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EFFECTS OF SODIUM CHLORIDE AND/OR TETRASODIUM PYROPHOSPHATE ON POSTMORTEM METABOLISM AND PROCESSING OF PRERIGOR AND POSTRIGOR GROUND BEEF

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EFFECTS OF SODIUM CHLORIDE AND/OR TETRASODIUM PYROPHOSPHATE ON POSTMORTEM METABOLISM AND PROCESSING OF PRERIGOR AND POSTRIGOR GROUND BEEF

By

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ABSTRACT

EFFECTS OF SODIUM CHLORIDE AND/OR TETRASODIUM PYROPHOSPHATE ON POSTMORTEM METABOLISM AND PROCESSING OF PRERIGOR AND POSTRIGOR GROUND BEEF

By

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The functional characteristics of prerigor and postrigor beef homogenates, as influenced by post-blending storage, sodium chloride (NaCl) and tetrasodium pyrophosphate (TSPP), were evaluated. Paired sternomandibularis were excised from market-weight steers immediately following bleeding and randomly labelled as prerigor or postrigor. Prerigor homogenates were evaluated at 0, 2, 4, 6, 12, 24, 48 and 96 hours. Postrigor muscles were stored 48 hours, processed similarly to prerigor muscles, and analyzed at 0, 24, and 48 hours. Response variables monitored during the study included water-holding capacity (WHC), R-values, pH and 1.0 M NaCl extractable protein (EP).

In the first study, NaCl treatments of 0, 0.5, 1.0, 2.0 and 4.0% were evaluated. Ultimate pH increased linearly ($P<0.05$) in prerigor homogenates with increasing NaCl concentration. EP and WHC values were higher ($P<0.05$) in prerigor homogenates containing 2.0 and 4.0% NaCl than postrigor homogenates treated in a similar manner. After 12 hours of storage, prerigor homogenates containing 0.5 and 1.0% NaCl had as high or higher WHC values than any

postrigor treatment. Results of this study indicated an advantage to using low NaCl concentrations in prerigor salted beef.

Nine combinations of TSPP at levels of 0, 0.25, or 0.5% and NaCl at levels of 0, 0.5, or 1.0% were evaluated in the second study. TSPP x NaCl interaction was not significant in postrigor ($P>0.25$) or prerigor ($P>0.10$) homogenates. In prerigor homogenates, increasing phosphate concentration increased the time required to reach ultimate pH. After 6 hours, no differences ($P>0.10$) were noted in EP or WHC values at different phosphate concentrations in prerigor homogenates. With increasing phosphate concentration in postrigor homogenates, there was an increase ($P<0.05$) in pH and EP values. Results of this study indicated limited advantages to using TSPP alone or in combination with NaCl in prerigor meat homogenates.

A final study evaluated TSPP binding to prerigor and postrigor muscle proteins indirectly by following labelled [P32] tetrasodium pyrophosphate disappearance from homogenates subjected to low ionic strength aqueous extraction. Results indicated that a large portion of the phosphate added to prerigor meat is physically entrapped and after 2 washings there are only small differences in phosphate retained.

To my wife, Marie; daughter, Andrea;

and son, Nicholas

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INTRODUCTION

Hot processing, fostered by attempts to reduce meat processing costs and to increase product quality, has received considerable attention over the last two decades. This procedure, which involves removal of muscles soon after slaughter and before conventional chilling, offers a number of potential advantages including reduction of energy input (Henrickson, 1975; 1981), cooling space, chilling time, and labor (Kastner, 1977; Cuthbertson, 1980). Furthermore, "hot" meat exhibits functional (water binding capacity, extractable protein, emulsification capacity) advantages when salted in the prerigor state (Hamm, 1981) and is ideal for utilization in processed meats and sausage manufacturing.

Although the terms "hot processing" and "prerigor processing" are often used interchangeably, all hot boning systems may not involve the use of prerigor meat (West, 1983). The major objective of hot processing of beef for production of fresh products is to obtain primal cuts with acceptable quality. In these systems, it is imperative to prevent extreme contraction and concomitant toughening associated with shortening of muscles excised while in the prerigor state. Therefore, many hot processing techniques

have been designed to avoid processing before the onset of rigor mortis (Kastner, 1983).

The superior functional properties of prerigor meat when used for production of sausage (salted) products are directly related to high concentrations of adenosine triphosphate (ATP) (Hamm, 1981). Thus, comminution and addition of sodium chloride (NaCl) to these products should occur before the onset of rigor mortis to obtain the beneficial functional advantages. The studies described herein will focus on the hot processing of prerigor beef.

Many meat processors, pressured by consumer groups, are making efforts to reduce sodium intake through the diet. This is because of the possible contribution of sodium to the development of hypertension that occurs in 10 to 20% of the United States population (Pearson and Wolzak, 1982). The concentration of NaCl necessary to achieve prerigor functional advantages is of particular interest. Hamm (1981) reported that in order to attain higher water-binding capacity in beef homogenates, the NaCl concentration of prerigor meat must be at least 1.8%. This concentration is in general agreement with the NaCl level of 2.0% necessary in prerigor salted pork (Poulanne and Terrell, 1983). However, Honikel (1986) indicated 1.0% NaCl will achieve the desired presalting effect in comminuted hot muscle. Furthermore, Coon (1982) reported that 0.5% NaCl produced greater water-holding capacity in prerigor meat than 0.0 or 3.0% NaCl in prerigor or postrigor meat. These results

leave sufficient uncertainties such that further investigation is merited.

The use of phosphates, as partial replacements of NaCl, provides another viable alternative for reduction of sodium levels in processed meat products. This is particularly pertinent as a 1982 USDA regulation permits the use of phosphates in a wider range of processed beef products. Although the effects of phosphates in postrigor meat are well documented (Schwartz and Mandigo, 1976; Offer and Trinick, 1983; Trout, 1984), there is a paucity of data regarding the effect of phosphates on the functional properties of prerigor meat.

Preblending is often used in the red meat industry to promote solubilization of proteins. This process involves blending some or all of the meat with part of the non-meat ingredients, primarily NaCl and sodium nitrite, prior to actual product fabrication. In prerigor preblending, time of further processing after salting is important. Although Hamm (1981) and Jolley et al. (1981) have extensively studied the effect of postmortem processing time on the functional properties of beef sternomandibularis, their research approach has been to salt, homogenize and analyze at various times postmortem. Evaluating individual presalted muscle homogenates over time would increase the limited knowledge regarding prerigor preblending.

The objectives of this study were:

1. To evaluate the effects of NaCl concentration on

prerigor and postrigor ground beef functional characteristics over time postmortem.

2. To evaluate the effects of TSPP and low concentrations (0, 0.5, 1.0 percent) of NaCl on the functional properties of prerigor and postrigor ground beef over time postmortem.
3. To assess the binding of TSPP to myofibrillar proteins in prerigor and postrigor ground beef.

LITERATURE REVIEW

Rigor mortis, Latin for "stiffness of death", has a profound effect on the quality and characteristics of meat products. Although recognized for hundreds of years our present knowledge concerning rigor mortis is based on the pioneering studies of rabbit psoas muscle by Bate-Smith and Bendall (1947;1949) and Hanson and Huxley's (1955) sliding filament concept of muscle contraction. This review will focus on the functional properties of comminuted beef as they are influenced by development of rigor mortis, post-blending storage time, and addition of NaCl and phosphates. Furthermore, current theories regarding water-binding capacity and protein extractability will be discussed.

Development of Rigor

Immediately after a well-rested animal is slaughtered, its muscles contain 5-10 umol ATP/g and have a pH of 6.9-7.2 (Hamm, 1982). Exsanguination results in removal of the oxygen-carrying blood supply and thus initiates the chain of events resulting in rigor. Unable to obtain oxygen, the muscle loses its oxidative metabolic potential, and within a very few minutes becomes anaerobic (Marsh, 1981). Muscle

cells then must rely on anaerobic metabolism and creatine phosphate (CP) for intracellular fuel (ATP) replenishment.

In anaerobic metabolism the storage carbohydrate, glycogen, is broken down to glucose-1-phosphate and follows the glycolytic pathway to pyruvate which is then reduced to lactate. The result is the production of 3 ATP molecules per hexose unit of glycogen (Jeacocke, 1984) and 1 proton (H+) per lactate formed (Bendall, 1973). Consequently, ATP is modestly replenished when compared to aerobic metabolism and in beef muscle the pH drops to about 5.5 after about 24 hours.

CP provides a second energy source to replenish ATP. Creatine phosphokinase (CPK) catalyzes the rephosphorylation of adenosine diphosphate (ADP) as long as supplies of CP last (Marsh, 1981):

$$ADP + CP \longrightarrow ATP + C$$

ATP levels remain fairly constant for a certain delay period after death (Bendall, 1973; Marsh, 1977; Honikel et al., 1981a). However, when the CP reserves are largely exhausted, ATP content also begins to decline. This is the initiation of the rapid phase of rigor onset which is accompanied by loss of muscle extensibility. This phase starts at an average ATP concentration in the tissue of about 1.0 umol/g (pH 5.9) and the ATP concentration decreases continuously until it has fallen below 0.1 umol/g (pH 5.5) (Honikel et al., 1981a). At these ATP concentrations, the muscle extensibility is only

approximately 5-10 percent of that in the prerigor tissue and rigor is established (Marsh, 1981). The relationship of pH, ATP, CP, and extensibility in beef muscle over postmortem time is shown in Figure 1.

Factors Influencing Rate of ATP Loss

The rate of ATP loss has a direct effect on meat quality and is especially important when one considers time of salt addition postmortem. The activity of muscle ATPases and the formation of ATP from ADP during glycolysis determine the rate and extent of the breakdown of ATP in muscle postmortem (Hamm, 1977). Three important ATPases that are involved in striated muscle hydrolytic reactions include the Na/K ATPase, the calcium pump, and the actomyosin ATPase (Bechtel and Best, 1985). Of these, the actomyosin ATPase requires the most ATP during a muscle contraction and governs the ATPase activity of the muscle tissue postmortem (Hamm, 1977).

Factors which influence the rate of ATP loss include muscle fiber type (Beatty et al., 1963; Beecher et al., 1965; Bechtel and Best, 1985), storage temperature (Marsh, 1954; Newbold and Scopes, 1967; Tarrant, 1977; Honikel et al., 1983), comminution (Newbold and Scopes, 1971a; Hamm, 1977; 1982), glycogen level (Bate-Smith and Bendall, 1949; Marsh, 1981), muscle location (Tarrant and Mothersill, 1977; Bendall, 1978; Hamm, 1982), electrical stimulation (Bendall

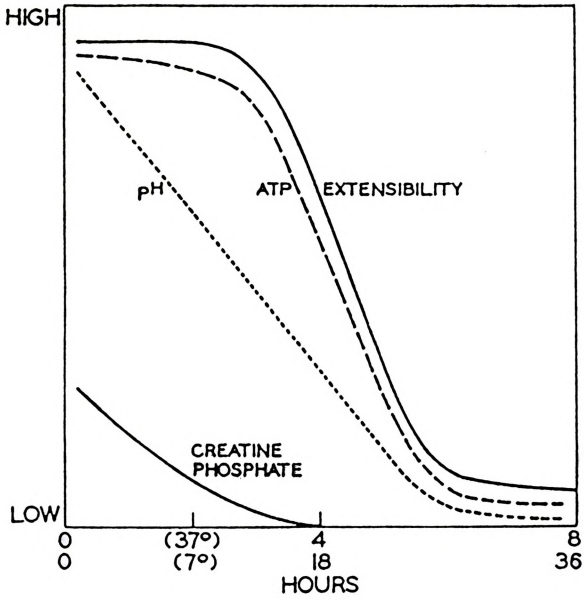

Figure 1. Relationship of pH, ATP, CP and extensibility in beef muscle over postmortem time at 7 and 37° C (Marsh, 1981).

et al., 1976; Bouton et al., 1980), and the addition of chloride salts (Newbold and Scopes, 1971a; Hamm, 1977) and phosphates (Newbold and Scopes, 1971a; 1971b; Hamm, 1977). Of these, the effects of comminution, storage temperature, and the addition of chloride salts and phosphates are relevant to the present study and will be reviewed.

Comminution

Comminution of beef muscles causes disruption of the muscle cell membrane (sarcolemma). The degree of disruption varies with the processing treatment (e.g. coarse grinding vs mincing vs emulsification). After comminution, the myofilaments are no longer restrained and myofibrillar swelling and thus water-holding capacity (WHC) is greatly enhanced (Hamm, 1982). In prerigor muscle, comminution, although beneficial for enhanced meat functional properties, accelerates the rate of postmortem metabolism.

Newbold and Scopes (1971a) found that ATPase activity and glycolytic metabolism were stimulated about three-fold by mincing. Furthermore, phosphorylase was stimulated more than phosphofructokinase, and nicotinamide adenine dinucleotide (NAD) was lost rapidly after mincing. These investigators suggested that the lack of NAD was responsible for the glyceraldehyde-3-phosphate dehydrogenase step being rate limiting for lactate production.

Similarly, Hamm and Van Hoof (1971), as cited by Hamm

(1977), found that accelerated breakdown of ATP and ADP resulted in a faster increase in inosine monophosphate (IMP) concentration in ground longissimus dorsi muscle. This acceleration of the breakdown of ATP by grinding caused a faster rate of glycolysis and pH decline. In spite of the increased glycolytic rate, the concentrations of glycogen, lactate (Figure 2), and pH of ground tissue were the same as those in intact muscle when compared at 72 hours. Hamm and Van Hoof (1971) hypothesized that the effect of grinding might be due to damage of the sarcoplasmic reticulum (SR), resulting in release of Ca++ ions from the SR membrane and causing an activation of the actomyosin ATPase.

Storage Temperature

The rate of postmortem metabolism in intact muscles or muscle homogenates is strongly influenced by tissue temperature. Lowering the temperature from 37 C (immediately after death) to 6-8 C results in a continuous decrease in the rate of postmortem glycolysis (Hamm, 1982). However, further decrease in temperature to the freezing point (about -1 C) causes an acceleration of metabolism in prerigor muscle (Jolley et al., 1981; Nuss and Wolfe, 1981). This phenomenon of accelerated metabolism at low temperatures is known as "cold shortening" (Locker and Hagyard, 1963) and is prevalent in ovine and bovine muscles which have a higher proportion of red to white fibers than

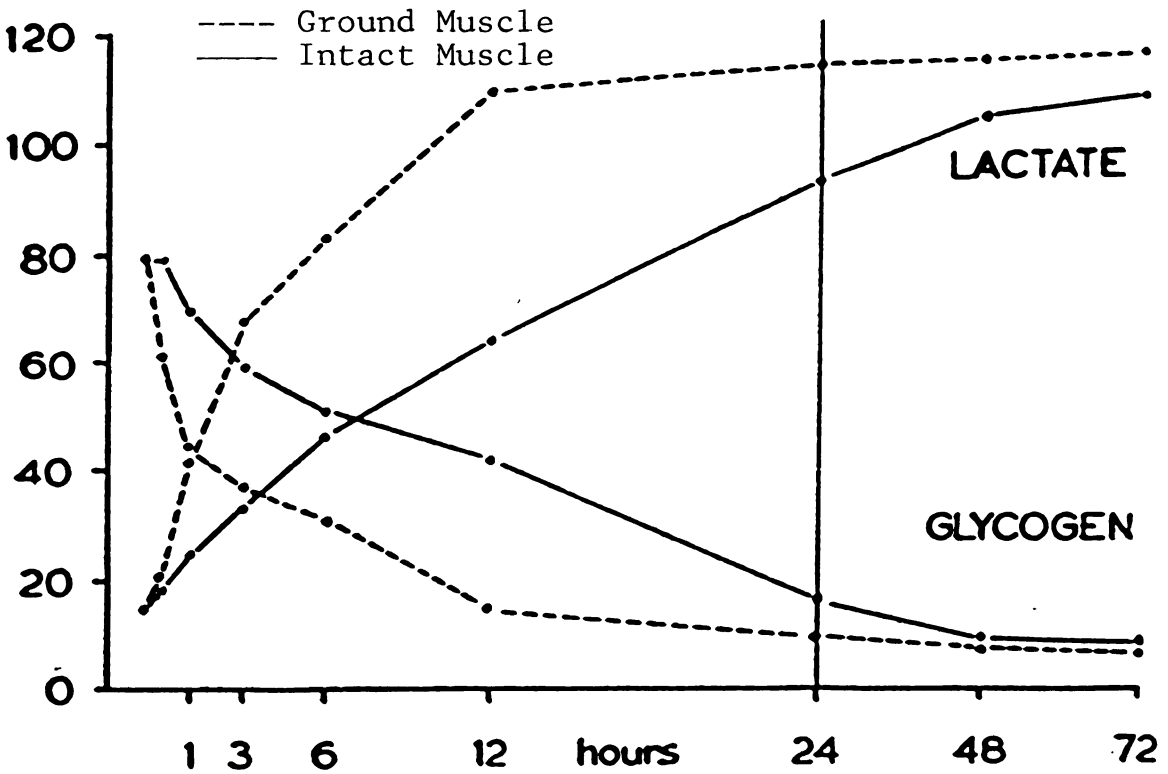

Figure 2. Influence of grinding of bovine muscle on glycogen breakdown and lactate accumulation postmortem (Hamm and VanHoof, 1971).

porcine muscle (Marsh and Leet, 1966; Locker et al., 1975; Cornforth et al., 1980). The difference in cold shortening between muscles which have predominately red or white fibers has been attributed to several factors: (1) Anoxic muscle mitochondria releasing Ca++; (2) Red fibers containing more mitochondria which release Ca++ into the sarcoplasm and thus stimulate muscle contraction; and (3) White fibers containing a more extensive SR system to reaccumulate the released Ca++ (Cornforth et al., 1980). Data presented in Figure 3 show the relationship between storage temperature and rate of pH fall in beef sternomandibularis.

