significantly higher ($P< 0.05$) than the pH's of blends made with either dendritic or evaporated and granulated NaCl. The reason for this higher pH is not known. However, the implications of such a difference are of the utmost importance. In the blends manufactured with flake NaCl the pH was in the basic range of the isoelectric point (pI) of the major myofibrillar proteins. This should theoretically result in a greater degree of solubilization of these proteins as has been hypothesized by Hamm (1960). The evaporated and granulated NaCl appeared to result in slightly higher blend pH's than did the dendritic NaCl, however this difference was not significant.

The volume of the soluble phases for blends manufactured with these three NaCl types are also shown in Table 7. Flake NaCl blends produced significantly less soluble phase than either the dendritic ($P<0.05$) or evaporated and granulated ($P<0.01$) NaCl types. These results indicate that

Table 7. Least squares means for blend pH and soluble phase properties as influenced by type of NaCl.

		NaCl Type			
		Flake	Dendritic	Evaporated Granulated	$s_{\bar{x}}$
pH		6.16[a]	5.94[b]	6.03[b]	0.022
Soluble phase volume	(ml)	6.9[a]	7.7[bd]	9.5[ce]	0.16
Soluble phase protein	(mg/ml)	34.63[ad]	29.42[bde]	22.91[cf]	1.24

[abc]Means with different superscripts within the same row are significantly different ($P<0.05$).

[def]Means with different superscripts within the same row are significantly different ($P<0.01$).

the moisture in the blends manufactured using flake NaCl was more tightly bound within the blend structure than was the moisture in the blends produced with the other two NaCl types. This phenomenon can most probably be attributed to a greater degree of hydration of the muscle proteins present in the flake NaCl blends. This theoretical improvement in muscle protein hydration is directly related to the increased pH of the flake NaCl blends which resulted in the protein molecules being more highly charged in these samples; therefore allowing for the increased hydration of these proteins. Theoretically this should result in improved binding of moisture and fat in the finished product.

Blends manufactured with dendritic NaCl produced less soluble phase than samples of blends produced with the evaporated and granulated NaCl ($P<0.01$) (Table 7). This result is the opposite of what would be expected from comparing the blend pH values and the precise difference is not fully understood. Although no data are available to substantiate this theory it is very possible that the evaporated and granulated NaCl is less pure than the dendritic NaCl thus resulting in the presence of numerous ions as impurities therefore decreasing the hydration of the muscle proteins.

Protein concentration in the soluble phase as affected by NaCl type are shown in Table 7. Protein concentration was higher in those blends which were made using flake NaCl

as compared to blends manufactured with dendritic NaCl ($P<0.05$) and blends produced using evaporated and granulated NaCl ($P<0.01$). Those blends prepared with dendritic NaCl also had a greater protein concentration in the soluble phase than blends manufactured with evaporated and granulated NaCl ($P<0.05$). During the course of data collection it was also noticed that the soluble phases extracted from blends produced using flake NaCl were thicker and more glue-like in nature than those from blends produced with dendritic or evaporated and granulated NaCl.

These protein concentration data correspond quite closely to their respective soluble phase volumes which were previously discussed in that blends with lower soluble protein concentrations exhibited greater soluble phase volumes. This relationship supports the theory that as soluble phase volume decreases the binding properties of the batter will be increased due to increased efficiency of protein utilization through the improvement of protein solubilization properties.

Although the total amounts of protein in the soluble phase correspond quite closely to one another there was probably a great deal of soluble protein which was entrapped within the batter matrix. This entrapped protein, although solubilized, could not be extracted in the soluble phase due to the improved hydration properties of these proteins thus

causing them to be strongly bound within the matrix of the batter. This theory represents one possibility for the explanation of the inverse relationship between soluble phase volume and protein concentration within the soluble phase.

Blend stability data as influenced by NaCl type are listed in Table 8. Blends produced using flake NaCl were found to be superior to the other blends regarding all aspects of stability which were measured.

Water loss was greater for those blends manufactured using evaporated and granulated NaCl as compared to dendritic and flake NaCl ($P<0.01$). Flake NaCl blends also exhibited a lower water loss than those blends made using dendritic NaCl ($P<0.01$). These data concur with and reinforce the projections made earlier concerning the improved binding properties which could be expected due to increased pH and the resulting increase in soluble protein concentration and subsequent decrease in soluble phase volume.

Fat loss was significantly less for the blends produced using flake NaCl as compared to those produced with dendritic NaCl ($P<0.05$) and evaporated and granulated NaCl ($P<0.01$). Blends manufactured utilizing the dendritic NaCl also exhibited improved fat binding properties as compared to those blends produced using evaporated and granulated NaCl ($P<0.01$).

Total loss data again revealed that blends produced using the flake NaCl were superior to those produced with

Table 8. Least squares means for blend stability parameters as influenced by type of NaCl.

		NaCl Type			
		Flake	Dendritic	Evaporated Granulated	$s_{\bar{x}}$
Water loss	(ml/35g)	0.79^{d}	1.18^{e}	1.70^{f}	0.06
Fat loss	(ml/35g)	0.14^{ad}	0.29^{bd}	0.81^{e}	0.03
Total loss	(ml/35g)	0.93^{ad}	1.47^{bd}	2.51^{e}	0.09

^{ab}Means with different superscripts within a row are significantly different ($P<0.05$).

^{def}Means with different superscripts within a row are significantly different ($P<0.01$).

dendritic NaCl ($P<0.05$) and blends manufactured with evaporated and granulated NaCl ($P<0.01$). The dendritic NaCl blends were again superior to those produced using evaporated and granulated NaCl ($P<0.01$).

These stability results suggest that the binding potentials of the inherent meat proteins in blends manufactured with flake NaCl were more efficiently utilized than were those in the blends produced using dendritic or evaporated and granulated NaCl samples. These data coincide quite closely with the soluble protein, soluble phase and pH data discussed earlier and lend credibility to the interpretation of those data.

The relative proportions of several myofibrillar proteins in the soluble phase as affected by NaCl type are shown in Table 9. There were no significant differences between the various NaCl types studied regarding the relative percentages of either myosin heavy chains, actin or α-actinin which were present in the soluble phase of the meat blends.

The proportions of soluble phase protein in various isoelectric point ranges as affected by NaCl type are shown in Table 10. These data again suggest that the relative composition of the soluble phase protein was essentially constant as no significant differences were noted.

These electrophoretic data in combination with data discussed previously show that it is the amount of soluble protein which is important in developing blend stability

Table 9. Least squares means for concentrations of selected myofibrillar proteins in the soluble phase as influenced by type of NaCl.

		NaCl Type			
		Flake	Dendritic	Evaporated Granulated	$s_{\bar{x}}$
Myosin Heavy Chains	(%)	20.59	23.62	17.38	3.57
Actin	(%)	15.63	13.39	17.24	2.91
α-actinin	(%)	4.82	4.53	5.23	1.03

Table 10. Least squares means for isoelectric distribution of proteins in the soluble phase as influenced by type of NaCl.

Isoelectric Point (pH)		NaCl Type			$s_{\bar{x}}$
		Flake	Dendritic	Evaporated Granulated	
4 - 5	(%)	5.32	5.39	5.26	0.94
5 - 6	(%)	49.29	51.80	51.43	0.81
6 - 7	(%)	13.46	13.97	14.05	0.63
7 - 8	(%)	29.69	27.45	28.71	0.87

rather than changes in the type of protein which is present. Since the relative proportions of the various proteins did not change it must therefore be the effect of protein concentration in the soluble phase which manifests itself in terms of improved blend stability through the improved efficiency of available protein utilization.

Effect of Blending Time

The effects of blending time on blend pH and the characteristics of the soluble phase are shown in Table 11. The pH's of the meat blends were not influenced by blending time. pH values remained constant at approximately six throughout ten minutes of blending.

As blending time increased the volume of the soluble phase decreased to a minimum at eight minutes of blending and then increased slightly at the ten minute sampling interval (Figure 12). All blend times produced significantly different soluble phase volumes ($P<0.05$) with the exception of the comparison between four and six minutes of blending (Table 11).

The concentration of protein in the soluble phase increased in a linear manner from zero to four minutes of blending (Figure 13). As was the case for soluble phase volume this trait again leveled off between four and six minutes of blending and then exhibited an increase at the eight minute sampling interval with a decrease obvious at

Table 11. Blend pH and soluble phase properties as influenced by blending time.

		Blending Time (min.)						
		0	2	4	6	8	10	$s_{\bar{x}}$
pH		6.00	6.02	6.01	6.02	6.02	6.03	0.029
Soluble phase volume	(ml)	14.5^{a}	10.1^{b}	7.6^{c}	6.9^{c}	3.4^{d}	5.7^{e}	0.21
Soluble phase protein	(mg/ml)	21.58^{a}	26.46^{b}	29.02^{c}	29.47^{c}	30.93^{d}	29.64^{c}	0.47

^{abcde}Means with different superscripts are significantly different ($P<0.05$).

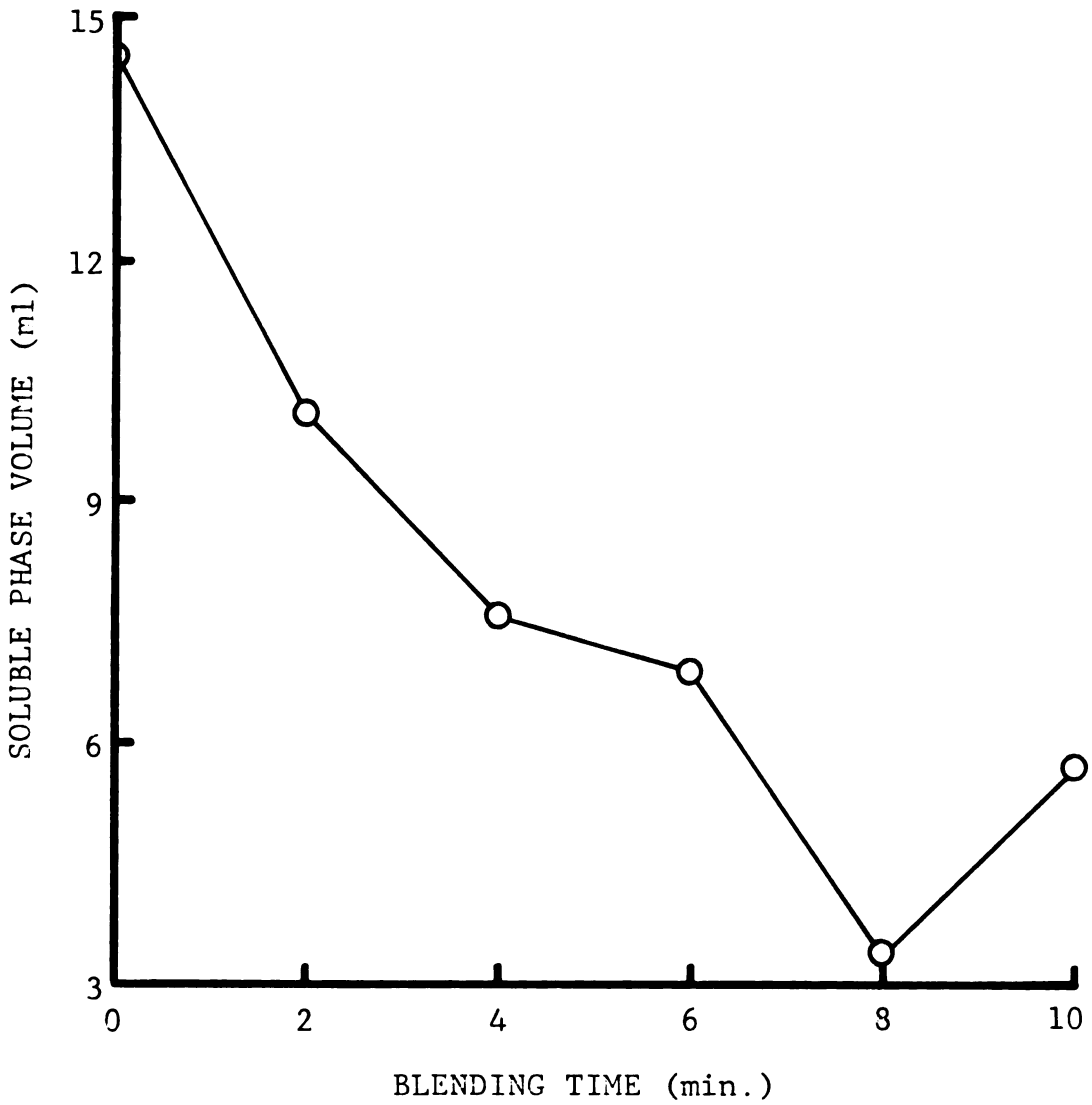


Figure 12. Influence of blending time on soluble phase volume.