Increasing temperature, along with increasing the rate of pH fall, increases the rate of ATP turnover. Tarrant and Mothersill (1977) observed a correlation coefficient of 0.97 between the rate of ATP turnover and muscle temperature when analyzing six major beef hindquarter muscles. Jolley et al. (1981) reported similar results with beef sternomandibularis. The relationship between storage temperature and ATP depletion rate in beef sternomandibularis is presented in Figure 4. Furthermore, a comparison of Figures 3 and 4 substantiates the relationship between the rate of pH fall, ATP depletion, and storage temperature.

Addition of Chloride Salts

NaCl exerts a stimulating effect on postmortem ATP

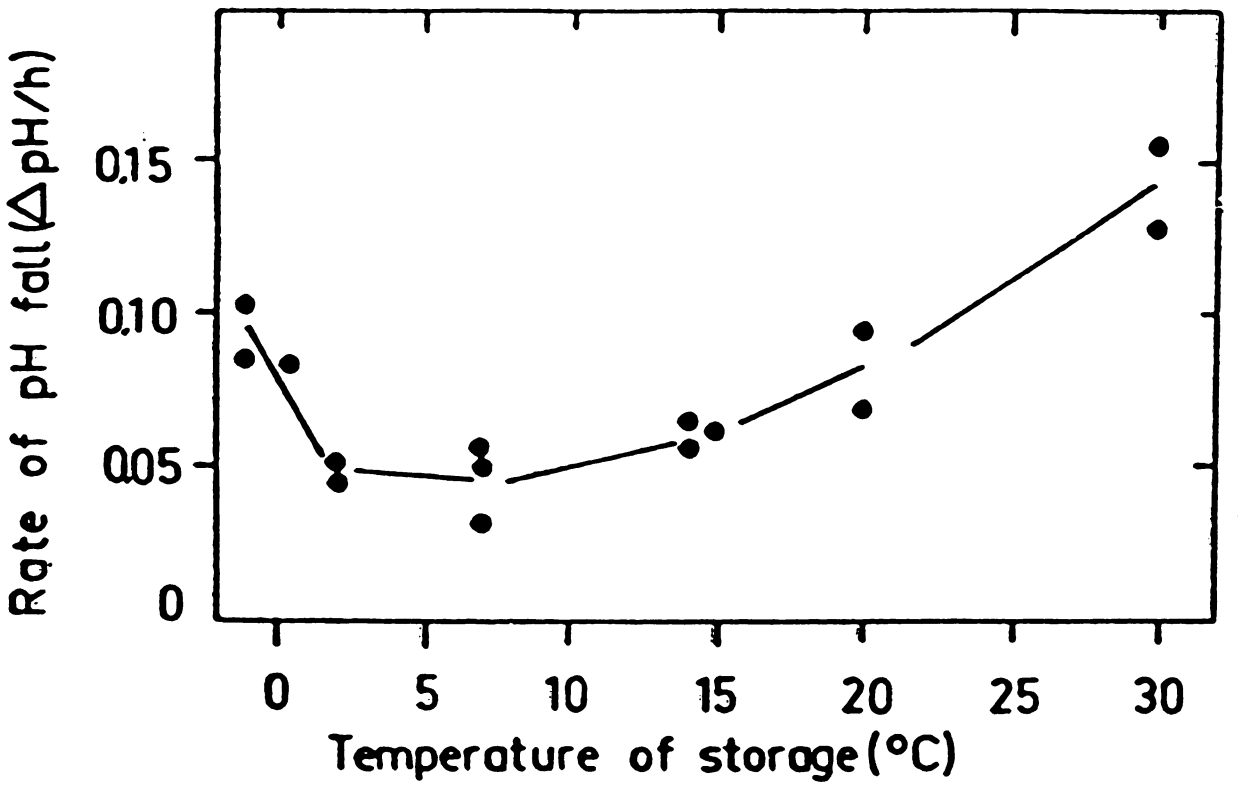

Figure 3. Relationship between temperature of storage and rate of pH fall between pH 6.8 and 6.1 in beef sternomandibularis (Jolley et al., 1981).

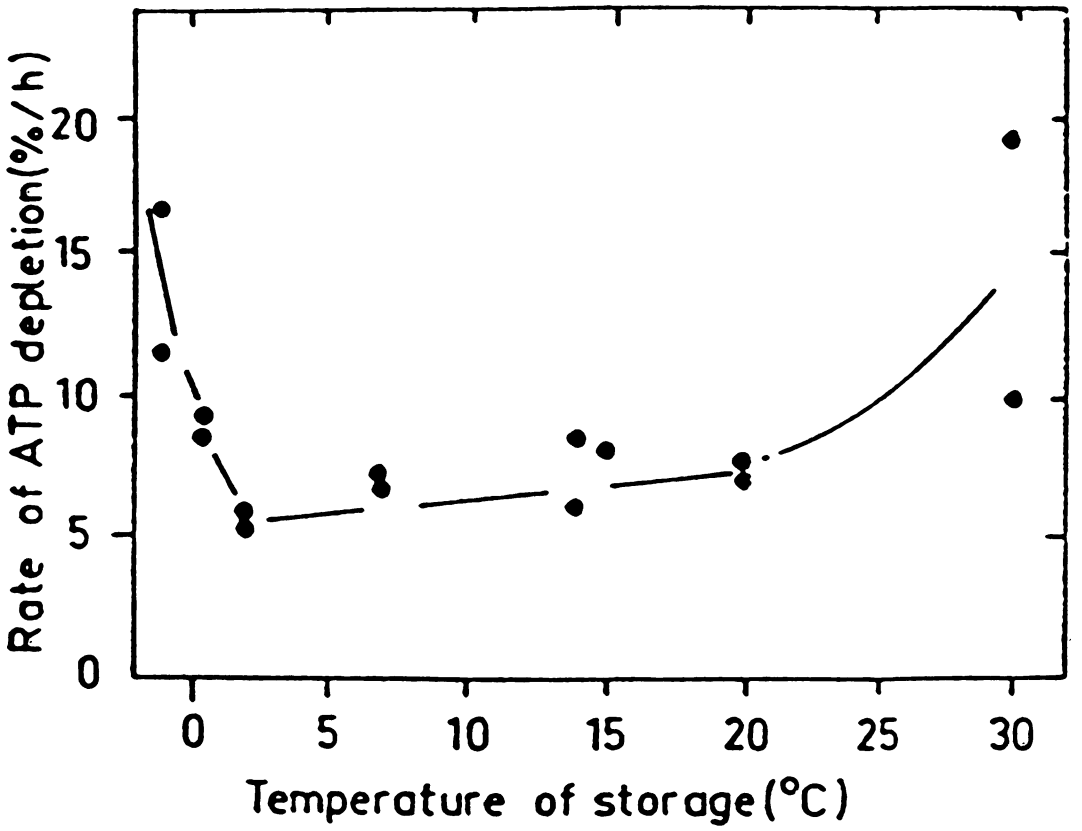

Figure 4. Relationship between temperature of storage and ATP depletion rate in beef sternomandibularis (Jolley et al., 1981).

hydrolysis in prerigor ground beef (Hamm, 1977) and in prerigor ground pork (Mroczek and Rutkowski, 1978; cited by Hamm, 1982). This salting effect is dictated by tissue temperature (Honikel and Hamm, 1978). In the presence of NaCl, the rates of ATP breakdown and lactate formation decrease continuously with falling temperature until the ground tissue is frozen. Thus, the "cold shortening" phenomenon is not observed in salted homogenates. Honikel and Hamm (1978) hypothesized that most of the calcium ions (Ca++) are released by the action of added NaCl. Sodium is then exchanged for calcium of the SR. Therefore, lowering the muscle temperature should not cause an additional release of calcium. Consequently, there should be no increased rate of ATP depletion. This theory was substantiated with the addition or non-addition of ethylene glycol tetraacetate (EGTA) to unsalted beef homogenates (Honikel and Hamm, 1978). When EGTA was absent, the homogenates underwent accelerated lactate formation at temperatures ranging from 0 to 6° C. However, when EGTA was added, the "cold shortening" effect was not observed and postmortem metabolism was similar to that of salted homogenates. This supports the work of Weiner and Pearson (1966) who demonstrated that ethylenediamine tetraacetate (EDTA) prevented "cold shortening" in prerigor muscle.

In addition to influencing the rate of ATP breakdown, NaCl also affects the extent of postmortem metabolism. Although NaCl stimulates the buildup of lactate initially in

sternomandibularis homogenates, there is an inhibition of glycogen breakdown and lactate formation after this period (Hamm, 1981). Numerous researchers (Newbold and Lee, 1965; Newbold and Scopes, 1971a; Hamm, 1977; 1981; Coon et al., 1983; Poulanne and Terrell, 1983) have observed higher ultimate pH values in prerigor salted tissues.

Hamm (1977) suggested that the inhibition of glycolysis in salted prerigor meat is due to the denaturation of glycolytic enzymes by the combined effect of low pH (<6) and high ionic strength. More specifically, Newbold and Lee (1965) found that the production of glucose-6-phosphate decreased when potassium chloride (KCl) was added to prerigor muscle homogenates. They hypothesized that a block in the glycolytic cycle had occurred at either the phosphoglucomutase or the phosphorylase step.

The necessity of low pH for inhibition of glycolysis has been refuted by Newbold and Scopes (1971a). Working with minced sternomandibularis, they found that as the concentration of KCl in the diluent increased, the pH fell more slowly and the ultimate muscle pH was higher. Their pH values ranged from approximately 5.9 with no KCl to approximately 6.3 with a 1.00 M KCl solution. They observed that dilution with KCl slowed the rate of glycolytic resynthesis of ATP but had little effect on ATPase activity. Other researchers have also cited ultimate pH values of greater than 6.0 in prerigor salted muscle tissue (Abu-Baker et al., 1982; Motycka and Bechtel, 1983; Poulanne

and Terrell, 1983). Therefore, the necessity of low pH for inhibition of glycolysis is uncertain.

Addition of Phosphates

The views regarding the effects of phosphates on postmortem metabolism in muscle tissues are limited and contradictory. Newbold and Scopes (1971a), analyzing diluted minced sternomandibularis, found that increasing inorganic phosphate (Pi) concentration resulted in a lower ultimate pH, the maximum effect usually being obtained when the diluent contained 50 mM Pi (approximately 0.3% potassium phosphate by weight). Lactate accumulated at a faster rate in Pi-treated samples, but due to a higher buffering capacity, the pH fell more slowly. Furthermore, they found that inclusion of 50 mM or 100 mM phosphate in the diluent accelerated the rate of glycolytic resynthesis of ATP, consequently allowing higher ATP levels to remain longer after comminution. In these studies, phosphorylase and phosphofructokinase activity were stimulated by Pi and had a greater cumulative activity. However, both these enzymes were not active at about pH 5.9 without phosphate (0.16M KCl only) and pH 5.7 in both the 50 mM and 100 mM Pi minces. They attributed the accelerated phosphorylase activity to the action of phosphorylase b. The Km of phosphorylase b decreased with increased Pi concentration, so that higher Pi concentrations increased activity of

phosphorylase b (Helmreich and Curri, 1964).

Van Hoof and Hamm (1973b), as cited by Hamm (1977), found that 0.5 and 1.0% TSPP accelerated the breakdown of ATP to IMP. This effect was not noted with 0.3% TSPP. These researchers found that glycogen breakdown increased and lactate production decreased when higher concentrations of phosphate were added to beef homogenates. They attributed this peculiarity to diphosphate inhibition of lactate dehydrogenase, consequently inhibiting lactate formation. These results are in contrast to those of Dalrymple and Hamm (1974) who found increased lactate concentrations at both 1 and 24 hours after addition of diphosphate. These researchers attributed the rapid ATP loss to a stimulation of phosphofructokinase by diphosphate addition and fructose-1, 6-diphosphate buildup.

Functional Characteristics of Comminuted Meat

The functional characteristics of comminuted meat have been of interest to the meat industry for many years and have been the topic of many research studies. Although different meat products vary greatly in composition and method of preparation, they still have specific properties which generally determine their eating quality. Factors affecting palatability include oxidative and bacteriological stability, texture parameters (primarily binding together of meat pieces), water-holding ability, and the amount and kind

of ingredients (e.g. spices) added. Hamm (1981) indicated that the decisive factor for producing a high quality sausage product is water-holding capacity (WHC). He found this property to be associated with increased binding, better taste, and prevention of fat cookout. Offer and Trinick (1983) stressed the importance of myofibrillar protein extraction in processed meat products. These proteins form a sticky exudate on the surface of meat pieces which serves to bind the pieces together after cooking. The functional characteristics of binding (due to protein extraction) and WHC are directly related. Thus, endogenous and exogenous factors affect both WHC and protein extractability. The following sections will address how WHC and protein extractability are affected by NaCl, phosphates, and rigor state.

Water in Meat

In order to more fully understand WHC as a functional characteristic of comminuted meat a review of the basic properties of the water associated with proteins is necessary.

Fennema (1977) has classified the water associated with proteins into three categories: (1) Constitutional water; (2) Interfacial water; and (3) Bulk phase water. Constitutional water is the most immobile type and is located in the interior of the protein molecule at specific

sites or simply in tiny interstitial regions. This type of water is present in quantities of approximately 0.3 grams per 100 grams of protein which is equivalent to less than 0.1% of the total tissue water. Interfacial water represents 5-15% of the total tissue water and is located at the surface of the proteins. This type of water, like constitutional water, exhibits a relatively restricted mobility (Hamm, 1972; cited by Hamm, 1986). On the other hand, bulk phase water can exist in a free or entrapped state. The water in the free state has properties similar to normal water or a dilute salt solution. However, the entrapped bulk phase water appears as water found in gels. Bulk phase water constitutes the majority of water found in muscle homogenates.

The many changes in WHC occurring during processing and storing of meat are determined by the extent to which bulk phase water is entrapped within the structure of the intact or comminuted tissue (Hamm, 1986). Myofibrillar proteins are considered to be the main water-binding constituents of meat tissue (Wismer-Pederson, 1978). These proteins are the main structural components of meat and occupy about 70% of the volume of lean meat (Offer and Trinick, 1983; Hamm, 1986). Myofibrils contain about 20% protein, with the remainder being water. Therefore, the majority of the bulk phase water is within the myofibrils in the interfilament spaces and in the filaments themselves (Hamm, 1986).

Water-holding Capacity

WHC, as described by Hamm (1960), means the ability of meat to retain its own or added water during application of any force (e.g. pressing, heating, or grinding). However, Trout and Schmidt (1983) used the term water-binding capacity (WBC) to describe the extent to which water is held or bound by cooked meat or meat products. This term included the specific terms used in the literature such as cook yield (raw meat % retained after cooking), cooking loss (inversely) and water-binding value.

Hamm (1986) in a review of WHC measurement methods listed five general types: (1) Drip determination methods; (2) Filter paper press methods; (3) Centrifugation methods; (4) Suction methods; and (5) Cooking loss methods. There are many methodological variations available when applying any of these general WHC type measurements. Furthermore, each type of WHC measurement has specific limitations and benefits. Therefore, when selecting a particular technique for a particular situation one must consider the purpose for which WHC data will be used.

Although the ability of meat to hold water in the cooked and uncooked state is not directly related (Wismer-Pederson, 1978; Trout and Schmidt, 1983), Tsai and Ockerman (1981) found high correlations ($r = 0.87$-0.95) when comparing cooked and uncooked methods of analyzing retained water in meat products. Furthermore, Wierbicki and Deatherage (1958) found parallel results when comparing a

cooked WHC measurement method to an uncooked WHC measurement method. For the purpose of this discussion, WHC will indicate measurements taken from either cooked or uncooked muscle tissue. The relative differences in WHC are maintained after heating (Hamm, 1960).

Extractable Proteins

Muscle proteins constitute 16-22 percent of the muscle mass and are generally categorized according to their solubilities and extractability in buffers of varying ionic strength (Forrest et al., 1975). These proteins include sarcoplasmic, myofibrillar, and stroma proteins.

Sarcoplasmic proteins are readily extractable in water or low ionic strength (IS) buffers (IS less than or equal to 0.1, Szent-Gyorgyi, 1951; IS = 0.15 or less, Forrest et al., 1975; IS = 0.06, Kramlich et al., 1980). This fraction contains the oxidative, glycolytic and lysosomal enzymes, water soluble albumens (e.g. myoglobin), and nucleoproteins (Kramlich et al., 1980). These proteins have isoelectric points between pH 6.0 and 7.0, molecular weights in the range 30,000 to 100,000 (Bendall, 1964), and amount to approximately 30% of the total muscle protein (Lawrie, 1974). Although the sarcoplasmic proteins are readily extractable in solutions of low ionic strength, various researchers have shown that they do not appreciably affect the binding qualities of sausages (Fukazawa et al., 1961b;

Ford et al., 1978; Siegal and Schmidt, 1979a).

Myofibrillar proteins constitute the majority (51%, Forrest et al., 1975; 52-56%, Goll et al., 1977; approximately 60%, Kramlich, 1978) of skeletal muscle proteins. These proteins, also known as the contractile proteins, function in the development of rigor mortis. The principal proteins in the myofibrillar fraction include myosin, actin, and actomyosin. In addition, this fraction contains troponin (C, T, I), tropomyosin, the actinins (alpha and beta), and other minor regulatory proteins (Kramlich et al., 1973). These proteins are known as salt-soluble proteins as they are soluble at an ionic strength of 0.3 or greater in KCl solutions (Kramlich et al., 1973).

In processing meat for production of sausage-type products, myofibrillar proteins are extracted to increase bind (water binding and meat particle binding). By increasing the amount of salt-soluble protein extracted, the processor can increase cook yields (Acton, 1972; Reynolds et al., 1978), improve emulsion stability in frankfurter-type products, and have a more desirable product due to enhanced binding characteristics in sectioned and formed products (Siegel et al., 1978). Goll et al. (1977) estimated that approximately 97% of the WHC and over 75% of the emulsifying ability of meat is due to myofibrillar proteins.

The binding properties of chunk-type products (Macfarlane et al., 1977; Ford et al., 1978) as well as

comminuted or emulsified sausages (Fukazawa et al., 1961a, b, c) have been shown to be dependent on the presence of myosin and actomyosin. When observing the exudate formed during massaging of cured hams, Siegal et al. (1978) found actin and myosin extraction to be a prerequisite for good binding ability. Turner et al. (1979) evaluating binding of meat pieces, demonstrated that myosin preparations had greater binding strength than actomyosin. However, Galluzzo and Regenstein (1978) when studying emulsifying capacity, found actomyosin alone performs like myosin, and when disassociated actin and myosin act independently. They concluded that actin contributes very little to a good emulsion formation and that myosin has superior emulsifying properties. Swift (1965) postulated that the binding qualities of myosin and actomyosin may be due to the alpha-helical content of myosin. According to Hamm (1966), the helical portions of the protein molecules in meat unravel during heating to form randomly organized chains. These chains produce random cross-links both with hydrogen and ionic bonds which may be responsible for the binding of comminuted meat pieces during denaturation by heat.

Stroma proteins are insoluble in neutral aqueous solvents and contribute the smallest quantity of protein in skeletal muscle (10-15%, Goll et al., 1977; approximately 16%, Forrest et al., 1975). Included are lipoproteins and mucoproteins from cell membranes and surfaces as well as connective tissue proteins (collagen, elastin, and

reticulin). Goll et al. (1977) indicated four important stromal protein characteristics that affect meat quality: (1) Stroma proteins lower meat tenderness (this is dependent on the amount and degree of crosslinking); (2) Stroma proteins decrease the emulsifying capacity of meat; (3) Stroma proteins, because of their low content of charged and hydrophilic amino acids, lower WHC of meat, and ; (4) The stromal protein fraction contains a low proportion of nutritionally essential amino acids and thus, lowers the nutritive value of the tissues. Stroma proteins adversely affect functional characteristics. Because of the low solubility of collagen and because it shrinks and converts to gelatin upon heating, it is desirable for finished sausages to have no more than 25% of their total protein present as collagen (Kramlich, 1978).

Relationship of WHC and Extractable Proteins

Water is retained by the myofibrils because of the three dimensional network of the filaments (Wismer-Pederson, 1978). Comminution of meat tissues causes partial or complete destruction of the sarcolemma. However, the water-holding three dimensional network is retained. Consequently, the myofilaments are no longer restrained and the myofibrillar system is transformed from a state of limited swelling to one of unlimited swelling (Hamm 1986). Offer and Trinick (1983) suggested that the changes in WHC of meat

are caused by changes in the volume of the myofibrils resulting from changes in the interfilament spacing. Figure 5 shows a diagram of their hypothesis explaining changes in WHC. On the left side is shown a transverse section of a myofibril. On the right-hand side the same myofibril is shown with an expanded filament lattice indicating increased swelling, i.e. WHC. This swelling of the protein network causes increases in the distances between molecules of the protein aggregates (Hamm, 1986). The greater the distance the lower the interactions between molecules. As the forces approach zero the proteins are solubolized. Thus, WHC (due to increased myofilament swelling) and protein solubility (extractability) are related.

Generally, as the amount of soluble protein increases, WHC also increases. Honikel et al. (1981a) demonstrated a close relationship between the change in solubility of myofibrillar proteins induced by postmortem metabolism and the WHC of salted beef tissue homogenates. Furthermore, Acton (1972) working with chicken loaves, found that increasing salt-soluble protein decreased cooking loss ($r = -0.90$) by decreasing particle size and increasing bind strength ($r = 0.90$). Similarly, Moore et al. (1976), when evaluating the effects of salt, phosphate and non-meat proteins on the binding strength and cook yield of beef rolls reported a highly significant ($P < .01$) correlation ($r = 0.64$) between cook yield and binding strength. Likewise, Cook (1967) utilizing a filter paper moisture

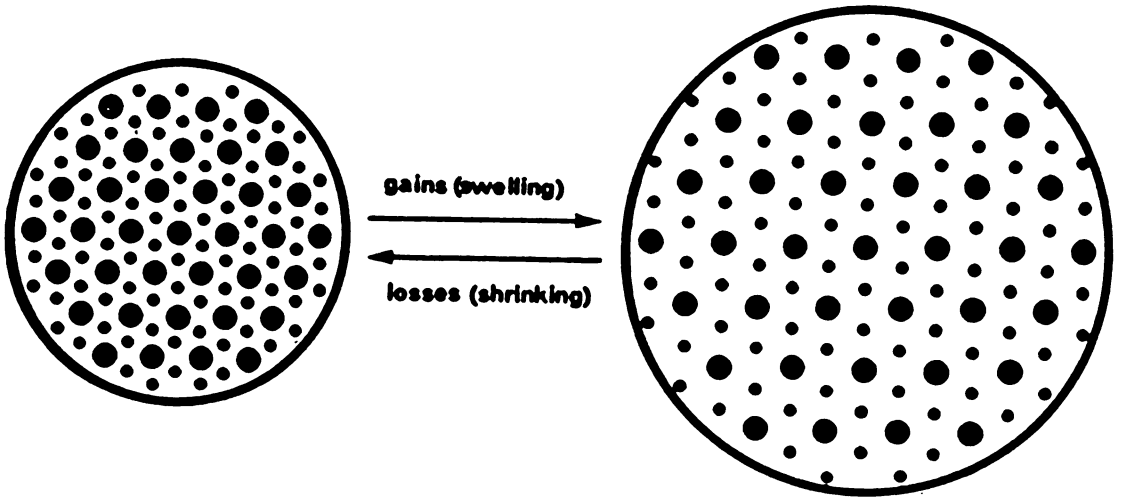

Figure 5. A hypothesis explaining changes in water-holding capacity (Offer and Trinick, 1983).

absorption technique found a highly significant correlation ($r = -0.51$) when comparing salt-soluble nitrogen and moisture released by pressing. These studies indicate that the salt-soluble proteins are required for binding and WHC. However, they did not directly compare the effects of water-soluble and salt-soluble proteins.

Acton and McCaskill (1972) evaluated cooking loss in chicken pectoral muscle cubes which were water-washed (partially removing sarcoplasmic proteins), salt-washed (partially removing myofibrillar proteins), or not washed. They found that salt-washed cubes had a reduced ability to retain moisture during cooking. Reynolds et al. (1978) when analyzing the effects of ultrasonic treatment on binding strength in cured ham rolls found that ultrasound decreased the amount of cookout, increased the potential for salt-soluble protein extraction and had no effect on water-soluble protein extraction. This, again, indicated that WHC was directly related to myofibrillar protein extraction. These results were substantiated by Morita et al. (1983) who found a close relationship between WHC and the amounts of myosin extracted. They used sodium dodecyl sulfate (SDS) gel electrophoresis densitograms to determine myosin concentration. However, Sayre and Briskey (1963), when evaluating protein solubility as influenced by pH and temperature, reported that sarcoplasmic protein solubility was more negatively correlated to expressible juice ratio than myofibrillar protein solubility. In this experiment,

samples for protein extraction were frozen and myofibrillar protein concentration was calculated as the difference between the amount of total soluble proteins and sarcoplasmic proteins.

These studies indicated that improved myofibrillar protein extractability is positively related to WHC when meat products are cooked. However, controversial views on the site of water uptake and its relationship to myofibrillar protein extraction have been expressed (Offer and Trinick, 1983; Hamm, 1986).

Offer and Trinick (1983), studying isolated rabbit psoas myofibrils, found that myofibrils are able to swell to at least twice their original volume in salt solutions and that this degree of swelling is more than enough to account for the water retention in meat processing. Electron microscopy indicated that maximum swelling occurred in the presence of pyrophosphate when only about a third of the length of the myosin A-band had been extracted. Therefore, they suggested that swelling of myofibrils, while often accompanied by extraction of myosin, is not related to it in a simple way. They hypothesized that water is held in meat by capillary action. The major portion is held in the interfilament spaces within the myofibrils, but a substantial part is also in the extracellular space and the spaces between myofibrils. Furthermore, they suggested that ultimate swelling (WHC) of myofibrils is partially dictated by the structural constraints, or crossbridges at the M- and

Z-lines. This supports the observations of Davey and Gilbert (1968) who indicated that increased WHC during aging is due to progressive weakening of the linkages between the filaments such as those making up the Z-line.

Voyle et al. (1984) found similar, although not as dramatic, results in pig longissimus dorsi tissue blocks. A reduction in density of myofibrils on meat surfaces when viewed by election microscopy indicated increased myofibril swelling. In addition, this swelling occurred in combination with myosin A-band extraction.

A second opinion which is generally more accepted is that extracted myosin forms a gel which is capable of holding water. Siegal and Schmidt (1979b) when studying myosin fractions as meat binders indicated that by solubilizing myosin the molecular interactions that are necessary to produce a three dimensional network of protein fibers are produced. This network was stated to give myosin gel greater strength and a higher WHC. Kotter and Fischer (1975), as cited by Offer and Trinick (1983), also reported that the majority of water uptake occurred in the form of a gel of the extracted myosin. Hamm (1986) in summarizing various studies indicated that the water-imbibing power of myosin in the thick filaments plays an important role in WHC of meat, particularily after mincing. He hypothesized that repulsive or attractive forces between adjacent molecules cause the protein network to swell or shrink. If the network is enlarged, more water can be immobilized within

the larger meshes, i.e., there is an increase in WHC.

Effects of NaCl in Postrigor Comminuted Meat

NaCl is the basic ingredient used in most processed meat products. Although mainly recognized for its flavor attributes by consumers, NaCl also enhances WHC and protein solubility. The mechanism by which NaCl affects meat functional characteristics has been of interest for many years.

Hamm (1960) reported that the pH where minimum WHC occurs is shifted to a more acidic value with the addition of NaCl (Figure 6). Because sodium acetate did not cause swelling, he concluded that the chloride ions (rather than the sodium ions) were bound to myofibrils to increase WHC. This binding increased the negative charges on the proteins and thus lowered their isoelectric point, the point at which positive and negative charges are equal and where proteins have minimum solubility and WHC (Figure 6). Addition of NaCl below the isoelectric point caused increased oppositely charged ions (decreased WHC) as the chloride ions were again preferentially bound. Furthermore, he hypothesized that because chloride ions were bound to the filaments the negative charges would cause an increased electrostatic repulsive force leading to swelling. These results were substantiated by Offer and Trinick (1983) working with isolated rabbit psoas myofibrils. They reported that

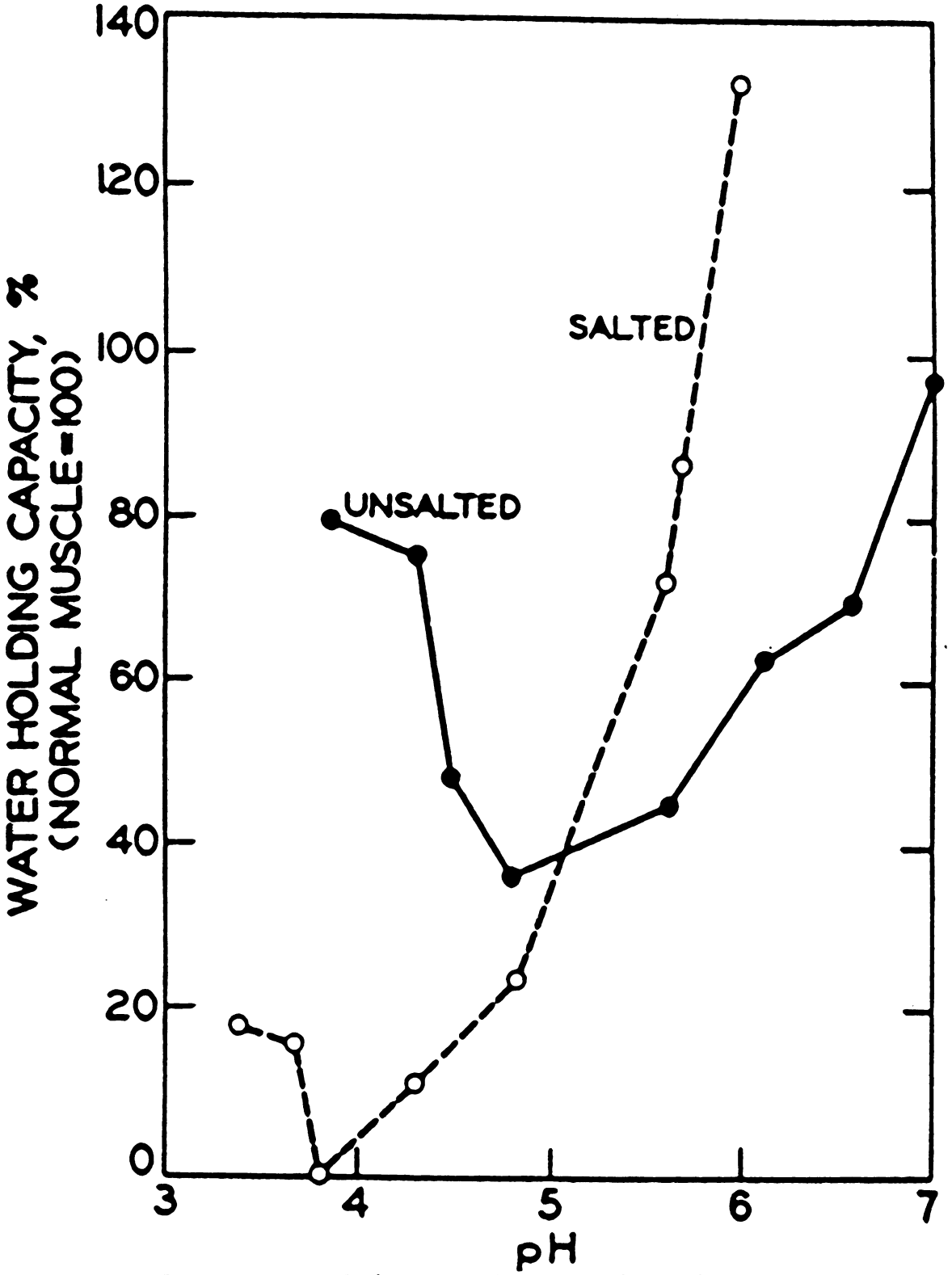

Figure 6. Effects of salting on the relationship between pH and WHC of beef muscle homogenates (Hamm, 1960).

increasing NaCl concentration increased swelling. At 0 to 0.5 M NaCl concentrations, only minor changes in diameter or band pattern of myofibrils were noticed on electron micrographs. However, a substantial increase in the diameter of the myofibrils was observed in micrographs of protein solutions containing 0.6 M NaCl. Further increases of NaCl to a 1.0 M concentration enhanced this swelling effect. Hamm (1957), as cited by Fennema (1977), indicated that when NaCl is added to ground beef (above the isoelectric pH) maximum WHC occurs at an IS of 0.8 - 1.0 or about 5% NaCl by weight. This again indicated the direct relationship between swelling and WHC. As swelling is increased by elevated concentrations of NaCl, protein extractability is also enhanced. Extraction yields of salt-soluble proteins in postrigor pork were evaluated by Bard (1965). By increasing salt concentration in the aqueous phase from 0 to about 10% he showed a linear increase in soluble protein extracted. Beyond this concentration protein solubility decreased due to a salting out effect.