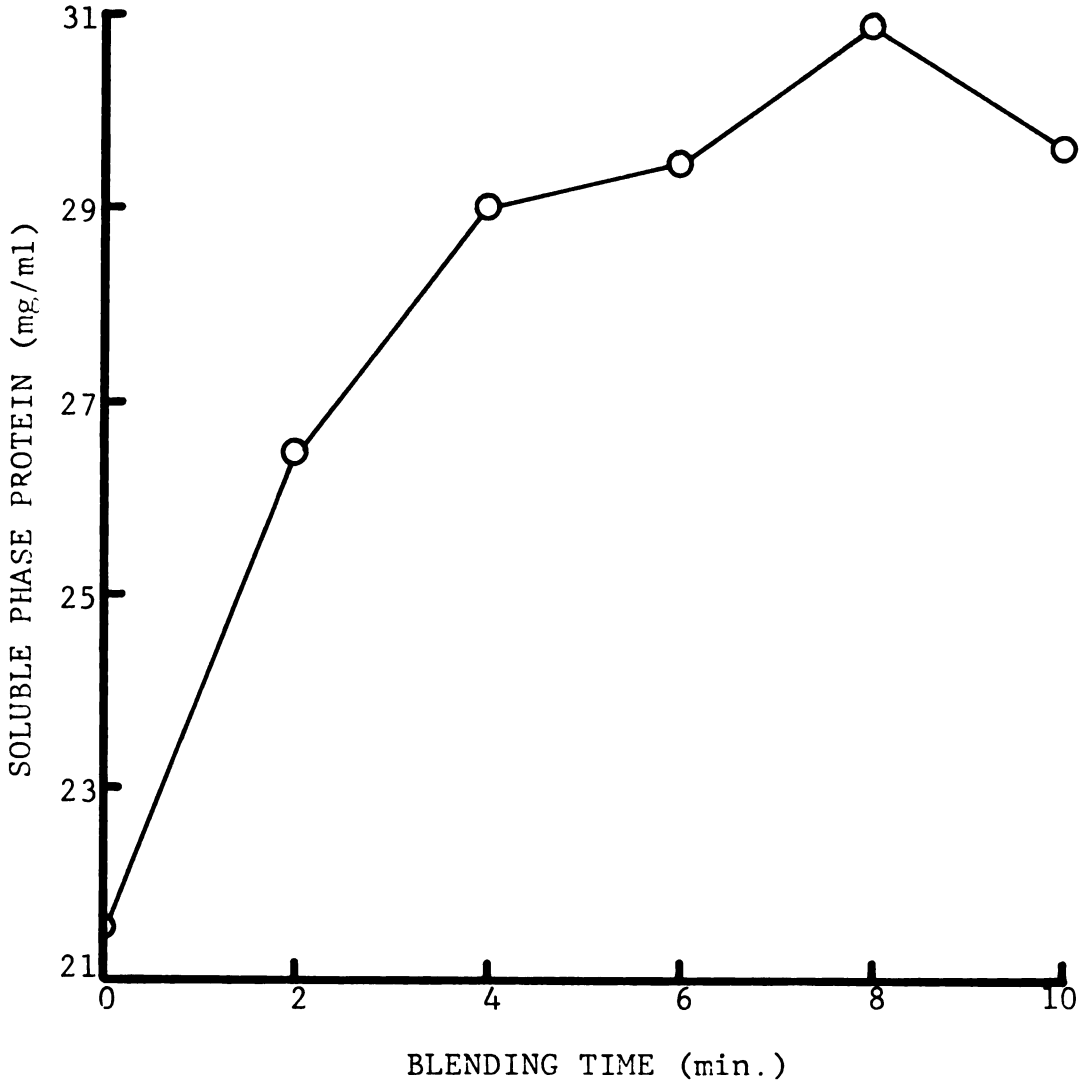


Figure 13. Influence of blending time on soluble phase protein concentration.

ten minutes of blending time. The mean comparisons revealed that all means were significantly different with the exception of the comparisons between four and six minutes, four and ten minutes and six and ten minutes ($P<0.05$).

When Figures 12 and 13 are compared it becomes apparent that soluble phase volume and protein concentration in the soluble phase responded to blending time in opposite but similar fashions. This is shown by the fact that as soluble phase volume decreased the protein concentration in this phase increased with both parameters reaching optimum levels at eight minutes of blending. This quite clearly illustrates the relationship between protein solubilization and the water retention properties of the blend, as has also been reported by Hamm (1960).

Blend stability parameters as influenced by blending time are shown in Table 12 and graphically in Figure 14. As blending time increased the amount of water lost during cooking decreased in a manner quite similiar to that which was previously discussed for the responses of soluble phase volume and protein concentration in the soluble phase. As in the case of these two parameters water loss during cooking reached a minimum at eight minutes of blending time. Unlike soluble phase volume and protein concentration in the soluble phase, however, water loss exhibited a significant ($P<0.05$) decrease when the blending time was extended from four to six minutes with no significant

Table 12. Least squares means for blend stability parameters as influenced by blending time.

		Blending Time (min.)						
		0	2	4	6	8	10	
Water loss	(ml/35g)	3.23^{g}	1.60^{h}	0.99^{ai}	0.62^{bi}	0.37^{bi}	0.51^{bi}	0.07
Fat loss	(ml/35g)	1.12^{a}	0.48^{b}	0.48^{b}	0.19^{c}	0.12^{c}	0.11^{c}	0.08
Total loss	(ml/35g)	4.35^{g}	2.08^{ha}	1.47^{b}	0.81^{ci}	0.49^{bi}	0.62^{bi}	0.07

^{abc}Means with different superscripts within a row are significantly different ($P<0.05$).

^{ghi}Means with different superscripts within a row are significantly different ($P<0.01$).

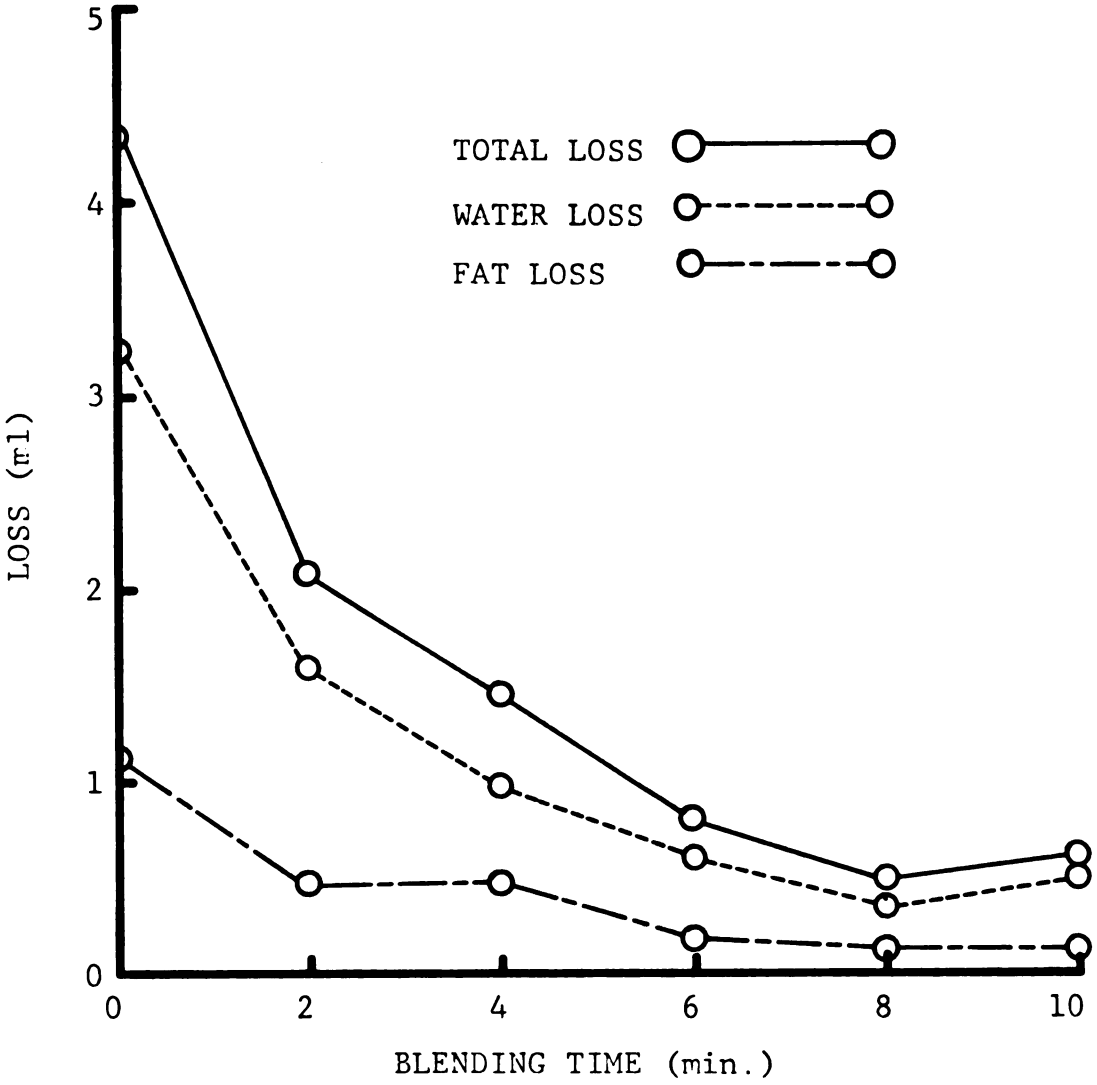


Figure 14. Influence of blending time on blend stability.

differences being found between the six, eight and ten minute blending times.

Fat loss responded to blending time by exhibiting significant decreases between zero and two and four and six minutes of blending ($P<0.05$). Again, as in the case of water loss, no differences were found to exist between the six, eight and ten minute blending times.

Total loss data showed the same basic pattern of change as that which was found for water loss. Decrease in water loss was at a maximum between zero and two minutes of blending. Following two minutes of blending the decrease in total loss occurred at a slower rate until a minimum total loss was reached at eight minutes of blending ($P<0.05$) and a subsequent increase in total loss was experienced between the eight and ten minute blending times ($P<0.05$).

Tables 13 and 14 show the breakdown of the soluble phase proteins by type of protein and isoelectric point range, respectively. As was the case concerning the effects of NaCl type no differences were found among the blending times studied regarding any of these parameters. This again implies that although more protein was solubilized as blending progressed the relative composition of the soluble proteins was basically unchanged. These results regarding the categorization of the proteins by isoelectric point range is probably due in part to the static condition which blend

Table 13. Least squares means for concentrations of selected myofibrillar proteins in the soluble phase as influenced by blending time.

		Blending Time (min.)						
		0	2	4	6	8	10	$s_{\bar{x}}$
Myosin Heavy Chains	(%)	20.81	20.25	20.53	20.94	20.02	20.63	0.90
Actin	(%)	15.49	14.82	16.04	15.63	15.21	15.33	1.14
α-actinin	(%)	4.71	4.82	4.89	4.81	4.91	5.02	0.49

Table 14. Least squares means for isoelectric distribution of proteins in the soluble phase as influenced by blending time.

Isoelectric Point (pH)		Blending Time (min.) 0	2	4	6	8	10	$s_{\bar{x}}$
4 - 5	(%)	5.36	5.37	5.25	5.28	5.30	5.36	0.68
5 - 6	(%)	50.79	50.64	50.86	50.74	50.95	51.06	0.82
6 - 7	(%)	13.79	13.80	13.88	13.74	13.94	13.83	0.23
7 - 8	(%)	28.87	28.88	28.03	28.54	28.33	29.01	0.73

pH maintained throughout the range of blending times studied.

All of these results concerning blending time strongly suggest that the optimum time of blending which is required to maximize protein solubilization and binding properties is in the range of six to ten minutes. Some of the data, in particular the total loss data, further suggest that in these model systems this optimum time exists at eight minutes of blending.

The decrease in the level of quality of most parameters studied in the blending time range of eight to ten minutes may be due to the increased level of mechanical energy which has been introduced into the system by further blending. This increased input of energy would theoretically result in a certain proportion of the proteins experiencing variable degrees of denaturation and therefore a subsequent decrease in solubilization and functionality as was seen in the previously discussed data. These results concerning the effects of blending time parallel those results previously reported concerning variable degrees of chopping (Brown and Toledo, 1975). Results on the electrophoretic separation of the soluble proteins are also in agreement with those results previously reported by Siegel et al. (1978a).

Effects of Post-Blending Storage Time

The means for the effects of post-blending storage time on the pH and solubility properties of sausage blends are shown in Table 15. The pH values of the blends did not change as storage time increased but rather maintained a constant value of approximately six. A small increase in pH value was always noted between days zero and one of storage, however, this increase was not large or consistent enough to be significant.

The volume of soluble phase (Figure 15) (Table 15) decreased in a linear fashion from zero to four days of post-blending storage. Following the minimum value which was obtained at four days of storage the blends exhibited an increase in soluble phase volume at day five of storage ($P<0.05$). All mean comparisons were found to be signficant ($P<0.05$).

The concentration of protein in the soluble phase (Figure 16) exhibited a linear increase from zero to three days of storage at which time protein concentration remained unchanged through day four ($P<0.05$). Protein concentration in the soluble phase then increased to a maximum at five days of post-blending storage ($P<0.05$).

The relationship between soluble phase volume and soluble protein concentration is less obvious in this case than it was in the case of blending time. However, as soluble

Table 15. Least squares means for blend pH and soluble phase properties as influenced by post-blending storage time.

		Post-Blending Storage Time (days)						
		0	1	2	3	4	5	$s_{\bar{x}}$
pH		5.97	6.02	6.03	6.03	6.04	6.04	0.029
Soluble phase volume	(ml)	14.5^{a}	11.5^{b}	7.7^{c}	5.9^{d}	3.7^{e}	5.0^{f}	0.21
Soluble phase protein	(mg/ml)	22.82^{a}	25.43^{b}	26.83^{c}	29.69^{d}	30.06^{d}	32.29^{e}	0.47

abcdef Means with different superscripts within a row are significantly different ($P<0.05$).

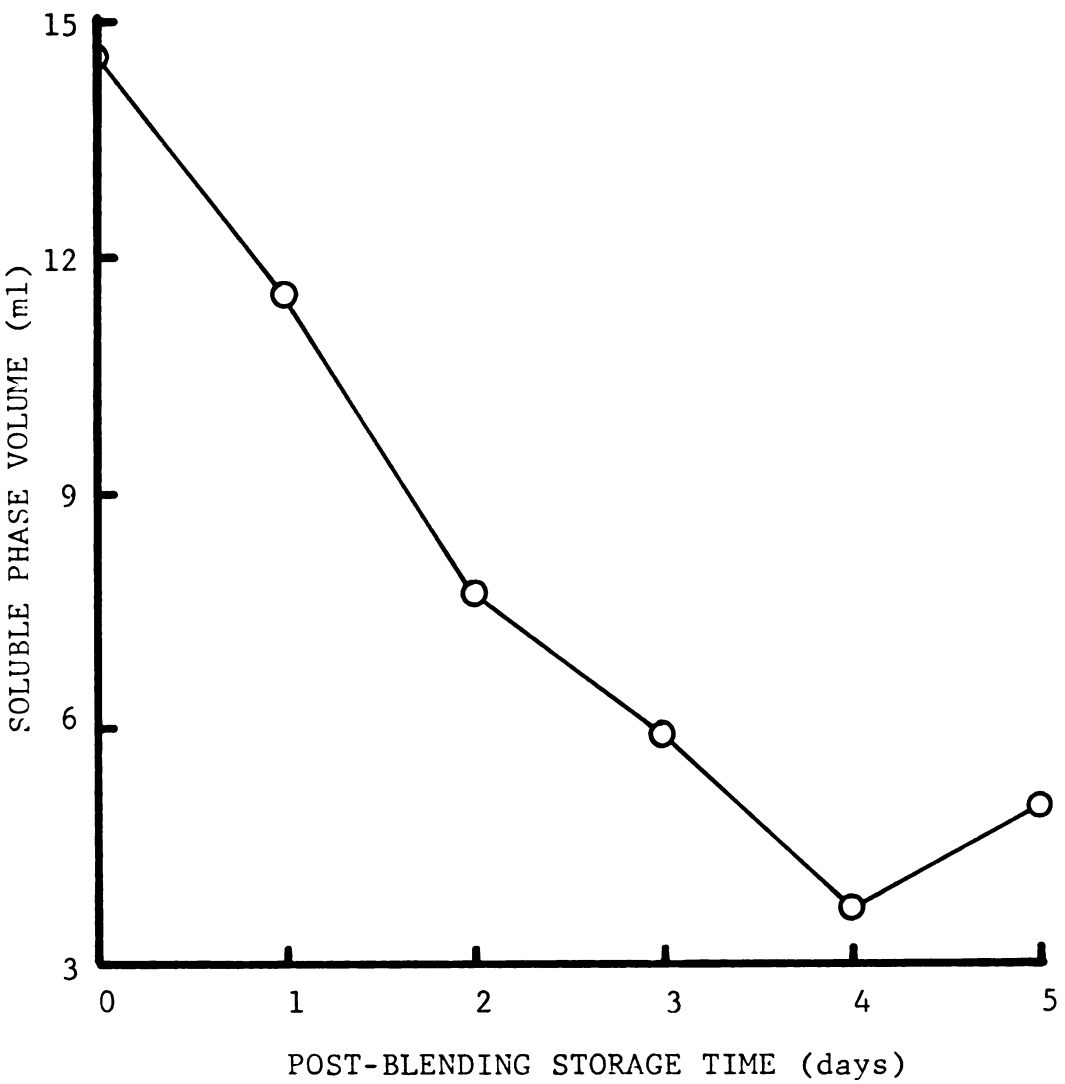


Figure 15. Influence of post-blending storage time on soluble phase volume.

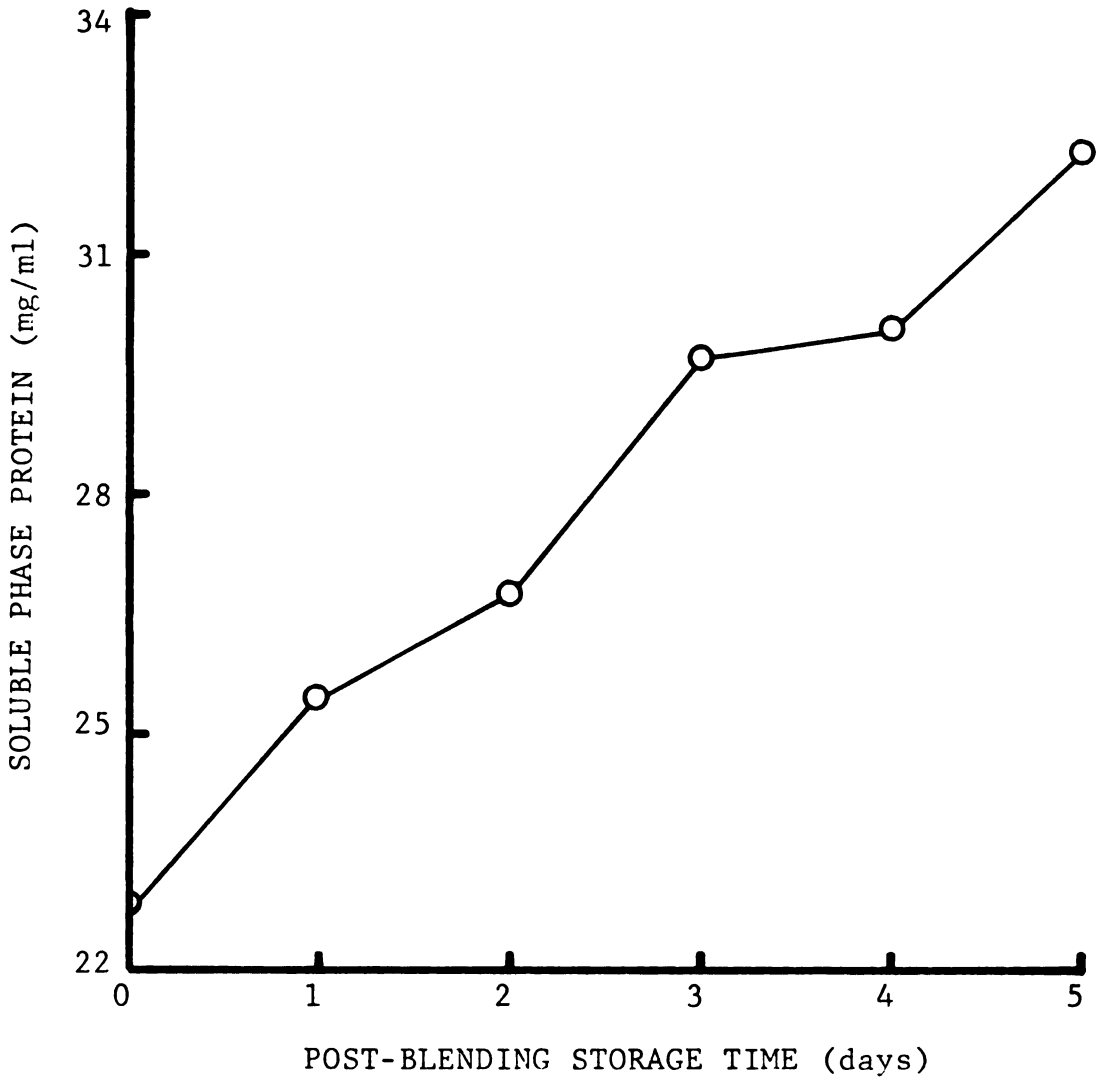


Figure 16. Influence of post-blending storage time on soluble phase protein concentration.

phase volume decreased the protein concentration increased and vice versa. An exception to this trend may be demonstrated by comparing the response of soluble protein concentration and soluble phase volume between four and five days of post-blending storage. Although the soluble protein concentration increased significantly ($P<0.05$) there was no corresponding decrease in soluble phase volume. This failure to behave in the previously established fashion may well have been due to a partial denaturation of the solubilized proteins during storage thus resulting in a decrease in functionality on a per unit protein basis therefore causing the unexpected increase in soluble phase volume. However, it should be noted at this point that this is merely conjecture and it should in no way be misconstrued as fact.