The effect of pH on WHC and protein extractability is well documented (Hamm, 1960; Hamm, 1986). As pH values are increased away from the isoelectric point of proteins WHC and protein solubility increase (Honikel et al., 1981a). In postrigor comminuted meat, the addition of NaCl has a minor effect, if any, on pH values. Mahon (1961) reported a slight decline (0.1 to 0.2) in pH values when adding NaCl to meat. He hypothesized that this was due to ion exchange

liberating hydrochloric acid. Shults and Wierbicki (1974) analyzed the effect of NaCl concentration on the pH of pork muscle. The pH values were found to increase as salt level was increased from 1 to 2%; however, there was a linear decline in pH values from 5 to 10% NaCl concentration. Furthermore, these variations in pH were not related to WHC changes found with increasing NaCl concentration. Thus, the probable mechanism by which NaCl affects postrigor meat functional properties is by contributing to the ionic strength of the system (Trout and Schmidt, 1984) and by enhancing myofibrillar filament swelling (Hamm, 1960; Offer and Trinick, 1983).

Effects of NaCl in Prerigor Comminuted Meat

Numerous researchers have shown functional advantages in prerigor versus postrigor salted meat homogenates. (Trautman, 1964; Acton and Saffle, 1969; Johnson and Henrickson, 1970; Kijowski et al., 1982; Poulanne and Terrell, 1983; Honikel, 1986). The same principles (electrostatic repulsion, moving away from the isoelectric point of proteins) which govern WHC and protein extractability in postrigor comminuted meat can be used to explain the effects of salt in prerigor meat (Hamm, 1960). However, in prerigor tissues the functional characteristics are accentuated because of the presence of ATP which causes the filament lattice to expand (Offer and Trinick, 1983).

Fischer et al. (1982), as cited by Hamm (1982), showed that NaCl prevents the interaction between actin and myosin filaments. This inhibition of rigor is probably due to a strong repulsion between adjacent molecules caused by the combined effects of ATP, high tissue pH, and high IS (Hamm, 1977; Hamm, 1982). Salt added to the prerigor muscle, therefore, causes irreversible changes in the conformation of myofibrillar proteins.

Honikel et al. (1981a) reported that the development of rigor mortis was accompanied by a decrease in WHC of homogenates salted at various times postmortem. Furthermore, rigor development caused a remarkable decrease in the solubility of myofibrillar proteins. They noted that between pH 6.6 and 6.1 (i.e., prerigor) a relatively small and linear decrease in the solubility of myofibrillar proteins occurred. However, below pH 6.1, during rigor mortis, a rapid decrease in protein solubility was observed. Furthermore, by adjusting the pH values of rigor homogenates to the pH values of prerigor homogenates they found increased solubility, but the values were much lower than those found in prerigor homogenates. Honikel et al. (1981a) attributed at least two-thirds of the rapid decrease in solubility, below pH 6.1, to the development of rigor mortis. Similarly, Honikel et al. (1981b) reported that between pH 6.8 and 6.1, the rate of change in cooking loss was 10.6% per pH unit, but after pH 5.9 was reached cooking losses increased 43% per pH unit.

In order to obtain the prerigor salting effect, the NaCl must penetrate the tissue before the ATP concentration has fallen to the concentration at which the onset of rigor mortis takes place (Hamm, 1982). Jolley et al. (1981) reported that salting meat at any time prior to the onset of rigor mortis will improve WHC. Temperature of storage postmortem should be chosen to induce low rates of ATP turnover. Muscles in that study were stored at various temperatures (-1, 7, 14, and 30 C). Comminution and salting (2% NaCl) of muscle portions was done throughout the storage period. Non-significant differences in area of expressible fluid (WHC) were noted until pH 5.9 was reached (Jolley et al., 1981).

The concentration of NaCl necessary to achieve the prerigor salting effect is of interest to meat processors as a result of pressure from consumer groups to reduce sodium levels in food products. Hamm (1981) reported that in order to attain higher water binding capacity in beef homogenates, the NaCl concentration of prerigor meat must be at least 1.8%. This concentration is in general agreement with the 2.0% NaCl level necessary in prerigor salted pork (Puolanne and Terrell, 1983). However, Honikel (1986) indicated 1.0% NaCl will achieve the presalting effect in comminuted hot muscle. Furthermore, Coon (1982) reported that 0.5% NaCl produced greater WHC in prerigor meat than 0.0 or 3.0% NaCl in prerigor or postrigor meat.

Although prerigor salted meat products have generally

been shown to have functional advantages when compared to postrigor products, there are studies which show no prerigor processing advantage. Stilwell et al. (1978) found non-significant (P>0.01) differences in emulsion capacity and WHC values in prerigor emulsion products when compared to postrigor products. Likewise, Coon et al. (1983) analyzing pre- and post-rigor sectioned and formed beef steaks at various salt concentrations (0, 0.5, 1.0%) reported non-significant (P>0.05) differences in cooking yields. In this study R values (the ratio of absorbance of a perchloric acid meat extract at 250 nm over the absorbance at 260 nm) of prerigor labeled samples were approximately 1.10 and 1.15 for the 0.5 and 1.0% NaCl treatments, respectively, at the 0 hour period. Honikel et al. (1981a) indicated that rigor mortis occurred when the R value was approximately 1.10. Thus, it is questionable in the study of Coon et al. (1983) whether samples labeled prerigor were actually in the prerigor state.

Influences of Phosphates in Comminuted Meat

Hamm (1986) estimated that over 400 publications exist on the effects and application of phosphates for meat and meat products. This wealth of information has often focused on the beneficial effects of phosphates on WHC (Hellendoorn, 1962; Shults et al., 1972; Shults and Wierbicki, 1973; Neer and Mandigo, 1977) and protein extractability (Turner et

al., 1979; Morita et al., 1983; Knipe et al., 1985). Brotskey and Everson (1973) summarized the effect of polyphosphates as being due to three factors: (1) pH; (2) IS; and (3) Specific polyphosphate-protein interactions.

pH

Alkaline phosphates are commonly used in meat products. Increased basicity is dependent on the specific phosphate used. Trout and Schmidt (1984) when investigating various phosphate types and concentrations found that the increase in pH of uncooked products ranged from 0.1 to 0.7. The pH increase of cooked products was 0.05 to 0.3 pH units. Similarly, Matlock et al. (1984) reported that 0.375% sodium tripolyphosphate (STPP) increased the pH of precooked frozen pork sausage by 0.25 pH units. Shults and Wierbicki (1973) indicated that TSPP increased pH more than STPP in chicken homogenates. Also, there was a direct relationship between pH and shrinkage and swelling of the meat. Hamm (1986) stated that sodium acid pyrophosphate (SAPP) lowers the pH of meat, and therefore exhibits little or no effect on WHC. Thus, the pH effect of alkaline phosphates is necessary for improved functional characteristics. The alkalinity raises the pH of meat systems, increasing the WHC of meat products which results in greater juiciness and texture (Ellinger, 1972).

The mechanism by which phosphates affect the

functional characteristics of meat products can be explained by the electrostatic theory of swelling protein systems. Increasing the pH on the basic side of the isoelectric point causes an increase in the net negative charge and a repulsion by equally charged negative groups (Hamm, 1986). This increases swelling (WHC), and hence protein extractability.

Ionic Strength

IS measures the concentration of charges in a solution:

$$IS = 1/2 \Sigma M Z^2$$

In this equation M equals the molarity of the ion, Z equals the net charge of the ion (regardless of sign), and Σ is a symbol meaning "the sum of " (Segel, 1968). In meat systems increasing the IS within certain limits causes electrostatic repulsion with resultant increased hydration (Hellendoorn, 1962; Brotsky and Everson, 1973). Since phosphates do not completely dissociate in solution (Trout, 1984), the assumption of 100% dissociation in the IS formula provides biased results for phosphates. Trout (1984) evaluated the effect of phosphate chain length, 1 to 20.8 (condensed phosphate molecules containing 1 to 20.8 phosphate atoms), and phosphate concentration (0.150 to 0.600%) on the degree of dissociation of phosphates using a sodium ion-selective electrode. He found that phosphate concentration had no effect on the degree of dissociation. However, increasing

phosphate chain length increased the degree of dissociation (Table 1). The dissociation % (DP) was then fitted to a polynomial equation (R^2 = 98.6%):

$$DP = 0.177\ (\bar{n})^2 - 6.74(\bar{n}) + 102.2$$

In this equation $\bar{n}$ equals average phosphate chain length. Furthermore, IS for phosphates was then calculated utilizing the degree of dissociation (D):

$$IS = 0.5\ ([Phos]\ (Dn)^2 + Dn\ [Phos] + [H+] + [Cl^-]$$

In this equation [Phos] equals the molar phosphate concentration, n equals the phosphate chain length plus 2, [H+] equals the hydrogen ion molar concentration and [Cl^-] equals the chloride ion molar concentration. When analyzing condensed phosphates with a chain length ranging from 1 to 20.8 there is a reduction in IS from 0.2 to 0.09 in a 0.5% phosphate solution. However, when phosphate dissociation is ignored there is an increase in IS from 0.21 to 0.60 (Trout and Schmidt, 1983). This phenomenon of varying dissociation was thought to be a possible solution to the question of why different phosphates generally affect functional properties in the following order: pyrophosphate > tripolyphosphate > tetrapolyphosphate > hexametaphosphate (Bendall, 1954; Shults et al., 1972; Trout and Schmidt, 1984).

Table 1. Common name, chain length, and dissociation (%) of phosphates (adapted from Trout, 1984).

Phosphate type	Average chain length number	Dissociation (%) RefA	RefB	RefC	RefD	RefD*
Disodium phosphate	1.0	94.9	100	100	100	100
Tetrasodium pyrophosphate	2.0	92.9	100	90.3	62.0	90.0
Sodium tripoly-phosphate	3.0	84.1	97.1	83.5	60.0	86.0
Sodium tetrapoly-phosphate	5.7	65.6	70.3	60.2	48.0	70.0
Sodium hexameta-phosphate	12.8	46.9	50.5	40.4	44.0	61.0
Sodium phosphate, glassy	20.8	38.0	42.7	34.0	----	----

-2
Phosphate concentration of 10^{-2} M.
RefA = Trout, 1984 using a sodium ion-selective electrode.
RefB = Schindewolf, 1953 using conductance and transference.
RefC = Schindewolf and Bonhoeffer, 1953 using ion-selective electrode.
RefD = Batra, 1965 using ion-selective electrode. -4
RefD* = Batra, 1965, phosphate concentration of 10^{-4} M.

The IS necessary to achieve enhanced functional properties in meat homogenates is greater than 0.1 (Hamm, 1960). The main function of ions in solution, at IS < 0.1, is the modification of the nature and thickness of the electrical double layer surrounding the protein molecule (Franks and Eagland, 1975). Meat hydration is maximum when the IS = 0.8 to 1.0 (Hamm, 1957; cited by Fennama, 1977). These figures correspond to 5% NaCl with no water added and approximately 6.5% NaCl with 30% added water. Trout and Schmidt (1984) indicated that an increase in WHC (both with and without phosphate) begins when the total IS is 0.4 and continues until the IS is 0.6. When comparing various phosphates they indicated 86.5% of the WHC variation could be explained in terms of the pH and IS of the homogenized meat. Furthermore, 95.4% of the variation was accounted for by pH and IS when analyzing varying levels of STPP. They reported that an IS equal to 0.57 and pH equal to 5.90 were the critical values necessary to achieve maximal cook yield.

Recently, Trout and Schmidt (1986) analyzed the role of pH, IS, and phosphate type on the functional properties of beef rolls. Their results indicated that at constant pH and IS (accounting for the degree of dissociation), different phosphates affected cooking yield differently. They hypothesized that changes in hydrophobic interactions were mainly responsible for salt-induced changes in meat protein functionality. It was suggested that one could accurately predict the cooking yield and tensile strength of meat

products using pH and molar NaCl and sodium phosphate concentration. This hypotheses was based on the premise that non-electrostatic effects of salt on protein conformation are proportional to the molar concentration of ions (Melander and Horvath, 1977).

Phosphate-Protein Interactions

Two major polyphosphate-protein interactions have been suggested to affect meat functional properties: (1) pyrophosphate cleavage of the actomyosin complex; and (2) phosphates binding to meat proteins.

The increase in WHC produced in the presence of pyrophosphate was suggested by Bendall (1954) to be due to the increased muscle protein solubility as a result of the pyrophosphate-induced dissociation of actomyosin. Pyrophosphate acts as an analog of ATP when dissociating actomyosin. In the presence of magnesium ions, pyrophosphate is bound to myosin, dissociates actomyosin into actin and myosin, and is hydrolyzed to orthophosphate (Granicher and Portzehl, 1964).

Hamm (1975) reported that the addition of diphosphate (0.5%) to prerigor meat had no effect on rheological characterictics. However, after 8 hours postmortem, when the breakdown of ATP had started an association of actin and myosin (rigor mortis), addition of diphosphate significantly affected rheological characteristics and caused an increase

in WHC. Hamm (1986) indicated that actomyosin dissociation by diphosphate exerts little effect on WHC or swelling if electrostatic repulsion between protein filaments is low (i.e., at the IP). With increasing pH, the effect of diphosphate on WHC is increased.

The ability to dissociate actomyosin is an innate characteristic of pyrophosphate. However, Yasui et al. (1964) reported that STPP is dephosphorylated to pyrophosphate in myofibrillar preparations. This hydrolysis has also been observed in meat homogenates (deMan, 1973).

The binding of phosphate anions to meat proteins is a second polyphosphate-protein interaction which has been hypothesized to possibly affect meat functional properties (Hamm, 1970). This non-specific binding is thought to increase electrostatic repulsion and therefore increase WHC. It is suggested that polyphosphates sequester divalent cations (e.g., Ca++) which are involved in bonds between adjacent myofilaments.

Lyons and Siebenthal (1966) evaluated binding of condensed phosphates by proteins and reported that binding increased with an increase in protein concentration at a given polyphosphate concentration. Also, binding was affected by the chain length of the polyphosphate. Binding increased with increasing chain length from 2 to 13 in gelatin samples.

Naus et al. (1968) showed that one mole of myosin bound two moles of pyrophosphate, but one mole of actomyosin

bound only one mole of pyrophosphate. They concluded that each myosin molecule had two phosphate binding sites, but that one site was made unavailable due to binding of actin. This indicates that the ability of meat to bind pyrophosphate may be different for the prerigor and rigor conditions.

Combined Effects of NaCl and Phosphates

Utilization of condensed phosphates lowers the concentration of NaCl required to obtain beneficial functional characteristics. Offer and Trinick (1983) showed that the presence of pyrophosphate (10mM) halved the concentration of NaCl needed to elicit swelling and water uptake. Furthermore, addition of pyrophosphate to salt solutions had the additional effect of enhancing myosin extraction. Neer and Mandigo (1977) reported synergistic effects of NaCl and STPP on smokehouse cook yields in a flaked, cured pork product. When comparing 0% phosphate (control), 0.5% STPP and 0.5% TSPP, Shults and Wierbicki (1974) indicated parallel trends in WHC with increasing NaCl concentrations. Maximum WHC occurred at approximately 2% NaCl. TSPP had a higher WHC then STPP over the range of NaCl concentrations analyzed.

Turner et al. (1979) studied the effect of various STPP and NaCl concentrations in myofibrillar protein extraction solutions on the yields of crude myosin. The yields of

crude myosin were greatest at a STPP concentration of approximately 0.25% in the extraction solution. Yields were increased when the NaCl concentration in the extraction solution was increased from 0.5 M to 1.0 M. Crude myosin extracted from prerigor muscle had greater binding strength than that extracted from postrigor muscle.

There is a paucity of data regarding the combined effects of salt and phosphate on prerigor meat functional properties. Pepper and Schmidt (1975) reported increased cook yields in hot boned beef rolls when 0.25% phosphate was included with 1.0% NaCl. However, there was no evaluation of the state of rigor in that study.

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CHAPTER I

Effect of Sodium Chloride Concentration on Postmortem Metabolism and Processing of Prerigor and Postrigor Ground Beef

ABSTRACT

Fifteen beef cattle of similar age and management history were randomly allotted by slaughter days into 3 groups. Paired sternomandibularis were removed immediately following bleeding and trimmed of visible fat and connective tissue. They were randomly labelled as prerigor or postrigor and assigned to 0, 0.5, 1.0, 2.0 or 4.0% NaCl treatments. Water-holding capacity (WHC), pH, the ratio of absorbance at 250 nm over the absorbance at 260 nm (R-values), and 1.0 M NaCl extractable protein (EP) were monitored over treatment times. R-values verified that 0 hour samples were in the prerigor or postrigor state. Ultimate pH increased linearly ($P<0.05$) in prerigor homogenates with increasing NaCl concentration. EP and WHC were higher ($P<0.05$) in prerigor than in postrigor homogenates with 2 and 4% NaCl at all time periods. Prerigor homogenates containing 0.5 and 1.0% NaCl had higher ($P<0.05$) WHC at 12, 24, 48, and 96 hours than similarly treated postrigor homogenates and as high or higher WHC than any postrigor treatment. Results of this study indicate an advantage to using low NaCl concentrations in prerigor salted beef.

INTRODUCTION

Hot processing, fostered by attempts to reduce meat processing costs and to increase product quality, has received considerable attention over the last two decades. This procedure which involves removal of muscle soon after slaughter, and before conventional chilling, offers a number of potential advantages: reduction of energy input (Henrickson, 1975; 1981), cooling space, chilling time, and labor (Kastner, 1977; Cuthbertson, 1980). Furthermore, "hot" meat exhibits functional (water binding capacity, extractable protein, emulsification capacity) advantages when salted in the prerigor state (Hamm, 1981) and is ideal for utilization in processed meats and sausage manufacturing. The sodium chloride (NaCl) concentration necessary to attain the "prerigor salting effect" has been studied. However, the experimental evidence is inconclusive.

Hamm (1981) reported that in order to attain higher water binding capacity in beef homogenates, the NaCl concentration of prerigor meat must be at least 1.8%. This finding is in general agreement with Puolanne and Terrell (1983) who found a 2.0% NaCl concentration necessary in prerigor salted pork. However, Honikel (1986) indicated that 1.0% NaCl will achieve the desired presalting effect in comminuted hot muscle. Furthermore, Coon (1982) reported that 0.5% NaCl produced greater water-holding capacity in

prerigor meat than 0.0 or 3.0% NaCl in prerigor or postrigor meat.

The possibility of prerigor processing with reduced NaCl concentrations producing postrigor equivalent or enhanced functional properties in comminuted meat products is of particular interest. Many meat processors, pressured by consumer groups, are making efforts to reduce sodium in the diet (Olson and Terrell, 1981). This is because of the possible contribution of sodium to development of hypertension that occurrs in 10 to 20% of the United States population (Pearson and Wolzak, 1982).

The objectives of this study were twofold: (1) To evaluate the effects of prerigor salting of ground beef and salt concentration on pH, water-holding capacity (WHC) and extractable protein (EP) over postmortem time; and (2) To compare prerigor samples to postrigor controls.

MATERIALS AND METHODS

Source of Meat

Fifteen beef cattle were slaughtered in three randomly allotted groups of five animals each at the Michigan State University Meat Laboratory. Paired sternomandibularis were removed immediately following bleeding, trimmed of visible fat and connective tissue, randomly labeled as prerigor or postrigor, and assigned to a 0, 0.5, 1.0, 2.0 or 4.0% NaCl treatment. Prerigor muscles were weighed, ground through a

4.0 mm plate (Kitchen Aid Food Grinder attachment, Model FG-A, Hobart Co., Troy, OH) into a beaker containing 50% (w/w) water and an appropriate NaCl concentration, mixed, reground and analyzed at 0 (immediately), 1 (pH only), 2, 4, 6, 12, 24, 48, and 96 hours. Postrigor muscles were restrained to prevent shortening, held 48 hours, processed in a similar manner to prerigor samples and analyzed at 0, 24, and 48 hours thereafter. All samples were held at room temperature (approximately 21° C) for 12 hours and then stored at 4° C for the remainder of the sampling periods.

pH and R-values

pH of the samples was determined by homogenizing in a Brinkman Polytron (Model PCU2, Brinkman Co., Westbury, NY) 1 g of sample with 10 mL of 5 mM Na-iodoacetate in 150 mM KCl (Bendall, 1973). The pH of the resultant suspension was measured with a Radiometer Model PHM 82 standard pH meter equipped with a Radiometer combination pH electrode (Model GK 2501, Radiometer, Copenhagen, Denmark). R-values were determined by homogenizing 2 g of meat with 10 mL of 1.0 M perchloric acid. The homogenate was filtered and 0.1 mL of the filtrate was added to 4.9 mL of 0.1 M potassium phosphate buffer pH 7.0. The absorption at 250 nm and 260 nm was measured (Bausch and Lomb Spectrophotometer) using the phosphate buffer as reference. The R-value was defined as the ratio of absorbance at 250 nm over the absorbance at 260 nm (Honikel and Fischer, 1977). Duplicate

determinations were made on each treatment replicate for pH and R-values.

Extractable Protein

Total protein content in muscle homogenates was determined by the Kjeldahl nitrogen method (AOAC, 1984). The procedure for extractable protein (EP) determination was a modification of the procedure used by Saffle and Galbreath (1964). Two 5 g samples of each treatment replicate were diluted with 20 mL of an appropriate unbuffered NaCl solution to attain a final concentration of 1.0 M NaCl. Samples were homogenized 3 seconds using the polytron, allowed to stand for 3 min, and homogenized again for 3 seconds. The slurry was centrifuged (RC2-B Sorval centrifuge, Norwalk, CT) at 16,000 x G for 12 min. After centrifugation, the fat layer floating on top was gently removed with a stainless-steel spatula. The supernatent was decanted and again centrifuged at 16,000 x G for 12 min. The second supernatent was analyzed for protein concentration by the Kjeldahl nitrogen method. EP was expressed as percent of the total meat protein.

Water-holding Capacity

Percent cook yield (raw meat weight % retained after cooking) was used as a measure of water-holding capacity (WHC). Five g of the unsalted or salted muscle homogenate were weighed into a preweighed corex centrifuge tube,

covered with a glass marble, and placed in a boiling water bath (Precision Scientific, Model 83, Chicago, IL) for 20 min. The tube was allowed to cool 15 min and the juice released by heating was drained off. The cooked meat sample was put on a Whatman number 1 filter paper, blotted, and placed back into the tube which was reweighed for determining the cook yield. This method is essentially the same as reported by Honikel et al. (1981). In this phase of the study, triplicate determinations were done.

Experimental Design and Statistical Analysis

The experimental design used was a randomized complete block (three blocks of five animals) with repeated measurements (sampling periods postmortem). The data were analyzed by analysis of variance. When the analysis of variance showed significant F values, either Tukey's multiple comparison test (when comparing treatments within the same period) or Scheffe's multiple comparison test (when comparing periods within the same treatment) were used to locate differences between treatment-period combination means (Gill, 1978b).

When comparing prerigor and postrigor data, postrigor values were averaged over time and subtracted from prerigor values at each prerigor time period. Confidence intervals, using treatment-period combination means and their estimated degrees of freedom, were calculated with Students t test (Gill, 1978a). Intervals including 0 indicated non-

significant differences at the specific type 1 error chosen.

RESULTS AND DISCUSSION

R-values and pH

The onset of rigor mortis occurs when R-values have reached a level of about 1.10 (Honikel et al., 1981). This corresponds to an ATP concentration of approximately 1 umol ATP/g wet tissue (Honikel and Fischer, 1977). According to this criterion, R-values in Table 1 indicate prerigor and postrigor conditions for 0 hour homogenates. Calkins et al. (1982) reported that at similar R-values, 0.88 and 1.36, the ratio of adenine nucleotides (adenosine tri-, di-, and monophosphate) to the concentration of inosine compounds (inosine monophosphate and inosine) was 0.70 and 0.11, respectively. These ratios of ATP are important as the superior functional properties of prerigor sausage (salted) products are directly related to high levels of ATP (Hamm, 1977).

The mean pH value of prerigor homogenates was 6.84 at the initial time of analysis (Figure 1) with no differences ($P>0.05$) among NaCl concentrations. These values concur with values (pH 6.84) found by Jolley et al. (1981) in beef sternomandibularis. A drop ($P<0.01$) in pH was noted for all homogenates from 0 to 1 hour and 1 to 2 hours. Furthermore, after 4 hours there was no change ($P>0.10$) in

Table 1 - R-values[a] for prerigor and postrigor homogenates at 0 hours.

Treatment	Rigor State	
	Prerigor	Postrigor
0% NaCl	0.88	1.35
0.5% NaCl	0.88	1.36
1.0% NaCl	0.90	1.35
2.0% NaCl	0.89	1.35
4.0% NaCl	0.91	1.37

[a] Data on the table are mean values from 3 animals with duplicate determinations per animal.

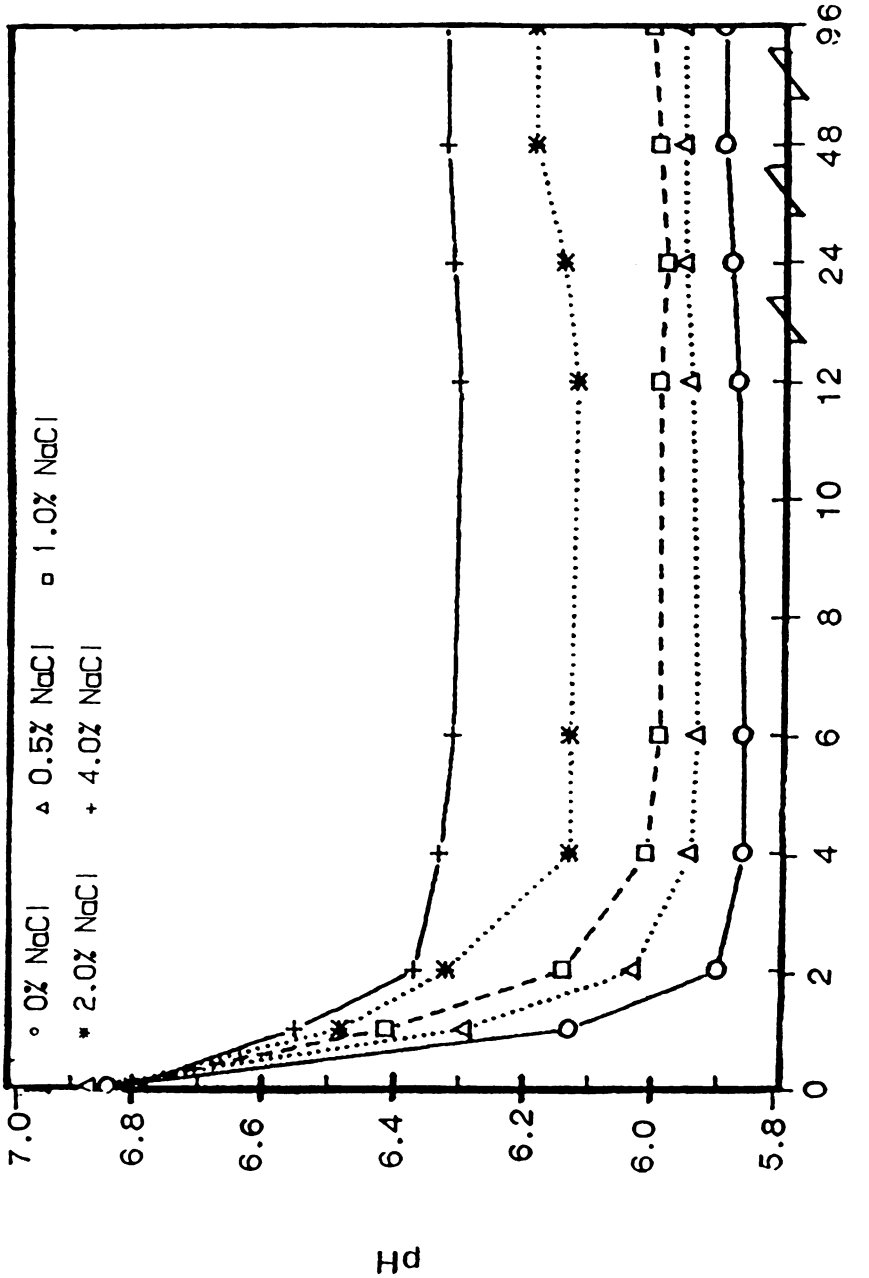

Figure 1. - Prerigor pH treatment means over postmortem time. Each point represents the means of 3 animals with duplicate determinations. MSD within periods and within treatments are 0.12 and 0.11, respectively, at $\alpha = 0.05$.

pH values for the remainder of the storage periods. With increasing NaCl concentrations there were higher ultimate pH values. This relationship between pH and salt concentration was linear ($P<0.005$) when analyzing regression lines with polynomial coefficients within time periods 4, 6, 12, 24, 48, and 96 hours.

The phenomenon of higher ultimate pH values in prerigor salted tissues has been observed by numerous researchers (Newbold and Lee, 1965; Newbold and Scopes, 1971; Hamm, 1977; Poulanne and Terrell, 1982; Coon et al., 1983). Hamm (1977) suggested that the inhibition of glycolysis in salted prerigor meat is due to the denaturation of glycolytic enzymes by the combined effect of low pH (<6.0) and high ionic strength. Contrary to this, our ultimate pH values in prerigor salted homogenates were higher (6.18 and 6.32) than pH 6.0 for the 2 and 4% NaCl treatments. Similarly, Newbold and Scopes (1971) found pH values ranging from approximately 5.8 with no KCl to 6.3 with a 1.0 M KCl solution. Partial explanation for these differences can be attributed to method of pH measurement. Bendall (1973) stated that the action of iodoacetate causes unavoidable pH shifts, resulting in an alkalinization of about 0.2 pH units at a pH of 7.0 and about 0.1 pH units at a pH of 6.0. In the present study, and in the study of Newbold and Scopes (1971), iodoacetate was used to inhibit glyceraldehyde-phosphate dehydrogenase.

The pH of postrigor homogenates were not different

($P>0.05$) over time or among treatments (Table 2). Therefore, postrigor pH values were averaged over time and compared to prerigor pH values (Table 3). Prerigor homogenates had higher ($P<0.01$) pH values than postrigor homogenates at all prerigor times and NaCl concentrations. Even grinding alone and water dilution of prerigor muscle limited the extent of pH decline as compared to any postrigor treatment. This observation has also been made in prerigor ground pork (Judge and Aberle, 1980). Lawrie (1979) reported that if muscles are exposed to oxygen during postmortem glycolysis, ATP is produced with such efficiency that rigor mortis is delayed and ultimate pH tends to rise. In the present study, grinding could have introduced the oxygen necessary to enhance aerobic metabolism. Elevated pH in meat homogenates retards the development of oxidative rancidity (Judge and Aberle, 1980; Yasosky et al., 1984). Therefore, the use of salted prerigor meat in oxidatively susceptible restructured products could delay or inhibit rancidity development.

WHC

Prerigor WHC values shown in Figure 2 indicate samples treated with 2 and 4% NaCl had a higher WHC than 0, 0.5 or 1.0% NaCl treatments. Furthermore, there was a decline ($P<0.01$) in WHC from 0 to 2 hours in all prerigor homogenates. The "prerigor salting effect" was the highest with the 4% salt concentration. The 2% NaCl homogenates

Table 2 - pH, WHC (percent yield), and EP (percent of total protein) of postrigor homogenates at various NaCl concentrations.

Treatment	Response Variable	Time of Analysis (h)[a] 0[b]	24	48	$\bar{X}$
0% NaCl	pH	5.63 cx	5.63 cx	5.63 cx	5.63
	WHC	52.76 cx	52.90 cx	54.36 cx	53.34
	SP	29.20 cx	29.47 cx	28.71 cx	29.13
0.5% NaCl	pH	5.59 cx	5.60 cx	5.60 cx	5.60
	WHC	53.23 cex	54.07 cdx	54.23 cx	53.84
	SP	30.75 cx	29.60 cx	29.93 cx	30.09
1.0% NaCl	pH	5.58 cx	5.60 cx	5.60 cx	5.59
	WHC	54.80 cdx	54.83 cdx	56.83 cy	55.49
	SP	28.69 cx	28.20 cx	28.28 cx	28.39
2.0% NaCl	pH	5.62 cx	5.63 cx	5.63 cx	5.63
	WHC	58.03 dx	57.47 dxy	56.00 cy	57.17
	SP	28.87 cx	29.08 cx	30.56 cx	29.50
4.0% NaCl	pH	5.59 cx	5.62 cx	5.59 cx	5.60
	WHC	56.76 dex	54.68 cdy	55.08 cxy	55.50
	SP	30.81 cx	30.83 cx	29.88 cx	30.50

[a] Time of analysis corresponds to hours after grinding of postrigor samples.

[b] Values represent means from 3 animals with duplicate determinations for pH and EP and triplicate determinations for WHC.

cde Within response variables, means in the same column bearing different superscripts are significantly different ($P<0.05$).

xy Means in the same row bearing different superscripts are significantly different ($P<0.05$). Standard errors for pH, WHC, and SP treatment period means were 0.04, 0.93, and 0.89, respectively.