The effects of post-blending storage time on the binding properties of the meat blends as established by blend stability tests are shown in Table 16. These same effects are also graphically illustrated in Figure 17. Total loss decreased from zero to one day of storage ($P<0.01$) and then remained unchanged throughout the remainder of the five day storage period. Total losses after one, two, three, four and five days of post-blending storage were significantly less ($P<0.05$) than for the initial storage time studied.

Table 16. Least squares means for blend stability parameters as influenced by post-blending storage time.

		Post-Blending Storage Time (days)						
		0	1	2	3	4	5	$s_{\bar{x}}$
Water loss	(ml/35g)	1.46^{g}	1.11^{h}	1.12^{h}	1.21^{h}	1.20^{h}	1.22^{h}	0.07
Fat loss	(ml/35g)	0.62^{a}	0.59^{a}	0.48^{a}	0.35^{b}	0.23^{b}	0.19^{b}	0.08
Total loss	(ml/35g)	2.08^{g}	1.70^{h}	1.60^{h}	1.56^{h}	1.43^{h}	1.41^{h}	0.07

^{ab}Means with different superscripts within a row are significantly different ($P<0.05$)

^{gh}Means with different superscripts within a row are significantly different ($P<0.01$).

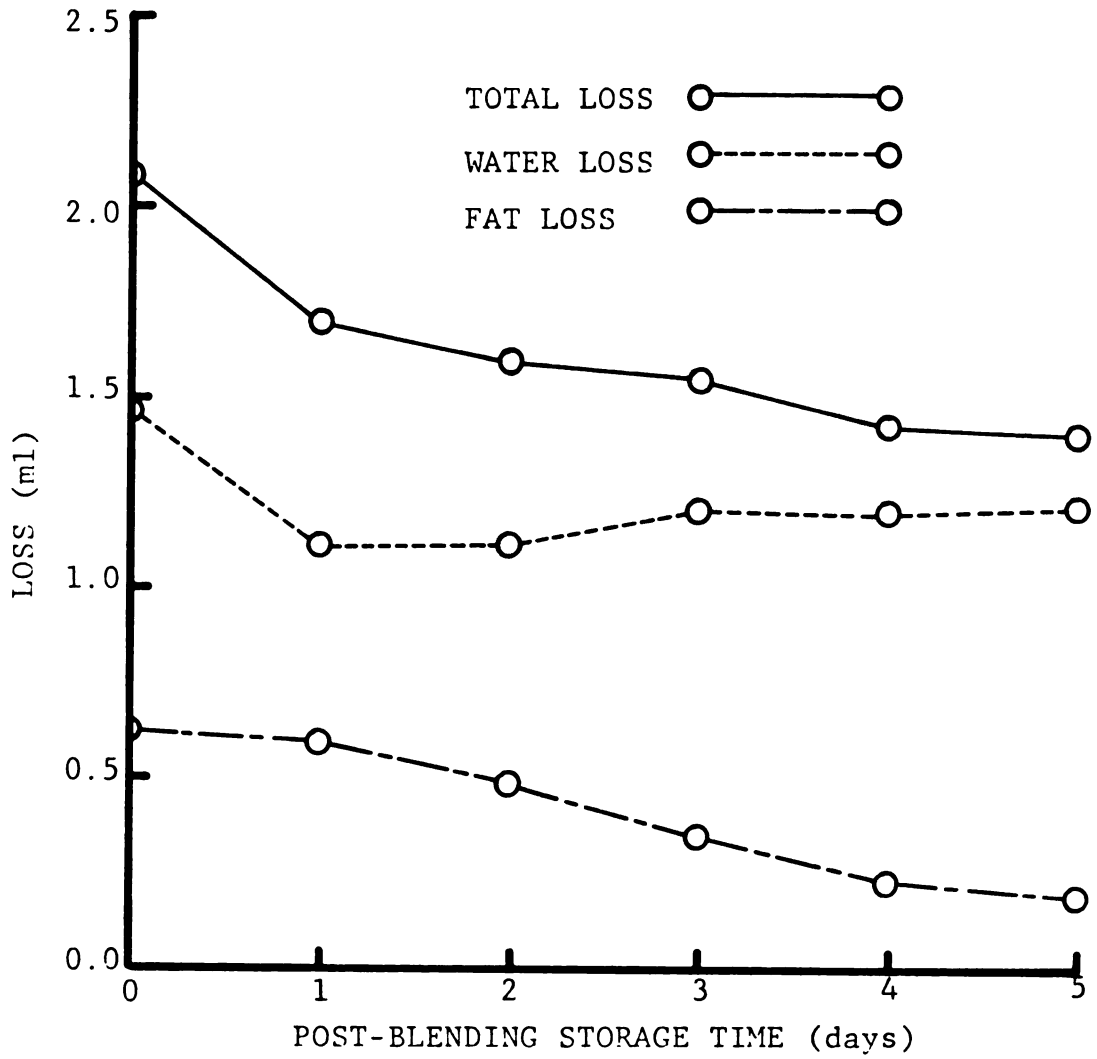


Figure 17. Influence of post-blending storage time on blend stability.

Water loss decreased ($P<0.01$) between zero and one day of storage and then remained constant through five days of post-blending storage. Whereas fat loss was unchanged after two days of post-blending storage and then experienced a latent linear decline from two to four days of storage. As in the case of total loss there was no difference between the fat losses for four and five days of storage ($P<0.05$).

These stability data indicate that water retention properties are the initial trait which is improved by post-blending storage with improved fat retention properties occurring at a later point in time. This phenomenon was quite possibly due to an increase in hydration in the basic range of the isoelectric points of the major muscle proteins caused by the binding of Cl^- ions from the NaCl as described by Hamm (1960). Once this muscle protein hydration had reached its maximum at one day of storage as indicated by the water loss data the proteins became more readily soluble in the actual meat system and therefore exerted the latent improvement shown in the fat retention properties of the meat blends.

The comparison of specific myofibrillar proteins and the partitioning of the soluble protein components by isoelectric point range are shown in Tables 17 and 18, respectively. As in the previous discussion of these data concerning NaCl type and blending time effects, no differences were noted for any of the traits evaluated. This once again

Table 17. Least squares means for concentrations of selected myofibrillar proteins in the soluble phase as influenced by post-blending storage time.

		Post-Blending Storage Time (days)						
		0	1	2	3	4	5	$s_{\bar{x}}$
Myosin Heavy Chains	(%)	20.32	20.20	20.56	20.93	20.86	20.31	0.90
Actin	(%)	15.30	15.32	15.14	15.64	15.71	15.41	1.14
α-actinin	(%)	5.08	4.88	4.72	4.82	4.94	4.62	0.49

Table 18. Least squares means for isoelectric distribution of proteins in the soluble phase as influenced by post-blending storage time.

Isoelectric Point (pH)		Post-Blending Storage Time (days)						$s_{\bar{x}}$
		0	1	2	3	4	5	
4 - 5	(%)	5.30	5.39	5.27	5.31	5.35	5.30	0.68
5 - 6	(%)	50.92	50.89	50.82	51.07	50.91	50.43	0.82
6 - 7	(%)	13.84	13.79	13.75	13.77	13.94	13.89	0.23
7 - 8	(%)	28.69	28.42	28.72	28.43	28.53	28.87	0.73

reinforces the theory that soluble protein composition is relatively constant. Therefore it is the concentration of solubilized protein which is important in determining the binding properties of the system rather than the type of protein which is solubilized. This in no way implies that all muscle proteins have the same ability to bind fat and water but rather that the overall binding ability per unit of total soluble protein is essentially constant due to consistent composition of the solubilized proteins.

As one compares all of the data presented regarding the effects of post-blending storage time it is evident that although soluble protein concentration was greatest following five days of post-blending storage that the additional soluble protein in this case lacked the ability to improve binding properties as evidenced by the blend stability data. Therefore, the optimum post-blending storage time appears to be four days in order to optimize the functionality of the available protein present in the system.

Blending Time by Post-Blending Storage Time Interaction

A graphical illustration of the effects of the significant ($P<0.01$) blending time by post-blending storage time interaction on protein concentration in the soluble phase is shown in Figure 18.

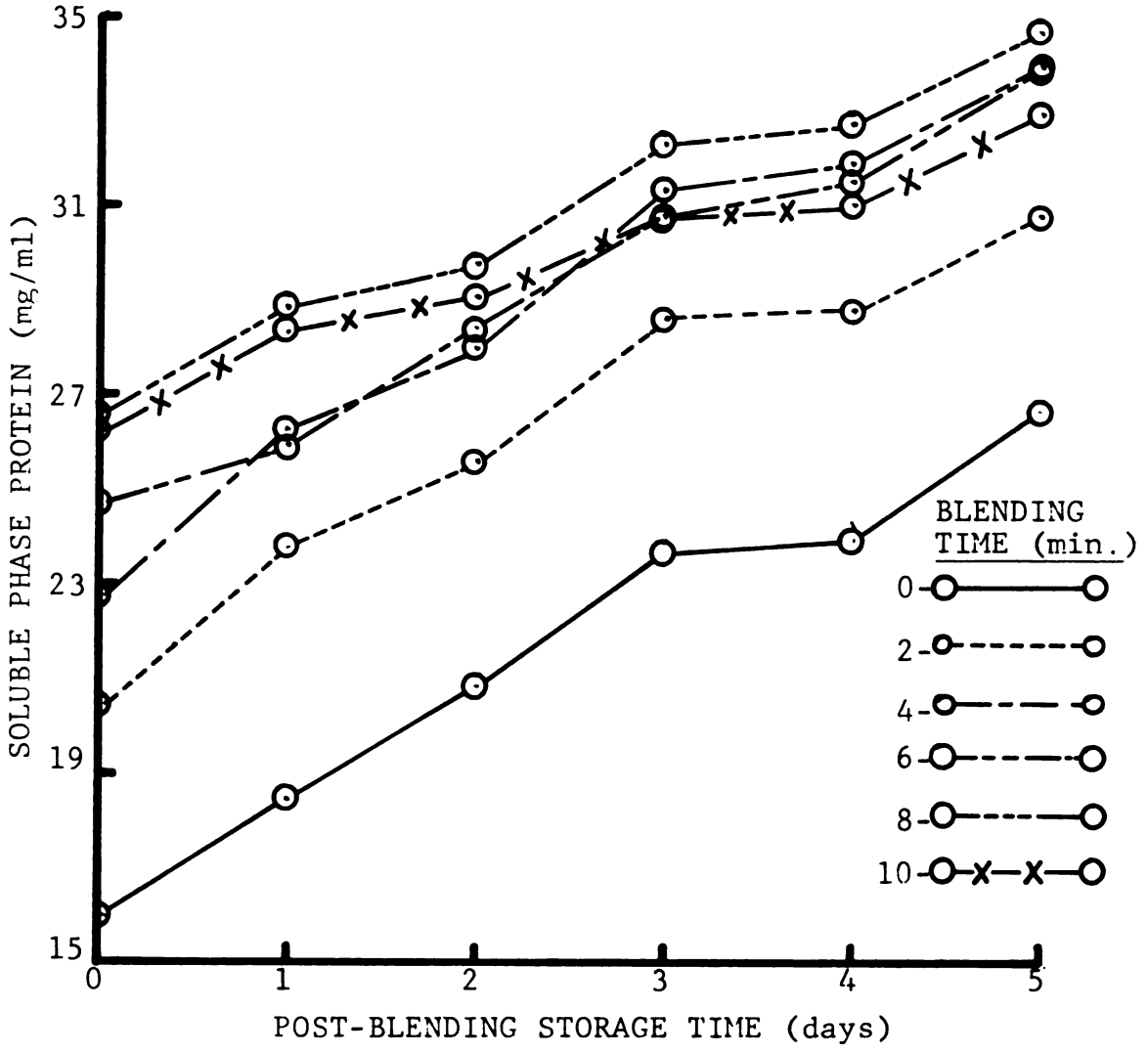


Figure 18. Influence of the interaction between blending time and post-blending storage time on soluble phase protein concentration.