Table 3 - pH, WHC (percent yield), and EP (percent of total protein) of prerigor minus postrigor values, averaged over time at various NaCl concentrations

Treatment	Response Variable	Time of Analysis (h)[a] 0	1	2	4	6	12	24	48	96
	pH[b]	1.2**	0.5**	0.3**	0.2**	0.2**	0.2**	0.2**	0.3**	0.3**
0% NaCl	WHC	8.6**	---	3.3*	2.5	3.2*	3.7*	4.6**	2.0	4.0**
	EP	17.1**	---	6.7**	-3.1	-1.1	0.9	0.8	0.3	0.7
	pH	1.3**	0.6**	0.4**	0.4**	0.3**	0.4**	0.4**	0.4**	0.4**
0.5% NaCl	WHC	8.2**	---	2.8*	3.3*	3.7*	4.2**	4.7**	4.9**	6.5**
	EP	19.0**	---	6.6**	1.4	2.6	4.0**	4.4**	7.1**	6.3*
	pH	1.3**	0.8**	0.5**	0.4**	0.4**	0.4**	0.4**	0.4**	0.4**
1.0% NaCl	WHC	9.1**	---	1.3	2.0	2.0	4.6**	5.2**	4.7**	6.6**
	EP	20.9**	---	10.7**	4.2**	6.4**	9.6**	11.2**	11.1**	14.0**
	pH	1.2**	0.9**	0.7**	0.5**	0.5**	0.5**	0.5**	0.6**	0.6**
2.0% NaCl	WHC	16.2**	---	6.5**	5.7**	4.9**	10.7**	8.4**	8.7**	7.0**
	EP	19.8**	---	17.4**	19.0**	19.5**	21.9**	23.7**	23.9**	23.6**
	pH	1.2**	0.9**	0.8**	0.7**	0.7**	0.7**	0.7**	0.7**	0.7**
4.0% NaCl	WHC	17.1**	---	13.2**	13.1**	13.7**	15.8**	15.9**	16.6**	16.2**
	EP	19.7**	---	18.7**	18.8**	19.9**	22.7**	24.3**	22.0**	23.5**

[a] Time of analysis corresponds to h after grinding of prerigor samples.

[b] Values represent means from 3 animals with duplicate determinations for pH and EP and triplicate determinations for WHC.

* Significantly different from 0 at $P<0.05$. ** Significantly different from 0 at $P<0.01$.

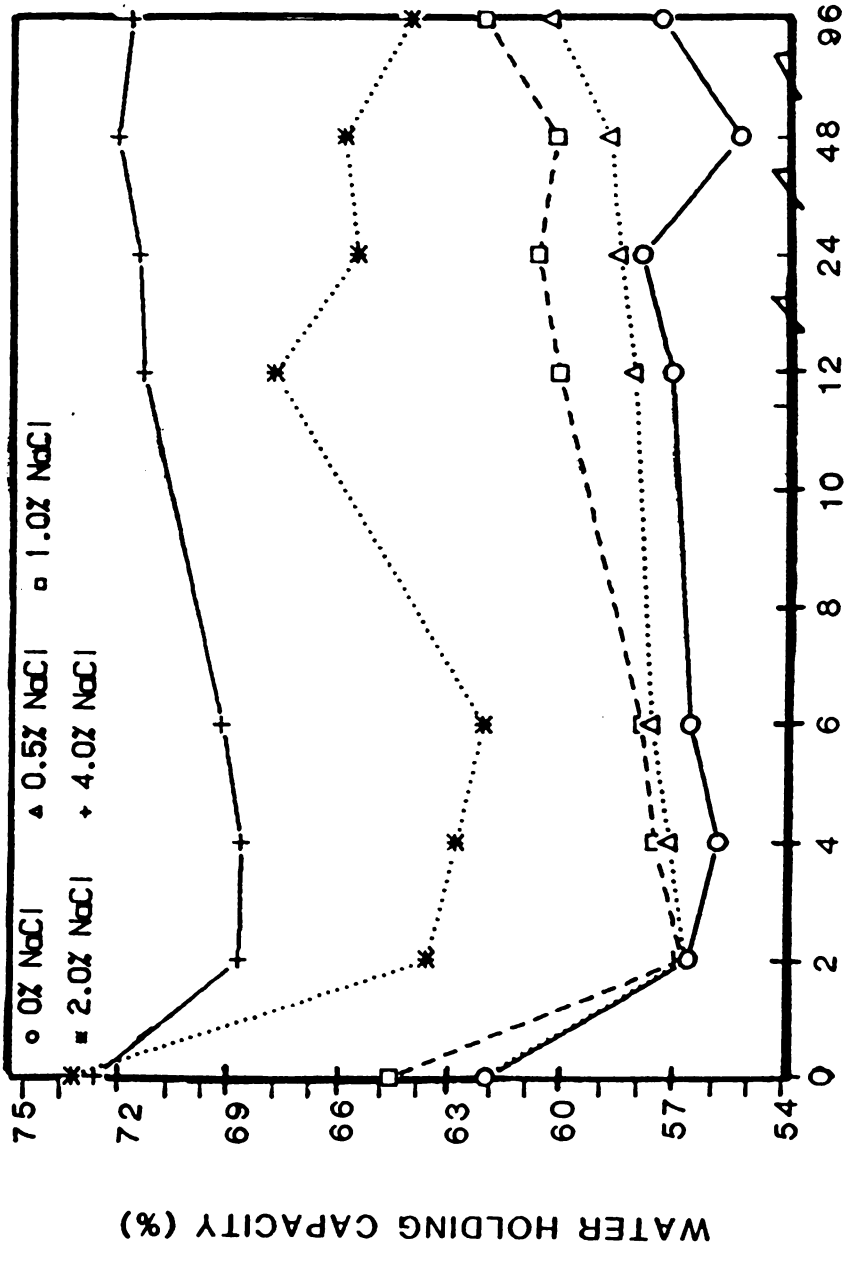

Figure 2. - Prerigor WHC treatment means over postmortem time. Each point represents the means of 3 animals with triplicate determinations. MSD within periods and within treatments are 4.49 and 3.49, respectively, at $\alpha = 0.05$.

had a higher ($P<0.01$) WHC than 0, 0.5 and 1.0% NaCl homogenates at all time periods, except 96 hours. However, with the 0.5 and 1.0% NaCl homogenates there was an increase ($P<0.05$) in WHC when comparing 2 and 96 hours. This increase in WHC over time was also noted in 2 and 4% NaCl homogenates, but the increase was not significant ($P>0.05$). These increases in WHC over time support the observations of Davey and Gilbert (1968). They suggested that increased WHC during aging is due to progressive weakening of the linkages between the myofibril filaments. Similarly, Offer and Trinick (1983) hypothesized that ultimate swelling (WHC) of myofibrils is partially dictated by the structural constraints at the M- and Z-lines.

The WHC of the 2 and 4% NaCl postrigor homogenates was higher ($P<0.05$) than the 0% NaCl homogenates at 0 hours (Table 2). However, at 48 hours the WHC among treatments could not be differentiated ($P>0.05$). This was due to the nonsignificant increase in WHC of the 0, 0.5 and 1.0% NaCl samples and the decline in WHC of the 2 and 4% NaCl homogenates. Data from Table 3 indicates WHC of prerigor homogenates was higher ($P<0.01$) than postrigor homogenates for the 2 and 4% NaCl treatments at all time periods. Furthermore, at 0, 12, 24, 48, and 96 hours, 0.5 and 1.0% NaCl treated prerigor homogenates had higher ($P<0.05$) WHC than similarly treated postrigor homogenates and as high or higher WHC than 2 or 4% NaCl treated postrigor homogenates (Table 2). Thus, although the largest "prerigor salting

effect" was observed with at least 2.0% NaCl added, 0.5 and 1.0% NaCl in prerigor homogenates had advantages over similar and higher NaCl concentrations in postrigor homogenates.

EP

By evaluating treatments at a uniform NaCl molarity, the differences in EP clearly reflect the prerigor salting effect. At 0 hours, no differences ($P>0.05$) were observed among the treatments (Figure 3). Henceforth, 2 and 4% NaCl homogenates had higher ($P<0.05$) EP than 0, 0.5 or 1.0% NaCl and did not change ($P>0.05$) over postmortem time. These data are contrary to the findings of Hamm and Grabowska (1979) who reported lower myofibrillar and total protein solubility in prerigor homogenates over postmortem time. The differences can possibly be attributed to the method of measuring EP. Hamm and Grabowska's (1979) protein extraction solution contained a lower concentration of NaCl and protein was measured by a dilution method. At lower (0, 0.5, and 1.0%) NaCl concentrations there was a drop ($P<0.05$) in EP from 0 to 2 hours and from 2 to 4 hours ($P>0.05$)(Figure 3). Furthermore, an "aging" effect was again noted with 0.5 and 1.0% NaCl homogenates as EP increased from 4 to 96 hours.

There were no differences ($P>0.05$) in EP among treatments or over postmortem time in postrigor homogenates (Table 2). This indicated that for postrigor treatments,

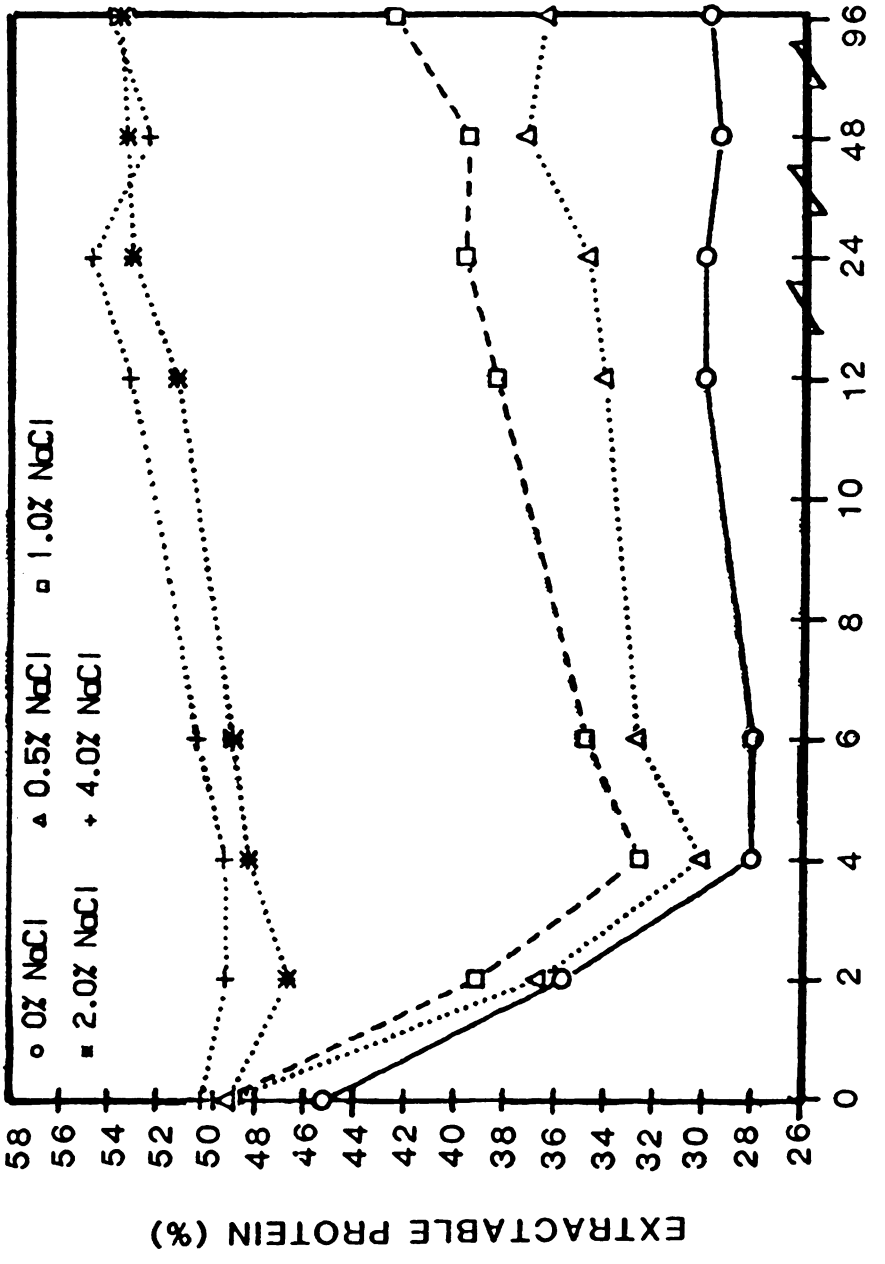

Figure 3. - Prerigor EP treatment means over postmortem time. Each point represents the means of 3 animals with duplicate determinations. MSD within periods and within treatments are 9.7 and 8.2%, respectively, at $\alpha = 0.05$.

protein extracted at uniform NaCl concentrations (1.0 M) was similar. All prerigor treatments had higher ($P<0.05$) EP than postrigor treatments at 0 and 2 hours and 2 and 4% NaCl homogenates had higher EP at all prerigor time periods. EP was higher ($P<0.01$) at 48 and at 6, 12, 24, 48, and 96 hours for 0.5 and 1.0% NaCl prerigor homogenates, respectively.

The values for EP are similar to those found by Acton and Saffle (1969) in prerigor and postrigor meat emulsions. They reported prerigor preblended soluble protein concentrations of 41.2% and postrigor soluble protein concentrations of 26.2%, in 3% w/w NaCl protein extraction solutions. Likewise, Johnson and Henrickson (1970) reported 69.9% more extractable salt-soluble protein in prerigor normal pH muscle than postrigor normal pH porcine muscle.

CONCLUSIONS

Immediately after grinding, prerigor meat homogenates have higher pH, WHC, and EP than postrigor homogenates. These advantages were evident even 96 hours after salting, although differences were not as great. In industrial applications it is doubtful whether the beneficial effects of low (0, 0.5, 1.0%) NaCl homogenates can be realized before the decline in functional properties noted at 2 and 4 hours. However, in preblending situations, 0.5 and 1.0% NaCl could have advantages, due to an accelerated "aging" effect, when comparing prerigor with postrigor homogenates. Additional experiments using low NaCl concentrations under

industrial processing practices are necessary to verify this hypothesis. Further studies utilizing salted prerigor meat in oxidatively susceptible products as an effort to delay or inhibit rancidity development are also warranted.

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Chapter II

Effect of Reduced Sodium Chloride Concentrations and Tetrasodium Pyrophosphate on Postmortem Metabolism and Processing of Prerigor and Postrigor Ground Beef

ABSTRACT

The effect of tetrasodium pyrophosphate (TSPP)(0, 0.25, 0.5% w/w) alone or in combination with salt (NaCl) (0, 0.5, 1.0% w/w) on water-holding capacity (WHC), pH, the ratio of absorbance at 250 nm over the absorbance at 260 nm (R-values) and 1.0 M NaCl extractable protein (EP) was studied in prerigor and postrigor sternomandibularis homogenates over time. R-values verified that 0 hour samples were in a prerigor or postrigor state. In prerigor homogenates, increasing phosphate concentration increased the time required to reach ultimate pH. Ultimate pH values of prerigor homogenates containing phosphate were lower ($P<0.05$) than homogenates containing 0% phosphate and similarly treated postrigor homogenates. After 6 hours, no differences ($P>0.10$) were noted in EP or WHC at different phosphate concentrations when averaged over NaCl concentrations in prerigor homogenates. With increasing phosphate concentration of postrigor homogenates, there was an increase ($P<0.05$) in pH and EP at the initial sampling time. However, 0 and 0.25% phosphate WHC values could not be differentiated ($P>0.10$). Results of this study indicate no advantages, after 6 hours postmortem, to using TSPP alone or in combination with NaCl in prerigor meat homogenates.

INTRODUCTION

Many meat processors, pressured by consumer groups, are making efforts to reduce sodium in the diet because of its possible contribution to development of hypertension that occurs in 10 to 20% of the population in the United States (Pearson and Wolzak, 1982). The use of phosphates, as a partial replacement of sodium chloride, provides a viable means for reduction of sodium levels in processed meat products. Renewed interest in this alternative has been fostered by a USDA regulation change allowing the use of phosphates in a much wider range of processed meat products (Federal Register, 1982).

The advantageous effects of inorganic, alkaline sodium phosphates on meat pH, protein solubility and WHC in postrigor meat tissues have been extensively studied (Bendall, 1954; Swift and Ellis, 1956; Shults and Wierbicki, 1973; Trout, 1984). Tetrasodium pyrophosphate (TSPP) is generally considered superior to sodium tripolyphosphate (STPP), tetrapolyphosphate and hexametaphosphate in enhancing meat functional characteristics (Bendall, 1954; Shults et al., 1972; Trout and Schmidt, 1984).

Furthermore, utilization of condensed phosphates lowers the concentration of NaCl required to obtain beneficial functional characteristics. Offer and Trinick (1983) showed that the presence of 10mM TSPP halved the concentration of NaCl needed to elicit swelling and water uptake in isolated

myofibrils. Neer and Mandigo (1977) reported synergistic effects of NaCl and STPP on smokehouse cook yields in a flaked, cured pork product. When comparing 0% phosphate, 0.5% TSPP and 0.5% STPP, Shults and Wierbicki (1974) found increasing WHC with increasing NaCl concentration. TSPP homogenates had higher WHC values than STPP homogenates over the range of NaCl concentrations analyzed. In these studies, the concentration of NaCl added was generally 2% or higher. Recently, we showed that prerigor homogenates treated with 0.5 or 1.0% NaCl have a higher WHC, after aging for 24 hours, than postrigor homogenates containing 2 or 4% NaCl (Chapter 1). The addition of phosphates to prerigor homogenates could enhance this WHC effect.

Although the effects of phosphates in postrigor meat are well documented there is a paucity of data regarding the effects of phosphates on prerigor meat functional properties. Therefore, the objectives of this study were: (1) To investigate the effects of TSPP alone or in combination with NaCl on the functional properties of prerigor and postrigor muscle homogenates; (2) To determine if the effects of low NaCl concentrations and TSPP are synergistic in prerigor and/or postrigor muscle homogenates; and (3) To substantiate the effects of NaCl alone in prerigor muscle homogenates.

MATERIALS AND METHODS

Source of Meat

Twenty seven beef cattle were slaughtered in nine allotted groups of three animals each at the Michigan State University Meat Laboratory. Prerigor and postrigor muscle homogenates were prepared following the procedure cited in Chapter 1 (page 61) and assigned to a NaCl (0, 0.5, 1.0% w/w) and TSPP (0, 0.25, 0.50 % w/w) combination treatment.

pH and R-values

pH and R-values were determined following the procedure cited in Chapter 1 (page 62).

Extractable Protein

Extractable protein was determined following the procedure cited in Chapter 1 (page 63). The ionic strength (IS) of the extraction solutions were not equivalent for all treatments because of the added phosphate in certain treatments. The differences in IS were small and are reported in Table 1.

Water-holding Capacity

Water-holding capacity values were determined following the procedure cited in Chapter 1 (page 63).

Table 1. Ionic strength[a] of homogenates and protein extraction solutions.

Treatment	Homogenates (IS)	EP Solutions (IS)
0% NaCl 0% Phos	0	1.0
0% NaCl 0.25% Phos	0.20	1.03
0% NaCl 0.50% Phos	0.40	1.07
0.5% NaCl 0% Phos	0.10	1.0
0.5% NaCl 0.25% Phos	0.30	1.03
0.5% NaCl 0.50% Phos	0.50	1.07
1.0% NaCl 0% Phos	0.20	1.0
1.0% NaCl 0.25% Phos	0.40	1.03
1.0% NaCl 0.50% Phos	0.60	1.07

[a] Ionic strength based on 83% water in meat homogenates, and calculated according to procedures given by Trout (1984).

Experimental Design and Statistical Analysis

The experimental design used was a factorial incomplete block (nine blocks of three animals) with repeated measurements (sampling periods postmortem). The criteria for statistical evaluation were the same as those cited in Chapter 1 (page 64).

RESULTS

The onset of rigor mortis occurs when R-values have reached a level of about 1.10 (Honikel et al., 1981). According to this criterion, R-values in Table 2 indicate prerigor and postrigor conditions for 0 hour homogenates.

The analysis of variance phosphate x salt and salt x time interactions and the salt main effect were not significant ($P>0.25$) for the postrigor response variables pH, WHC and EP. Therefore, phosphate effects were evaluated averaged over NaCl concentration (e.g. 0% phosphate included the averaged values from three treatments 0% phosphate 0% NaCl, 0% phosphate 0.5% NaCl, and 0% phosphate 1.0% NaCl). Data in Figures 1, 2, and 3 generally indicated that increasing the phosphate concentration of postrigor homogenates caused increases H, WHC, and EP. Homogenates containing 0.5% phosphate had the highest values ($P<0.01$) for each response variable at all time periods. EP and pH values for 0.25% phosphate treated homogenates were higher ($P<0.01$) than 0% phosphate treated homogenates at the

Table 2. R-values[a] for prerigor and postrigor homogenates at 0 hours.

Treatment	Rigor State	
	Prerigor	Postrigor
0.0% NaCl 0.00% Phos	0.87	1.35
0.0% NaCl 0.25% Phos	0.88	1.35
0.0% NaCl 0.50% Phos	0.92	1.36
0.5% NaCl 0.00% Phos	0.87	1.35
0.5% NaCl 0.25% Phos	0.94	1.37
0.5% NaCl 0.50% Phos	0.86	1.36
0.0% NaCl 0.00% Phos	0.91	1.37
0.0% NaCl 0.25% Phos	0.92	1.35
0.0% NaCl 0.50% Phos	0.92	1.36

[a] Values represent means from 3 animals with duplicate determinations.

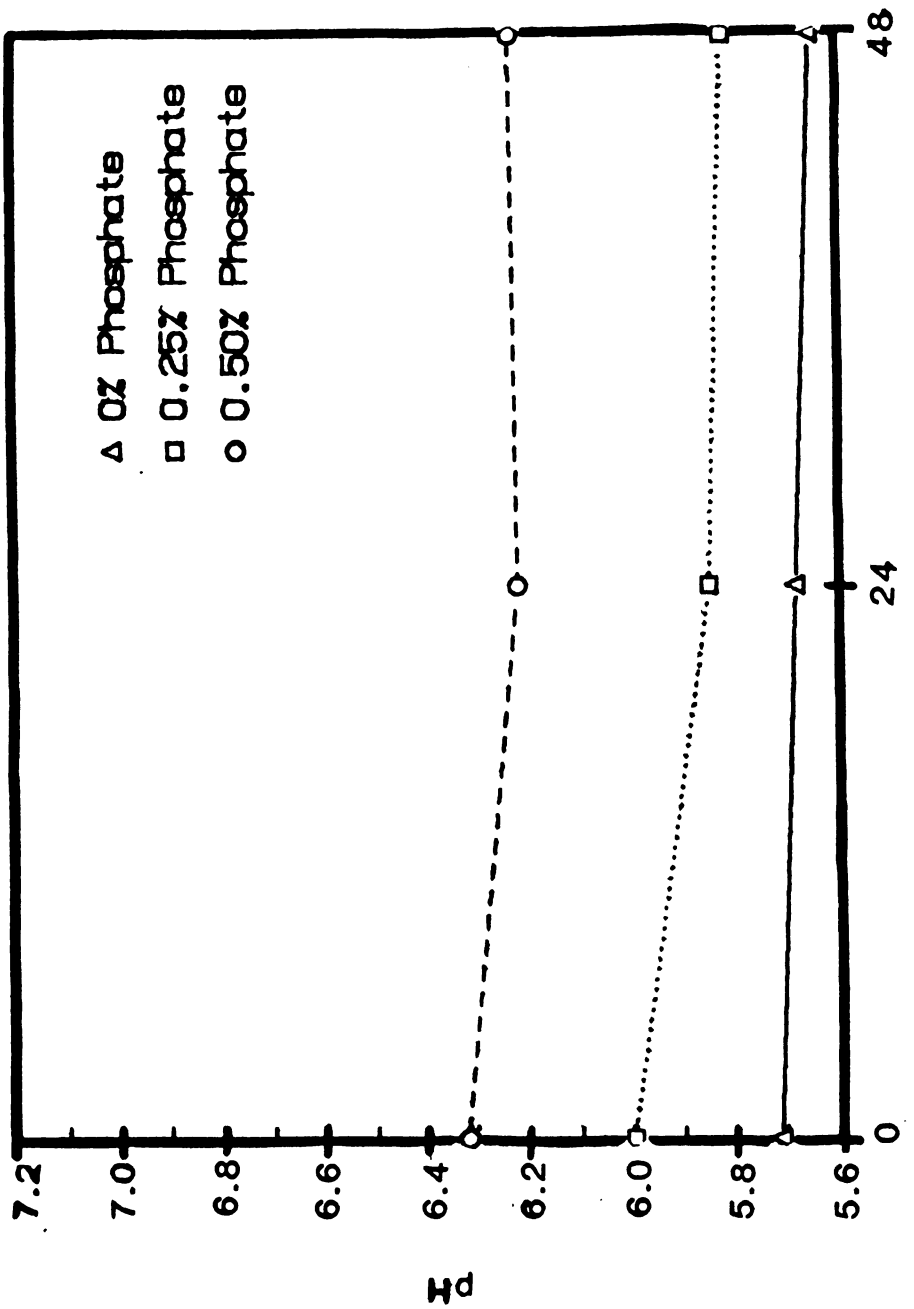


Figure 1.- Postrigor pH means averaged over NaCl concentration. Each point represents the means of 9 animals with duplicate determinations. MSD within periods and within treatments are 0.13 and 0.04, respectively, at $\alpha = 0.05$.

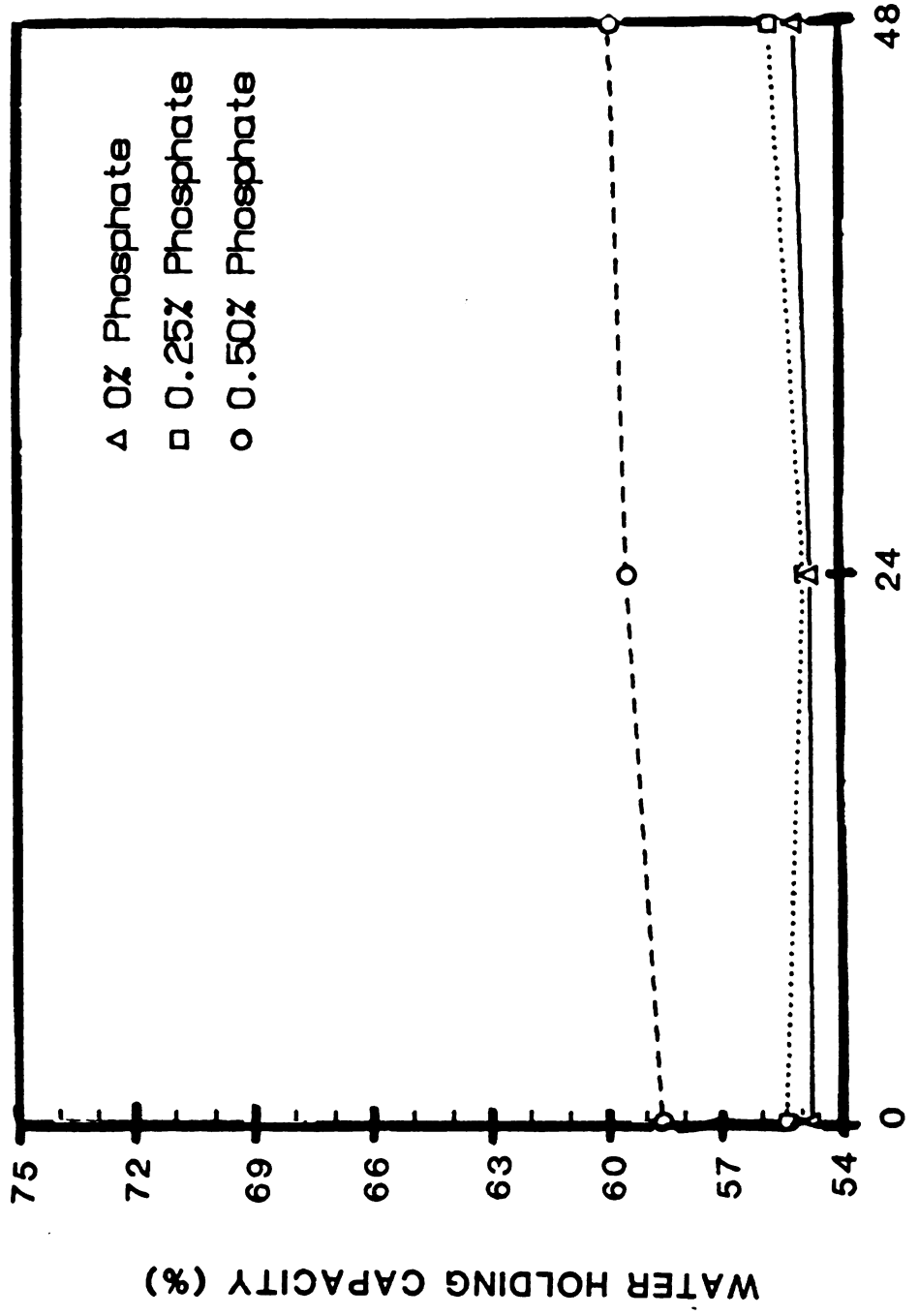


Figure 2.- Postrigor WHC means averaged over NaCl concentration. Each point represents the means of 9 animals with triplicate determinations. MSD within periods and within treatments are 2.72 and 1.00% respectively, at $\alpha = 0.05$.

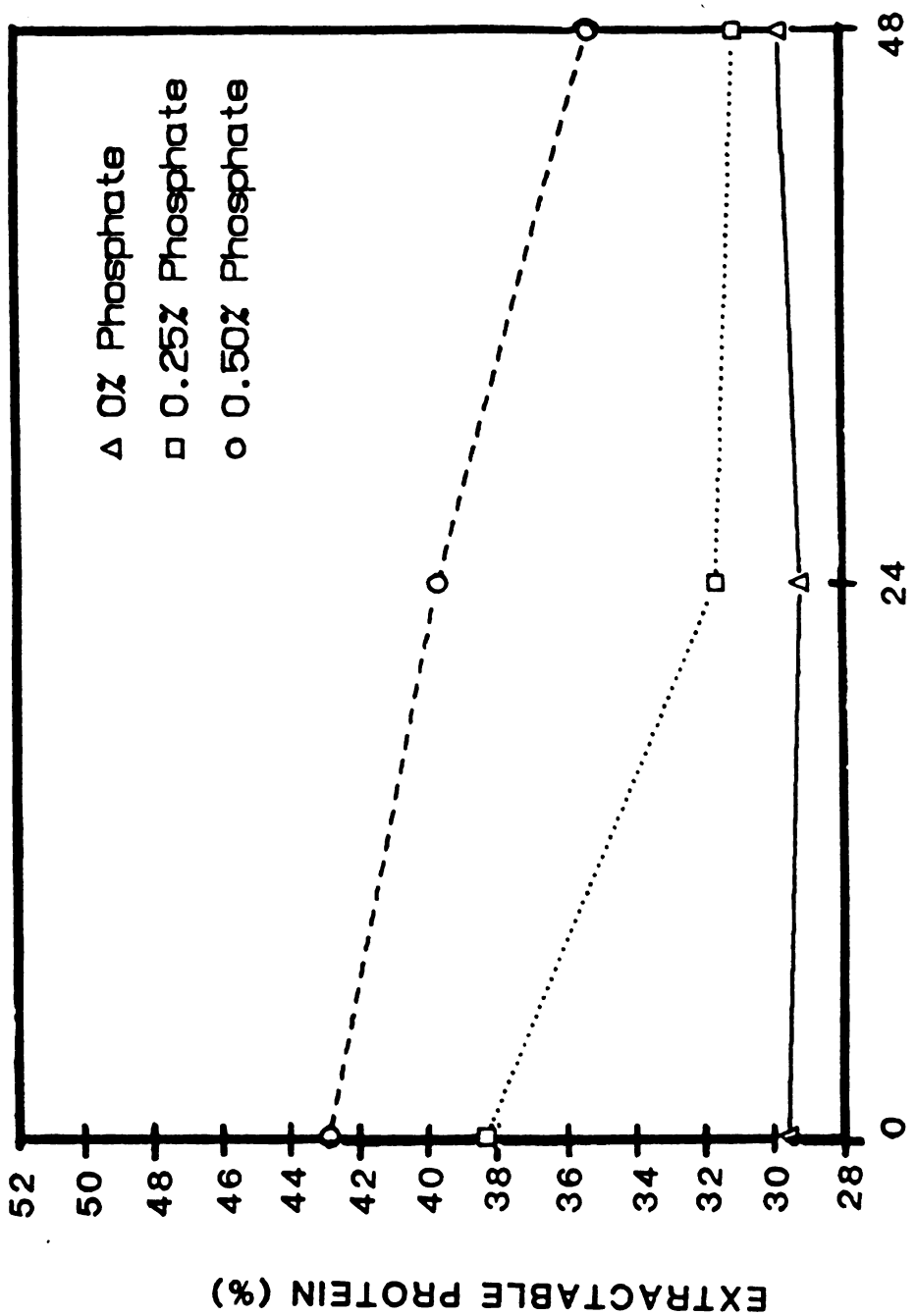


Figure 3.- Postrigor EP means averaged over NaCl concentration. Each point represents the means of 9 animals with duplicate determinations. MSD within periods and within treatments are 3.21 and 2.48%, respectively, at $\alpha = 0.05$.

0 hour sampling period, but EP values for the same comparison could not be differentiated ($P>0.10$) at 24 or 48 hours. No differences ($P>0.10$) were noted when comparing WHC values between treatments containing 0 and 0.25% phosphate. Furthermore, from 0 to 48 hours a decline ($P<0.01$) in pH and EP occurred for homogenates containing phosphate.

As in the postrigor analysis, in the prerigor analysis of variance the phosphate x salt interaction was not significant ($P>0.10$) for the pH, WHC and EP response variables. However, in addition to phosphate having a significant effect in prerigor homogenates, the salt main effect and salt x time interaction were also significant ($P < 0.10$). This indicated that salt and phosphate acted independently when exerting a significant effect on WHC, pH, and EP in prerigor homogenates. Therefore, the effects of phosphates were evaluated averaged over NaCl concentration (Figures 4, 5, and 6) and NaCl effects were evaluated averaged over phosphate concentration (Figures 7, 8, and 9).