The zero, two and eight minute blending time treatments all responded to post-blending storage in the same manner regarding soluble phase protein concentration. However, the four, six and ten minute treatments exhibited a high degree of interaction as illustrated by the frequent intersection of the lines representing these treatments. This suggests that the same degree of protein solubilization can be attained by storing meat blended for four,six or ten minutes for three days. It is also worthwhile to note from Figure 18 that the eight minute blending time resulted in superior protein solubilization at all storage times studied.

None of the other interaction terms from this study were found to be significant.

Part II

Effects of Blending Time

The effects of blending time on the proximate composition and yields of experimental bolognas are shown in Table 19. None of the parameters studied were influenced by blending times. These results coincide with results previously discussed concerning blend stability parameters and blending times as all of the blending times studied were in the plateau portion of blend stability responses to blending time as determined in part I of this project.

Table 19. Yields and proximate composition of experimental bologna as influenced by blending time.

		Blending Time (min.)			
		6	8	10	$s_{\bar{x}}$
Yield	(%)	95.20	95.27	94.66	0.33
Moisture	(%)	54.57	55.39	55.40	0.23
Fat	(%)	29.57	28.58	29.00	0.37
Protein	(%)	11.29	11.26	10.98	0.21
Moisture:Protein		4.85	4.94	5.05	0.091

Effects of Post-Blending Storage Time

The means for the effects of post-blending storage time on the proximate composition and yield of experimental bologonas is shown in Table 20. Yields were significantly greater ($P<0.01$) in those blends stored for one day as compared to both the zero time and five day blends. This result was no doubt due to a sharp increase in the quantity of solubilized protein present between the initial blending and one day of storage. The difference between one and five days of post-blending storage was probably due to the decrease in the binding efficiency of proteins following prolonged storage as was indicated by data discussed in phase I of this study.

Moisture and fat values were significantly greater ($P<0.05$) in products manufactured from meat blends stored for one day as compared to the blends stored for five days post-blending. These differences were primarily due to a sampling error which occurred in the analysis of the regular pork trimmings utilized in the formulation of these products. However, since the moisture:protein ration was unaffected by this sampling error this did not greatly influence the corresponding yield data.

Table 20. Yields and proximate composition of experimental bologna as influenced by post-blending storage time.

		Post-Blending Storage Time (days)			
		0	1	5	$s_{\bar{x}}$
Yield	(%)	94.49[d]	95.77[e]	94.87[d]	0.12
Moisture	(%)	54.08[a]	55.17[ab]	56.12[b]	0.41
Fat	(%)	30.65[a]	29.01[ab]	27.50[b]	0.56
Protein	(%)	10.68[a]	11.25[ab]	11.61[b]	0.19
Moisture:Protein		5.08	4.91	4.85	0.076

[ab]Means with different superscripts are significantly different ($P<0.05$).

[de]Means with different superscripts are significantly different ($P<0.01$).

Blending Time by Post-Blending Storage Time Interaction

Yields of experimental bolognas were affected by an interaction between blending time and post-blending storage time ($P<0.10$). This interaction is graphically depicted in Figure 19. Bolgonas produced from blends blended for eight and ten minutes showed a greater increase in yield due to one day of storage than those produced from six minutes blends. The decreases in yield which were then realized due to storage for five days were also correspondingly greater for these blends.

This trend may very well be due to greater amounts of protein being solubilized due to blending in the eight and ten minute blends, therefore causing these blends to achieve their maximum yields with less post-blending storage than the six minute blends. This phenomenon would also logically result in an earlier decline in binding properties due to the partial denaturation of these proteins at higher concentrations thereby explaining the lower yield values observed following five days of post-blending storage.

Sensory Evaluation Results

Table 21 contains the number of correct responses for each of the triangle comparisons conducted.

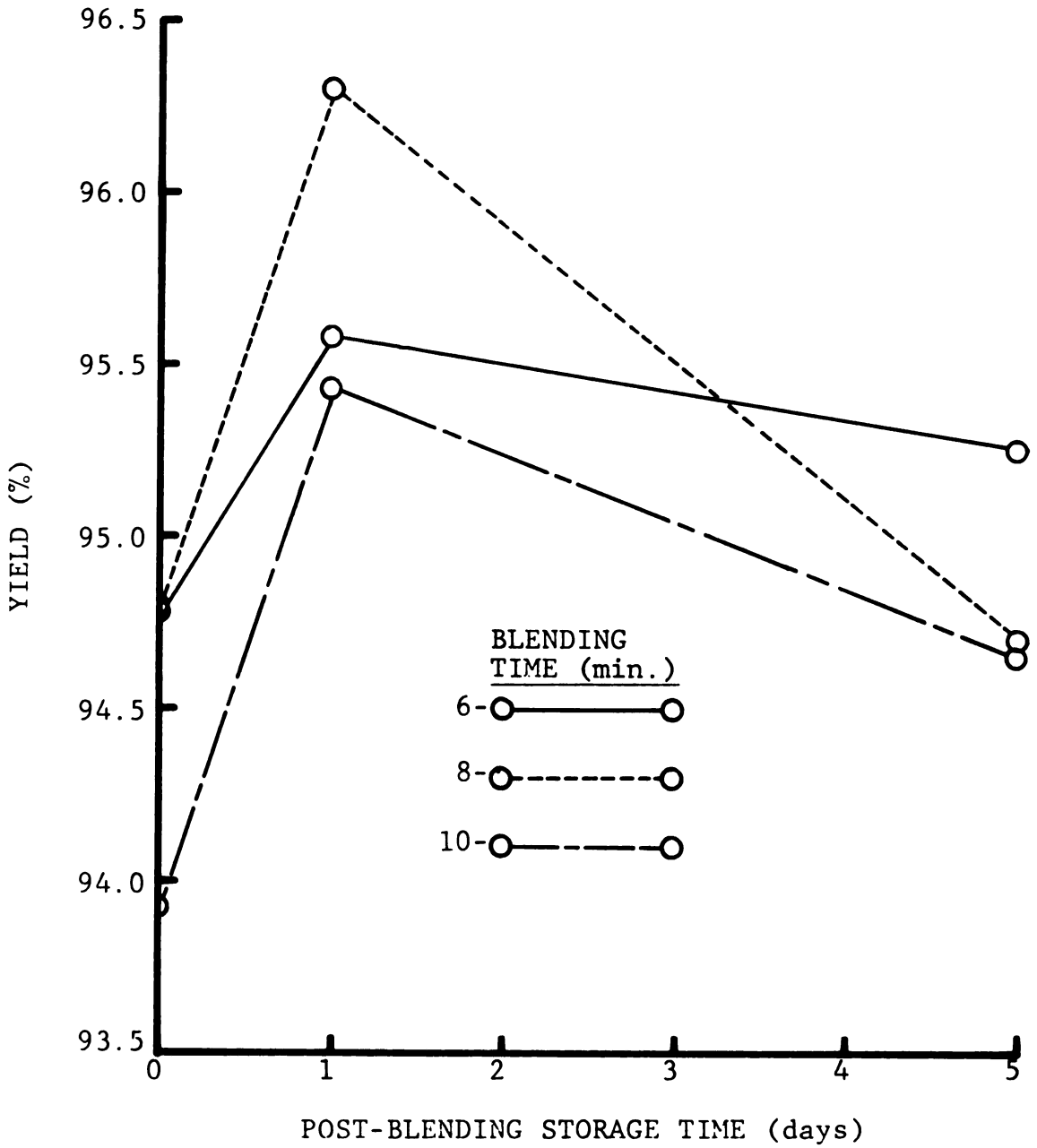


Figure 19. Influence of the interaction between blending time and post-blending storage on the yield of experimental bolognas.

Table 21. Number of correct judgements obtained by triangle difference testing of experimental bologna.

Blending Time (min.)		Post-Blending Storage Time (days)	
Comparison	[1]No. Correct	Comparison	[1]No. Correct
6 vs. 8	9	0 vs. 1	9
6 vs. 10	8	0 vs. 5	8
8 vs. 10	6	1 vs. 5	9

[1]Number of judges for each comparison equals 20.

None of the comparisons were found to be different by the judges. This strongly suggests that neither blending time nor post-blending storage time exerts an appreciable effect on the sensory quality of bologna.

SUMMARY

Flake NaCl produced red meat blends which possessed superior fat and water binding properties as compared to blends which were manufactured using dendritic or evaporated/granulated NaCl ($P<0.05$). The reason for this difference in the binding properties of blends produced with these experimental NaCl samples appears to lie in the relationship between blend pH and protein solubilization in that as pH increased both soluble protein concentration and blend stability parameters also improved.

The response of binding properties to variations in the time of blending was also quite clear in part I of this project. The concentration of protein in the soluble phase increased as blending time was extended until a maximum in soluble phase protein concentration was attained at eight minutes of blending. This phenomenon was also quite closely paralleled by the response of blend stability traits to extended blending times. The optimum blend stability parameters were achieved using a blending time of eight minutes also.

Storage time exerted a somewhat different effect on the properties of the blends utilized in this study. As in the case of blending time, as post-blending storage time

increased the concentration of protein in the soluble phase also increased until a maximum was reached after five days of storage. However, blend stability parameters showed very little change as storage time exceeded one day therefore, the efficiency of utilization of the soluble proteins was lower with extended post-blending storage times.

Post-blending storage time and blending time had a significant ($P<0.05$) combined effect on the protein concentration in the soluble phase of meat blends. It was found that blending times of four, six and ten minutes possessed virtually identical soluble phase protein concentrations when stored for two to three days post-blending. It was also noted from the analysis of this interaction that a blending time of eight minutes possessed superior soluble phase protein concentrations at all post-blending storage times studied.

Data reported regarding the composition of the soluble protein showed no differences attributable to any of the treatments studied. This result implies that the functionality and fat and water binding properties of the soluble proteins are dependent on the concentration of the solubilized protein in the blend rather than on some more involved effect relating to protein type.

Part II of this study concentrated on the more promising treatments studied in part I. These were blending

time and post-blending storage time. Blending times of six, eight and ten minutes and storage times of zero, one and five days post-blending were utilized in a pilot plant bologna production system.

Yield data on these experimental bolognas revealed that a post-blending storage period of one day produced a product with a greater yield ($P<0.01$) than those products manufactured from blends stored for zero and five days post-blending. These data seemingly concur with those obtained in part I which showed that the most rapid improvement in binding parameters and protein solubilization occurred during the first day of post-blending storage.

Blending time and post-blending storage produced a significant interaction ($P<0.10$). This interaction suggested that a blending time of eight minutes combined with a post-blending storage period of one day produced maximum yields.

The results of sensory difference testing revealed that panelists were unable to differentiate between the various blending times and post-blending storage intervals utilized in this study.

Preblending systems designed around a blending time of six to eight minutes and a storage interval of one day post-blending utilizing the equipment and processing parameters specified in this study should result in the maximization of the utilization of muscle proteins. This should

therefore enable the processor to utilize a greater proportion of lower cost meat ingredients in sausage formulations thus decreasing the finished product cost while maintaining the same level of quality as that which is characteristic of products which are not produced via preblending systems.

BIBLIOGRAPHY

BIBLIOGRAPHY

Ackerman, S.A., Swift, C.E., Carroll, R.J. and Townsend, W.E. 1971. Effects of types of fat and of rates and temperatures of comminution on dispersion of lipids in frankfurters. J. Food Sci. 36:266.

Acton, J.C. 1973. Composition and properties of extruded, texturized poultry meat. J. Food Sci. 38:571.

AOAC. 1970. "Official Methods of Analysis," (11th Ed.) Association of Official Agricultural Chemists. Washington, D.C.

Bard, J.C. 1965. Some factors influencing extractability of salt soluble proteins. Proc. Meat Ind. Research Conf., p. 96. Am. Meat Inst. Foundation, Chicago, IL.

Baumann, G. and Chrambach, A. 1976. A highly crosslinked, transparent polyacrylamide gel with improved mechanical stability for use in isoelectric focusing and isotachophoresis. Anal. Biochem. 70:32.

Becher, P. 1965. "Emulsions: Theory and Practice," 2nd ed. Reinhold Publishing Corp., New York.

Belohlavy, R. 1975. The effects of mechanical mixing time and salt on the chemical and physical properties of flaked, formed and sectioned meat products. M.S. Thesis. University of Nebraska, Lincoln.

Borchert, L.L., Greaser, M.L., Bard, J.C., Cassens, R.G. and Briskey, E.J. 1967. Electron microscopy of a meat emulsion. J. Food Sci. 32:419.

Borton, R.J., Webb, N.B. and Bratzler, L.J. 1968. Emulsifying capacities and emulsion stability of dilute meat slurries from various meat trimmings. Food Technol. 22:94.

Brown D.D. and Toledo, R.T. 1975. Relationship between chopping temperatures and fat and water binding in comminuted meat batters. J. Food Sci. 40:1061.

Buttkus, H. 1970. Accelerated denaturation of myosin in frozen solution. J. Food Sci. 35:558.

Cann, J.R. and Phelps, E.A. 1955. Binding of salt ions by bovine γ-pseudoglobulin. J. Am. Chem. Soc. 77:4266.

Carpenter, J.A. and Saffle, R.L. 1964. A simple method of estimating the emulsifying capacity of various sausage meats. J. Food Sci. 29:774.

Cassidy, R.D., Ockerman, H.W., Krol, B., van Roon, P.S., Plimpton, R.F. and Cahill, V.R. 1978. Effect of tumbling method, phosphate level and final cook temperature on histological characteristics of tumbled porcine muscle tissue. J. Food Sci. 43:1514.

Florini, J.R., Brivio, R.P. and Battelle, B.A.M. 1971. Isoelectric focusing of myosin in dilute polyacrylamide gels. Anal. Biochem. 40:345.

Froning, G.W. 1970. Poultry meat sources and their emulsifying characteristics as related to processing variables. Poultry Sci. 49:1625.

Froning, G.W. and Janky, D. 1971. Effect of pH and salt preblending on emulsifying characteristics of mechanically deboned turkey frame meat. Poultry Sci. 50:1206.

Froning, G.W. and Neelakantan, S. 1971. Emulsifying characteristics of prerigor and postrigor poultry muscle. Poultry Sci. 50:839.

Fukazawa, T., Hashimoto, Y. and Yasui, T. 1961a. Effect of storage conditions on some physico-chemical properties in experimental sausage prepared from fibrils. J. Food Sci. 26:331.

Fukazawa, T., Hashimoto, Y. and Yasui, T. 1961b. Effect of some proteins on the binding quality of an experimental sausage. J. Food Sci. 26:541.

Fukazawa, T., Hashimoto, Y. and Yasui, T. 1961c. The relationship between the components of myofibrillar protein and the effect of various phosphates that influence the binding quality of sausage. J. Food Sci. 26:550.

Galluzzo, S.J. and Regenstein, J.M. 1978a. Emulsion capacity and timed emulsification of chicken breast muscle myosin. J. Food Sci. 43:1757.

Galluzzo, S.J. and Regenstein, J.M. 1978b. Role of chicken breast muscle proteins in meat emulsion formation: myosin, actin and synthetic actomyosin. J. Food Sci. 43:1761.

Galluzzo, S.J. and Regenstein, J.M. 1978c. Role of chicken breast muscle proteins in meat emulsion formation: natural actomyosin, contracted and uncontracted myofibrils. J. Food Sci. 43:1766.

Goa, J. 1953. A micro biuret method for protein determination: determination of protein in cerebrospinal fluid. Scand. J. Clin. Lab. Invest. 5:218.

Goll, D.E., Henderson, D.W. and Kline, E.A. 1964. Post mortem changes in physical and chemical properties of bovine muscle. J. Food Sci. 29:590.

Gorbatov, A.V. and Gorbatov, V.M. 1974. Advances in sausage meat rheology. J. Texture Studies 4:406.

Gorbatov, V.M., Spirin, E.E. and Gorbatov, A.V. 1972. Some aspects of the theory of comminuted meat adhesion. J. Food Technol. 7:1.

Gorovsky, M.A., Carlson, K. and Rosenbaum, J.L. 1970. Simple method for quantitive densitometry of polyacrylamide gels using fast green. Anal. Biochem. 35:359.

Hamm, R. 1957. Uber das Wasserbindungsvermogen des Saugetiermuskels. III. Mitt. Die Wirkun von Neutralsalzen. Z. Lebensm.-Untersuch. u. Forsch. 106:281. (German).

Hamm, R. 1960. Biochemistry of meat hydration. Adv. Food Res. 10:355.

Hamm, R. 1973. Die Bedeutung des Wasserbindungsvermogens des Fleishes bei der Bruhwurstherstellung. Fleischwirtschaft 25:72. (German).

Hamm, R. and Grau, R. 1958. Uber das Wasserbindungsvermogen des Saugetiermuskels. IV. Mitt. Die Wirkung von Neutralsalzen bei Hitzedenaturierung. Z. Lebensm. Untersuch. u. Forsch. 108:28. (German).

Hansen, L.J. 1960. Emulsion formation in finely comminuted sausage. Food Technol. 14:565.

Hargus, G.L., Froning, G.W., Mebus, C.A., Neelakantan, S. and Hartung, T.E. 1970. Effect of processing variables on stability and protein extractability of turkey meat emulsions. J. Food Sci. 49:1625.

Hegarty, G.R., Bratzler, L.J. and Pearson, A.M. 1963. Studies on the emulsifying properties of some intracellular beef muscle proteins. J. Food Sci. 28:663.

Helmer, R.L. and Saffle, R.L. 1963. Effect of chopping temperature on the stability of sausage emulsion. Food Technol. 17:1195.

Hwang, P.A. and Carpenter, J.A. 1975. Effect of pork hearts, additives and pH adjustment on properties of meat loaves. J. Food Sci. 40:741.

Johnson, H.R. 1975. Physical and chemical influences on meat emulsion stability. Ph.D. Dissertation. Purdue University, West Lafayette, IN.

Johnson, H.R., Aberle, E.D., Forrest, J.C., Haugh, C.G. and Judge, M.D. 1977. Physical and chemical influences on meat emulsion stability in a model emulsitator. J. Food Sci. 42:523.

Johnston, J. 1979. Personal communication.

Krause, R.J., Ockerman, H.W., Krol, B., Moerman, P.C. and Plimpton, R.F. 1978a. Influence of tumbling, tumbling time, trim and sodium tripolyphosphate on quality and yield of cured hams. J. Food Sci. 43:853.

Krause, R.J., Plimpton, R.F., Ockerman, H.W. and Cahill, V.R. 1978b. Influence of tumbling and sodium tripolyphosphate on salt and nitrite distribution in porcine muscle. J. Food Sci. 43:190.

Lehninger, A.L. 1975. "Biochemistry," 2nd ed. Worth Publishers Inc., New York.

Maesso, E.R., Baker, R.C., Bourne, M.C. and Vadehra, D.V. 1970a. Effect of some physical and chemical treatments on the binding quality of poultry loaves. J. Food Sci. 35:440.

Maesso, E.R., Baker, R.C. and Vadehra, D.V. 1970b. The effect of vacuum, pressure, pH and different meat types on the binding ability of poultry meat. Poultry Sci. 49:697.

Maurer, A.J. and Baker, R.C. 1966. The relationship between collagen content and emulsifying capacity of poultry meat. Poultry Sci. 45:1317.

Maurer, A.J., Baker, R.C. and Vadehra, D.V. 1969a. Kind and concentration of soluble protein extract and their effect on the emulsifying capacity of poultry meat. Food Technol. 23:575.

Maurer, A.J., Baker, R.C. and Vadehra, D.V. 1969b. The influence of type of poultry and carcass part on the extractability and emulsifying capacity of salt-soluble proteins. Poultry Sci. 48:994.

Meyer, J.A., Brown, W.L., Giltner, N.E. and Guinn, J.R. 1964. Effect of emulsifiers on the stability of sausage emulsions. Food Technol. 18:1796.

Morrison, G.S., Webb, N.B., Blumer, T.N., Ivey, F.J. and Haq, A. 1971. Relationship between composition and stability of sausage type emulsions. J. Food Sci. 36:426.

McCready, S.T. and Cunningham, F.E. 1971. Salt-soluble proteins of poultry meat. Poultry Sci. 50:243.

Neer, K.L. 1975. The effects of salt, sodium tripolyphosphate and frozen storage on flaked, cured pork. Ph.D. Dissertation. University of Nebraska,Lincoln.

Ockerman, H.W., Plimpton, R.F., Cahill, V.R. and Parrett, N.A. 1978. Influence of short term tumbling, salt and phosphate on cured canned pork. J. Food Sci. 43:878.

O'Farrell, P.H. 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250:4007.

Pepper, F.H. and Schmidt, G.R. 1975. Effect of blending time, salt, phosphate and hot-boned beef on binding strength and cook yield of beef rolls. J. Food Sci. 40:227.

Porzio, M.A. and Pearson, A.M. 1977. Improved resolution of myofibrillar proteins with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Biochim. Biophys. Acta. 490:27.

Potter, J.D. 1974. The content of troponin, tropomyosin, actin and myosin in rabbit skeletal muscle myofibrils. Arch. Biochem. Biophys. 162:436.

Saffle, R.L. 1965. Some comments on emulsifying properties of muscle proteins. Proc. Meat Ind. Research Conf., p.94. Am. Meat Inst. Foundation, Chicago, IL.

Saffle, R.L. 1968. Meat emulsions. Adv. Food Res. 16:105.

Schellman, J.A. 1953. The application of Bjerrums' theory of ion association on binding of anions by proteins. J. Phys. Chem. 57:472.

Schmidt, G.R. 1979. New methods in meat processing. Proc. Meat Ind. Research Conf., p. 31. Am. Meat Inst. Foundation, Chicago, IL.

Siegel, D.G., Theno, D.M. and Schmidt, G.R. 1978a. Meat massaging: the effects of salt, phosphate and massaging on the presence of specific skeletal muscle proteins in the exudate of a sectioned and formed ham. J. Food Sci. 43:327.

Siegel, D.G., Theno, D.M. and Schmidt, G.R. 1978b. Meat massaging: the effects of salt, phosphate and massaging on cooking loss, binding strength and exudate composition in sectioned and formed ham. J. Food Sci. 43:331.

Snedecor, G.W. and Cochran, W.G. 1967. " Statistical Analysis," (6th Ed.). Iowa State University Press, Ames, IA.

Starr, L.D. 1979. What's new in the old technologies: cooking, chopping and converting. Proc. Meat Ind. Research Conf., p.41. Am. Meat Inst. Foundation, Chicago, IL.

Swift, C.E., Lockett, C. and Fryar, A.J. 1961. Comminuted meat emulsions: the capacity of meats for emulsifying fat. Food Technol. 15:468.

Swift, C.E. and Sulzbacher, W.L. 1963. Factors affecting meat proteins as emulsion stabilizers. Food Technol. 17:224.

Theno, D.M. and Schmidt, G.R. 1978. Microstructural comparisons of three commercial frankfurters. J. Food Sci. 43:845.

Theno, D.M., Siegel, D.G. and Schmidt, G.R. 1977. Meat massaging techniques. Proc. Meat Ind. Research Conf., p.53. Am. Meat Inst. Foundation, Chicago, IL.

Theno, D.M., Siegel, D.G. and Schmidt, G.R. 1978a. Meat massaging: effects of salt and phosphate on the microstructural composition of the muscle exudate. J. Food Sci. 43:483.

Theno, D.M., Siegel, D.G. and Schmidt, G.R. 1978b. Meat massaging: effects of salt and phosphate on the ultrastructure of cured porcine muscle. J. Food Sci. 43:488.

Townsend, W.E., Ackerman, S.A., Witnauer, L.P., Ralm, W.E. and Swift, C.E. 1971. Effects of types and levels of fat and rates and temperatures of comminution on the processing characteristics of frankfurters. J. Food Sci. 36:261.

van Eerd, J.D. 1971. Meat emulsion stability: influence of hydrophilic-lipophilic balance, salt concentration and blending with surfactants. J. Food Sci. 36:1121.

Webb, N.B. 1968. Preformulation of sausage raw material. Proc. 21st Ann. Reciprocal Meat Conference, p. 371. National Live Stock and Meat Board, Chicago, IL.

Weber, H.H. and Portzehl, H. 1952. Muscle contraction and fibrous muscle proteins. Adv. Protein Res. 7:162.

Weiss, G.M. 1974. Ham tumbling and massaging: A special report. Western Meat Industry. October: 10.

APPENDICES

APPENDIX A. Manufacturer's analysis of experimental NaCl types.

	Flake	Dendritic	Evaporated/ Granulated
NaCl (%)	99.95	99.9	99.6
Sodium Sulfate (%)	0.00	0.08	
Calcium Carbonate (%)	0.013	0.013	
Magnesium Sulfate (%)	0.00	0.007	
Moisture (%)	0.10	0.05	0.07
Total $Ca^{+}Mg$ as Ca (ppm)		55.0	
Cu (ppm)	0.4	0.3	0.02
Fe (ppm)	0.7		
Free		0.7	1.0
Complexed		2.0	0.9
Sodium Ferrocyanide (ppm)		11.0	5.0
Calcium Silicate (%)		0.25	
Calcium Sulfate (%)	0.02		0.11
Calcium Chloride (%)			0.03
Magnesium Chloride (%)	0.00		0.02
Sodium Sulfate	0.00		
Polysorbate 80 (%)	0.001		

APPENDIX B. Micro-biuret procedure.

Reagents

I. Biuret-Dissolve 173 g sodium citrate and 100 g sodium carbonate in about 500 ml of water with gentle warming. Dissolve 17.3 g copper sulfate pentahydrate in 100 ml of water and add to the first solution. Make up to 1000 ml with water. Discard if reddish precipitate forms.

II. Sodium hydroxide - 6% w/v

Sample

0.1 - 4.0 mg protein in 4 ml 3% NaOH.

Test

I. To a sample containing 0.1 - 4.0 mg protein in up to 2 ml volume add 2 ml 6% NaOH.

II. Add 0.2 ml biuret reagent, mix and allow to stand 15 minutes at room temperature.

III. Read absorbance at 540 nm.

Standard Curve

Develop a standard curve using a protein source with a composition similar to that which will be present in the experimental samples.

APPENDIX C. Myofibril preparation procedure.

Reagents

I. 0.25 M sucrose, 0.05 M Tris, 1 mM EDTA; adjust to pH 7.6 with HCl.

II. 0.05 M Tris, 1 mM EDTA; adjust to pH 7.6 with HCl.

III. 0.15 M KCl
pre-cool all reagents to 4°C

Sample Preparation

Carefully trim the muscle sample of excess fat and grind or otherwise macerate the tissue at 4°C.

Isolation

I. Suspend the macerated tissue in 10 volumes (v/w) of reagent I. Blend in a Waring blender for 30 seconds.

II. Centrifuge the slurry at 1000 x g for 10 minutes.

III. Decant the supernatant and resuspend the sedimented myofibrils in 5 volumes (v/w) of reagent I.

IV. Centrifuge at 1000 x g for 10 minutes.

V. Decant the supernatant and resuspend the sedimented myofibrils in 5 volumes (v/w) of reagent II by homogenizing in a Waring blender for 15 seconds.

VI. Pass the suspension through four layers of cheese-cloth to remove connective tissue.

VII. Centrifuge at 1000 x g for 10 minutes.

VIII. Decant the supernatant and resuspend the sedimented myofibrils in 5 volumes (v/w) of reagent III.

IX. Centrifuge at 1000 x g for 10 minutes.

X. Repeat step VIII.

XI. Decant the supernatant and resuspend the sedimented myofibrils in 5 volumes (v/w) of reagent III.

APPENDIX D. Sodium dodecyl sulfate polyacrylamide gel electrophoresis procedure.

Reagents

I. Dissolve 25 g of acrylamide and 0.25 g bis in 50 ml of distilled, deionized water, bring to 100 ml with water, filter and store at 4°C.

II. Dissolve 121 g of Trizma base and 225 g of glycine in 1500 ml of distilled, deionized water. Bring to 200 ml with water and store at 4°C.

III. Dissolve 12.5 g of sodium dodecyl sulfate (SDS) and 0.465 g EDTA in 400 ml of deionized, distilled water and bring to 500 ml with water.

IV. Dissolve 0.1 g of ammonium persulfate in 5 ml of deionized, distilled water and bring to 10 ml with water. This solution must be prepared fresh daily.

V. Dissolve 0.61 g of Trizma base in 40 ml of reagent III. Add 0.5 ml of 2-mercaptoethanol, 20 ml of glycerol and 0.01 g of pyronin Y. When the pyronin Y is completely dissolved adjust the pH to 7.2 with HCl, bring the solution to volume with distilled deionized water and store at 4°C.

VI. Disperse 12.5 g of glycerol in 10 ml of distilled, deionized water and bring to volume of 25 ml with water.

Gel Preparation

I. Combine: 10 ml reagent I, 5 ml reagent II, 2.5 ml reagent VI, 1.0 ml reagent III, 10 µl TEMED and 5.5 ml deionized, distilled water.

II. Deaerate with a water aspirator.

III. Add 1.0 ml of reagent IV and quickly transfer the solution to running tubes.

IV. Overlay the gels with water and allow to polymerize.

Sample Preparation

I. Combine sample aliquot with aliquot of reagent V such that the ratio of reagent V to protein is >4:1.

II. Heat at 100°C for 5 minutes.

APPENDIX D. (continued)

Electrophoresis

I. Remove the water from the gels and place them in the electrophoresis chamber.

II. Fill the lower buffer chamber with a solution consisting of: 100 ml reagent II, 40 ml reagent III and 860 ml of deionized distilled water.

III. Place prepared samples onto the gels.

IV. Overlay the samples with the solution prepared in Electrophoresis step II.

V. Fill the upper chamber with the solution prepared in Electrophoresis step II.

VI. Electrophorese at 0.5 mA/gel until the tracking dye is 0.5 cm from the end of the gel.

VII. Remove the gels from the tubes.

APPENDIX E. Isoelectric focusing in polyacrylamide gels procedure.

Reagents

I. Dissolve 5.7 g of urea in distilled,deionized water. When urea is dissolved add 0.2g of Triton X-100, 0.5 ml of carrier ampholytes and 0.5 ml of 2-mercaptoethanol. Bring to a volume of 10 ml with water and store frozen.

II. Disperse 10 g of Triton X-100 in distilled,deionized water. Bring to 100 ml with water and store frozen.

III. Dissolve 7.08 g of acrylamide and 1.25 g of DATD in distilled, deionized water and bring to a volume of 25 ml with water and store in a sealed amber container at 4°C.

IV. Dissolve 1.0 g of ammonium persulfate in deionized, distilled water and bring to a volume of 10 ml with water. Prepare fresh daily.

Gel Preparation

I. Dissolve 5.5 g of urea in a solution consisting of 2.0 ml of solution II, 1.33 ml of solution III, 0.5 ml of carrier ampholytes and 2.0 ml distilled, deionized water. Swirl until urea is completely dissolved.

II. Bring volume to 10 ml with water.

III. Add 7.0 µl of TEMED.

IV. Deaerate using a water aspirator.

V. Add 10.0 µl of solution IV and quickly transfer to prepared gel tubes.

VI. Overlay the gels with water and allow to polymerize.

APPENDIX E. (continued)

Sample Preparation

I. Combine protein sample with an aliquot of solution I sufficient to yield a final protein concentration of less than 1 mg/ml.

II. Vortex briefly.

Electrophoresis

I. Place polymerized gels in the apparatus and fill the anodic chamber with 0.02 M H_3PO_4 and the cathodic chamber with 0.04 M NaOH.

II. Pre-run the gels using the following schedule:
200 V for 30 minutes
300 V for 15 minutes
400 V for 15 minutes.

III. Remove the upper electrolyte and apply the protein samples.

IV. Refill the upper reservoir with the appropriate electrolyte.

V. Electrophorese for 16 hours at 400 V.

VI. Remove gels from tubes.

VII. Stain and/or profile.

APPENDIX F. 1. Analysis of variance for soluble phase protein concentration

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	18568.3480		
Due to Main Plot Units	5	16798.7090		
NaCl Type (NT)	2	16466.5880	8233.2944	74.37**
Error A	3	322.1216	110.7072	
Due to Sub-Plot Units	210	1769.6390		
Blending Time	5	102.2796	20.4559	2.98*
Post-Blending Storage	5	117.3812	23.4762	3.42**
BT x PBS	25	302.0336	12.0813	1.76*
BT x NT	10	87.1779	8.7178	1.27
PBS x NT	10	89.9236	8.9924	1.31
BT x PBS x NT	50	350.0844	7.0017	1.02
Error B	105	720.7620	6.8644	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$

APPENDIX F.2. Analysis of variance for water loss.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	128.8885		
Due to Main Plot Units	5	59.9063		
NaCl Type (NT)	2	59.1287	25.9644	114.06**
Error A	3	0.7776	0.2592	
Due to Sub-Plot Units	210	68.9822		
Blending Time	5	33.8864	6.7773	38.42**
Post-Blending Storage	5	2.3726	0.4745	2.69*
BT x PBS	25	4.5865	0.1835	1.04
BT x NT	10	1.9228	0.1922	1.09
PBS x NT	10	2.1344	0.2134	1.21
BT x PBS x NT	50	5.5566	0.1111	0.63
Error B	105	18.5220	0.1764	

*denotes values of P< 0.05
**denotes values of P< 0.01

APPENDIX F.3. Analysis of variance for fat loss.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	58.7421		
Due to Main Plot Units	5	2.2019		
NaCl Type (NT)	2	2.0075	1.0038	15.49*
Error A	3	0.1944	0.0648	
Due to Sub-Plot Units	210	56.5401		
Blending Time	5	3.4675	0.6935	3.01*
Post-Blending Storage	5	3.0413	0.6083	2.64*
BT x PBS	25	6.9696	0.2788	1.21
BT x NT	10	3.7555	0.3756	1.63
PBS x NT	10	2.3270	0.2327	1.01
BT x PBS x NT	50	12.7872	0.2557	1.11
Error B	105	24.1920	0.2304	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$

APPENDIX F. 4. Analysis of variance for total loss.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	82.7333		
Due to Main Plot Unit	5	26.6406		
NaCl Type (NT)	2	24.8910	12.4455	21.34*
Error A	3	1.7496	0.5832	
Due to Sub-Plot Unit	210	56.0927		
Blending Time	5	13.9987	2.7997	15.72**
Post-Blending Storage	5	6.1890	1.2378	6.95**
BT x PBS	25	5.5211	0.2208	1.24
BT x NT	10	2.4044	0.2404	1.35
PBS x NT	10	1.7988	0.1799	1.01
BT x PBS x NT	50	7.4802	0.1496	0.84
Error B	105	18.7005	0.1781	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$

APPENDIX F.5. Analysis of variance for pH of blend.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	8.00012		
Due to Main Plot Units	5	0.81061		
NaCl Type (NT)	2	0.70606	0.35303	10.13*
Error A	3	0.10455	0.03485	
Due to Sub-Plot Units	210	7.18951		
Blending Time	5	0.31860	0.06372	2.10
Post-Blending Storage	5	0.21635	0.04527	1.50
BT x PBS	25	0.25075	0.01003	0.33
BT x NT	10	0.26590	0.02659	0.88
PBS x NT	10	0.30001	0.03000	1.00
NT x PBS x BT	50	2.65850	0.05317	1.76**
Error B	105	3.17940	0.03028	

*denotes values of P < 0.05
**denotes values of P < 0.01

APPENDIX F. 6. Analysis of variance for soluble phase volume.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	466.7292		
Due to Main Plot Units	5	128.1764		
NaCl Type (NT)	2	122.6468	61.3234	33.27**
Error A	3	5.5296	1.8432	
Due to Sub-Plot Units	210	338.5528		
Blending Time	5	27.7365	5.5473	3.49**
Post-Blending Storage	5	25.0105	5.0021	3.15*
BT x PBS	25	39.0975	1.5639	0.99
BT x NT	10	9.4361	0.9436	0.59
PBS x NT	10	8.7492	0.8749	0.55
BT x PBS x NT	50	61.8250	1.2365	0.78
Error B	105	166.6980	1.5876	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$

APPENDIX F. 7. Analysis of variance for % Actin

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	11519.8053		
Due to Main Plot Units	5	2065.3474		
NaCl Type (NT)	2	912.7498	456.3749	1.19
Error A	3	1152.5976	384.1992	
Due to Sub-Plot Units	210	9454.4576		
Blending Time	5	243.7155	48.7431	1.04
Post-Blending Storage	5	236.5734	47.3146	1.01
BT x PBS	25	986.7802	39.4712	0.84
BT x NT	10	466.4934	46.6493	1.00
PBS x NT	10	491.1020	149.1102	1.05
BT x PBS x NT	50	2117.3050	42.3461	0.91
Error B	105	4912.4881	46.7856	

*denotes values of P < 0.05
**denotes values of P < 0.01

APPENDIX F. 8. Analysis of variance for % myosin heavy chains.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	10253.466		
Due to Main Plot Units	5	4838.3986		
NaCl Type (NT)	2	2085.5002	1042.7501	1.14
Error A	3	2752.8984	917.6328	
Due to Sub-Plot Units	210	5415.0677		
Blending Time	5	168.7465	33.7493	1.16
Post-Blending Storage	5	107.4925	21.4985	0.74
BT x PBS	25	716.3575	28.6543	0.98
BT x NT	10	311.5372	31.1537	1.07
PBS x NT	10	220.4789	22.0479	0.76
BT x PBS x NT	50	828.6551	16.5731	0.57
Error B	105	3061.8000	29.1600	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$

APPENDIX F. 9. Analysis of variance for % α-actinin.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	2202.8017		
Due to Main Plot Units	5	354.5815		
NaCl Type (NT)	2	125.4271	62.7135	0.82
Error A	3	229.1544	76.3848	
Due to Sub-Plot Units	210	1848.2202		
Blending Time	5	31.5892	6.3178	0.73
Post-Blending Storage	5	52.1655	10.4331	1.21
BT x PBS	25	225.1052	9.0042	1.04
BT x NT	10	89.6110	8.9611	1.04
PBS x NT	10	85.3111	8.5311	0.99
BT x PBS x NT	50	456.8601	9.1372	1.06
Error B	105	907.5781	8.6436	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$

APPENDIX F. 10. Analysis of variance for % protein in pI range of pH 4-5.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	4086.1231		
Due to Main Plot Units	5	390.6218		
NaCl Type (NT)	2	199.7642	99.8821	1.57
Error A	3	190.8576	63.6192	
Due to Sub-Plot Units	210	3695.5013		
Blending Time	5	73.2442	14.6488	0.88
Post-Blending Storage	5	85.7290	17.1458	1.03
BT x PBS	25	461.9376	18.4775	1.11
BT x NT	10	161.4700	16.1470	0.97
PBS x NT	10	199.7572	19.9757	1.20
BT x PBS x NT	50	965.4912	19.3098	1.16
Error B	105	1747.8721	16.6464	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$

APPENDIX F. 11. Analysis of variance for % protein in pI range of pH 5-6.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	5328.6656		
Due to Main Plot Units	5	268.3187		
NaCl Type (NT)	2	126.6011	63.3005	1.34
Error A	3	141.7176	47.2392	
Due to Sub-Plot Units	210	5060.3469		
Blending Time	5	107.7185	21.5437	0.89
Post-Blending Storage	5	122.2423	24.4485	1.01
BT x PBS	25	732.2436	29.2897	1.21
BT x NT	10	242.0630	24.2063	1.00
PBS x NT	10	213.0163	21.3016	0.88
BT x PBS x NT	50	1101.3912	22.0278	0.91
Error B	105	2541.6720	24.2064	

*denotes values of P < 0.05
**denotes values of P < 0.01

APPENDIX F. 12. Analysis of variance for % protein in pI range of pH 6-7.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	1048.8174		
Due to Main Plot Units	5	142.8841		
NaCl Type (NT)	2	57.1537	28.5767	1.00
Error A	3	85.7304	28.5768	
Due to Sub-Plot Units	210	905.9333		
Blending Time	5	38.2784	7.6557	2.01
Post-Blending Storage	5	35.4218	7.0844	1.86
BT x PBS	25	133.3084	5.3323	1.40
BT x NT	10	60.5600	6.0560	1.59
PBS x NT	10	46.0861	4.6086	1.21
BT x PBS x NT	50	192.3444	3.8469	1.01
Error B	105	399.9242	3.8088	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$

APPENDIX F. 13. Analysis of % protein in pI range of pH 7-8

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	215	4654.3662		
Due to Main Plot Units	5	385.8373		
NaCl Type (NT)	2	222.3469	111.1735	2.04
Error A	3	163.4904	54.4968	
Due to Sub-Plot Units	210	4268.5289		
Blending Time	5	180.3334	36.0667	1.88
Post-Blending Storage	5	200.4770	40.0954	2.09
BT x PBS	25	580.3281	23.2131	1.21
BT x NT	10	274.3369	27.4337	1.43
PBS x NT	10	193.7624	19.3763	1.01
BT x PBS x NT	50	824.9292	16.4986	0.86
Error B	105	2014.3620	19.1844	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$

APPENDIX F. 14. Analysis of variance for yield of experimental bolognas.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	17	10.8979		
Due to Main Plot Units	5	6.5209		
Blending Time	2	4.5607	2.2804	3.49
Error A	3	1.9602	0.6534	
Due to Sub-Plot Units	12	4.3770		
Post-Blending Storage	2	2.6836	1.3418	15.53*
BT x PBS	4	1.1750	0.2938	3.40
Error B	6	0.5184	0.0864	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$

APPENDIX F. 15. Analysis of variance for moisture content of experimental bolognas.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	17			
Due to Main Plot Units	5	3.4977		
Blending Time	2	2.5455	1.2728	4.01
Error A	3	0.9522	0.3174	
Due to Sub-Plot Units	12	23.2180		
Post-Blending Storage	2	11.1551	5.5776	5.53*
BT x PBS	4	6.0113	1.5028	1.49
Error B	6	6.0516	1.0086	

*denotes values of P < 0.05
**denotes values of P < 0.01

APPENDIX F. 16. Analysis of variance for fat content of experimental bolognas.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	17	53.5427		
Due to Main Plot Units	5	11.3517		
Blending Time	2	8.8875	4.4438	5.41
Error A	3	2.4642	0.8214	
Due to Sub-Plot Units	12	42.1910		
Post-Blending Storage	2	22.8426	11.4213	6.07*
BT x PBS	4	8.0588	2.0147	1.07
Error B	6	11.2896	1.8816	

*denotes values of P < 0.05
**denotes values of P < 0.01

APPENDIX F. 17. Analysis of variance for protein content of experimental bolognas.

SOURCE	DEGREES OF FREEDOM	SS	MA	F
TOTAL	17	6.7509		
Due to Main Plot Units	5	1.6088		
Blending Time	2	0.8150	0.4075	1.54
Error A	3	0.7938	0.2646	
Due to Sub-Plot Units	12	5.1421		
Post-Blending Storage	2	2.9154	1.4577	6.73*
BT x PBS	4	0.9270	0.2318	1.07
Error B	6	1.2996	0.2166	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$

APPENDIX F. 18. Analysis of variance for moisture:protein of experimental bolognas.

SOURCE	DEGREES OF FREEDOM	SS	MS	F
TOTAL	17	1.0840		
Due to Main Plot Units	5	0.2574		
Blending Time	2	0.1083	0.0542	1.09
Error A	3	0.1491	0.0497	
Due to Sub-Plot Units	12	0.8266		
Post-Blending Storage	2	0.1992	0.0996	2.87
BT x PBS	4	0.4192	0.1048	3.02
Error B	6	0.2082	0.0347	

*denotes values of $P < 0.05$
**denotes values of $P < 0.01$