At the 0 hour period in prerigor homogenates, with increasing alkaline phosphate concentration, pH (Figure 4) and WHC (Figure 5) values increased ($P<0.01$). Furthermore, treatments containing 0% phosphate reached their ultimate pH values after 4 hours, whereas 0.25 and 0.5% phosphate homogenates did not reach ultimate values until 6 and 12 hours, respectively (Figure 4). After 12 hours the

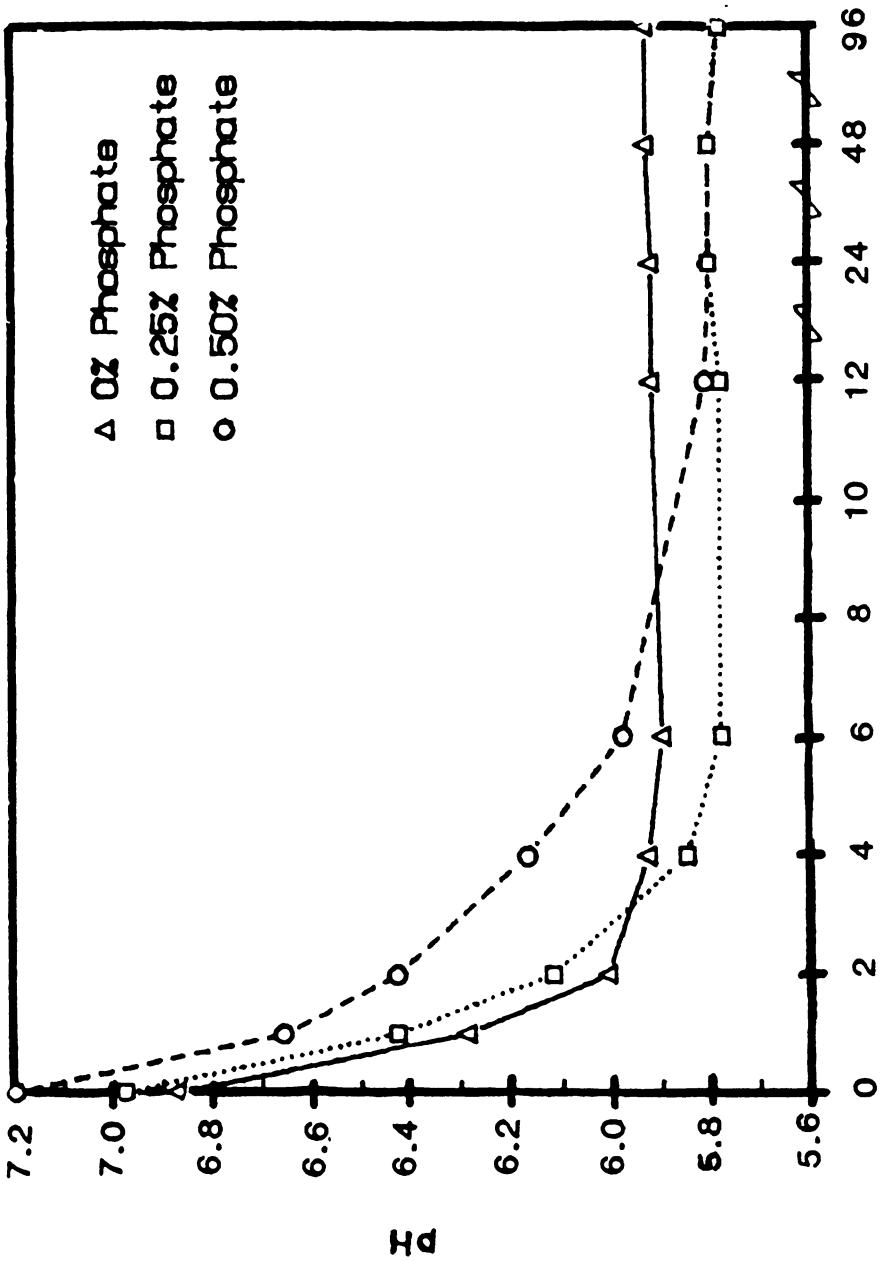


Figure 4.- Prerigor pH means averaged over NaCl concentration. Each point represents the means of 9 animals with duplicate determinations. MSD within periods and within treatments are 0.11 and 0.08 respectively, at $\alpha = 0.05$.

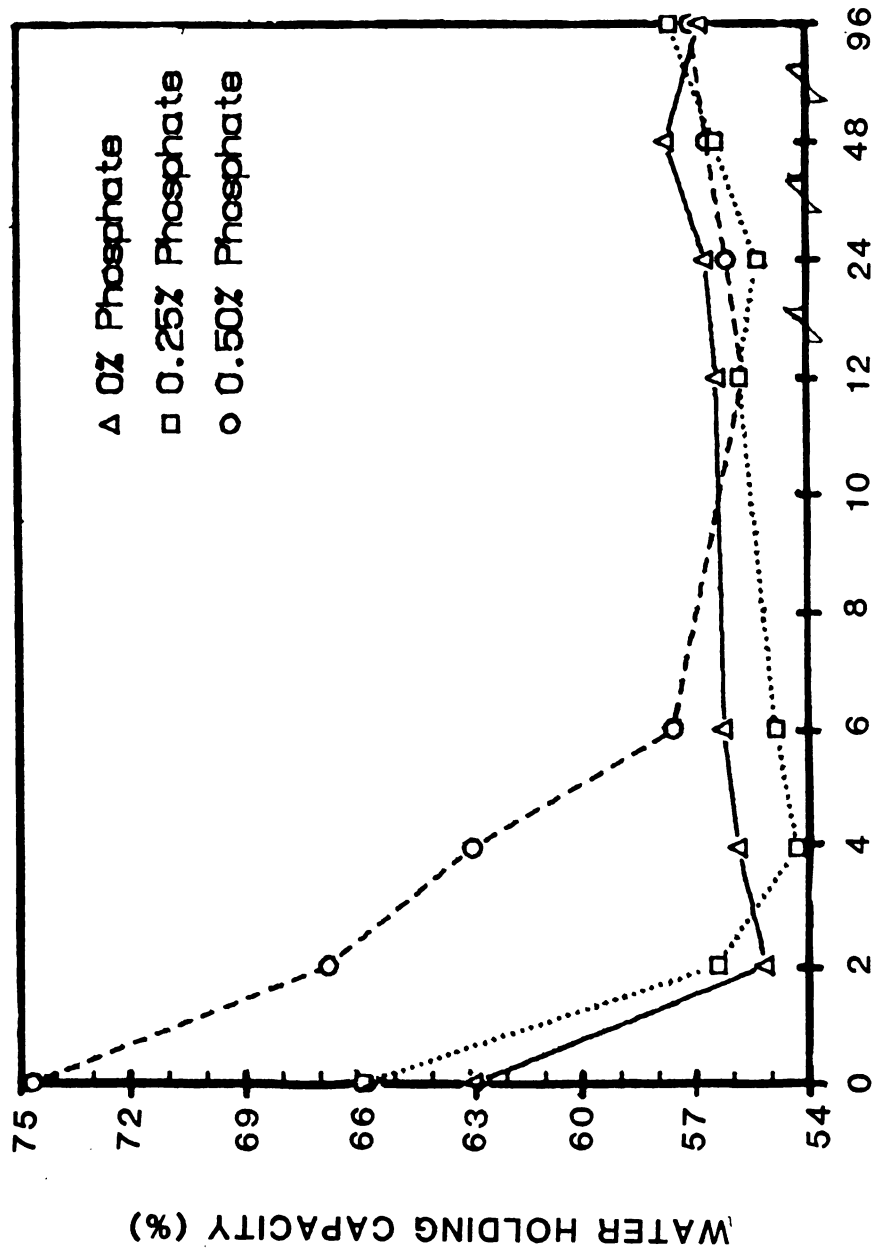
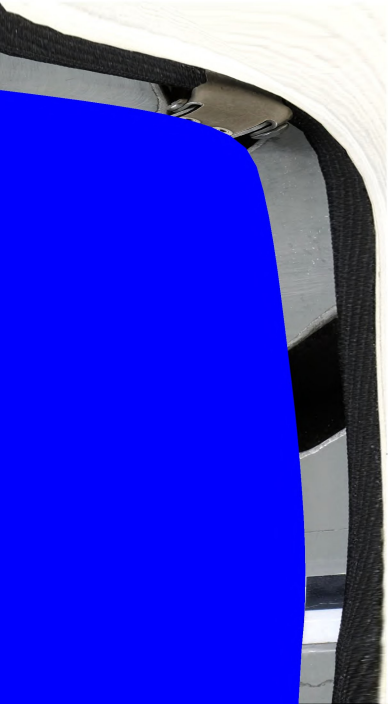


Figure 5.- Prerigor WHC means averaged over NaCl concentration. Each point represents the means of 9 animals with triplicate determinations. MSD within periods and within treatments are 2.85 and 1.92%, respectively, at $\alpha = 0.05$.



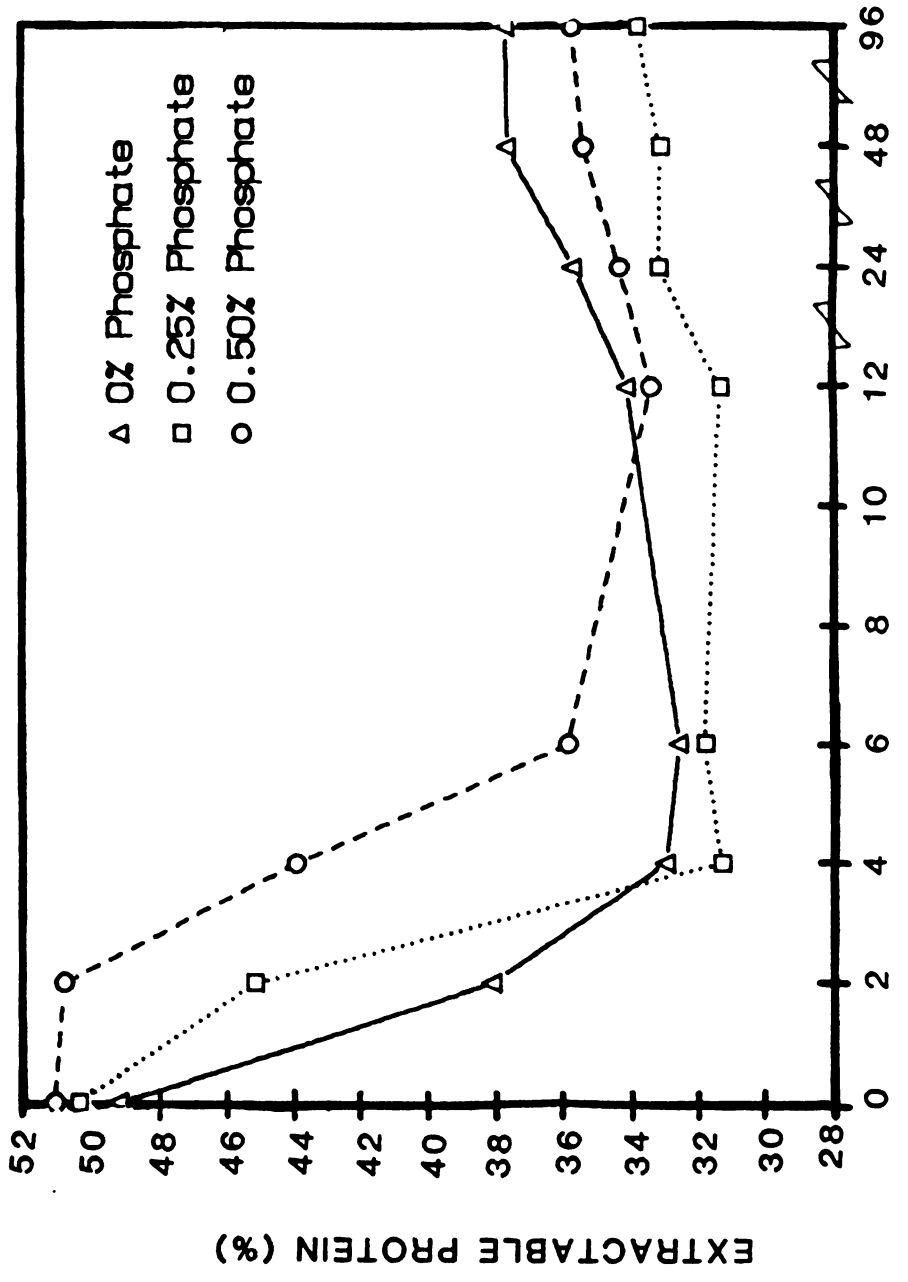


Figure 6.- Prerigor EP means averaged over NaCl concentration. Each point represents the means of 9 animals with duplicate determinations. MSD within periods and within treatments are 3.75 and 3.6%, respectively, at $\alpha = 0.05$.

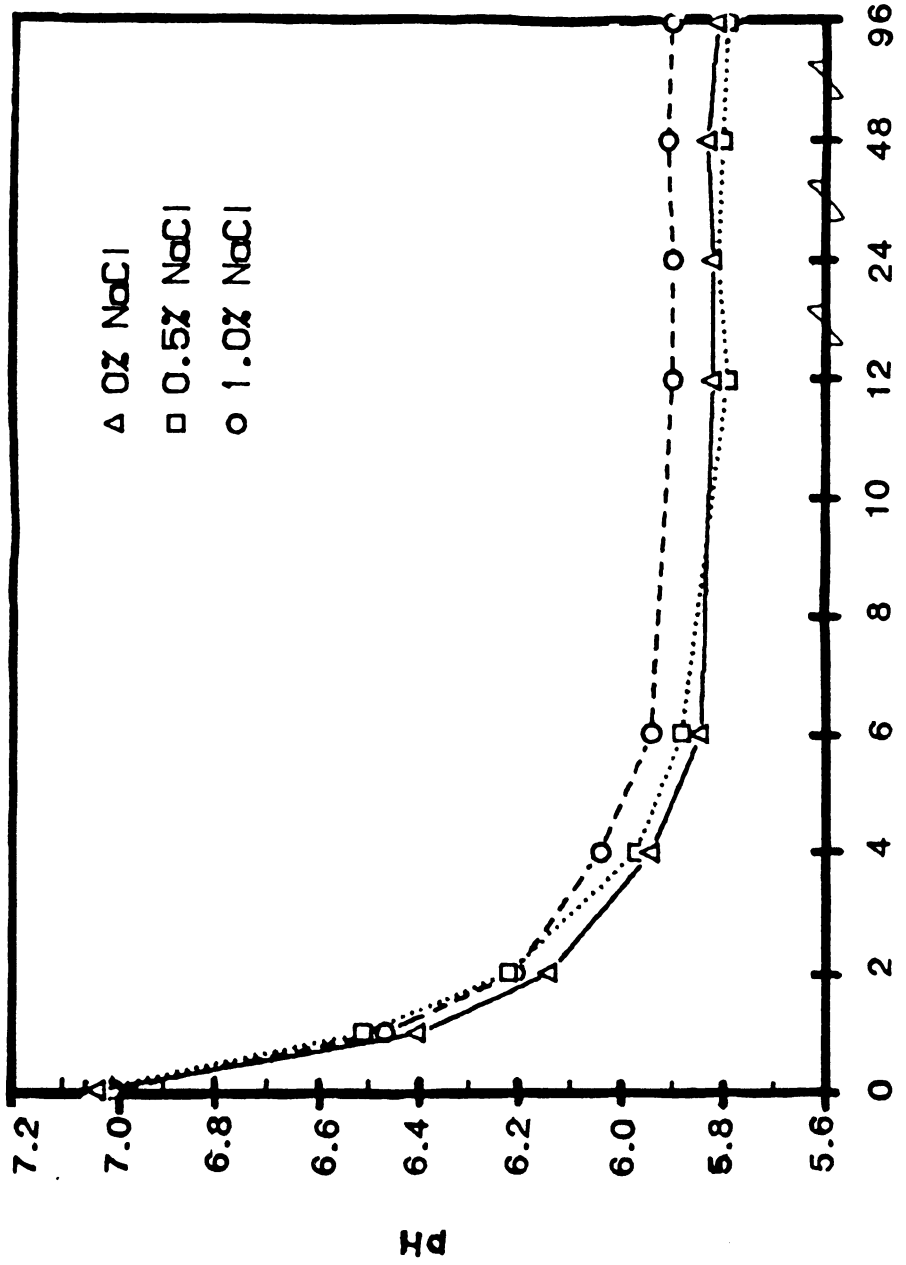


Figure 7.- Prerigor pH means averaged over phosphate concentration. Each point represents the means of 9 animals with duplicate determinations. MSD within periods and within treatments are 0.11 and 0.08, respectively, at $\alpha = 0.05$.

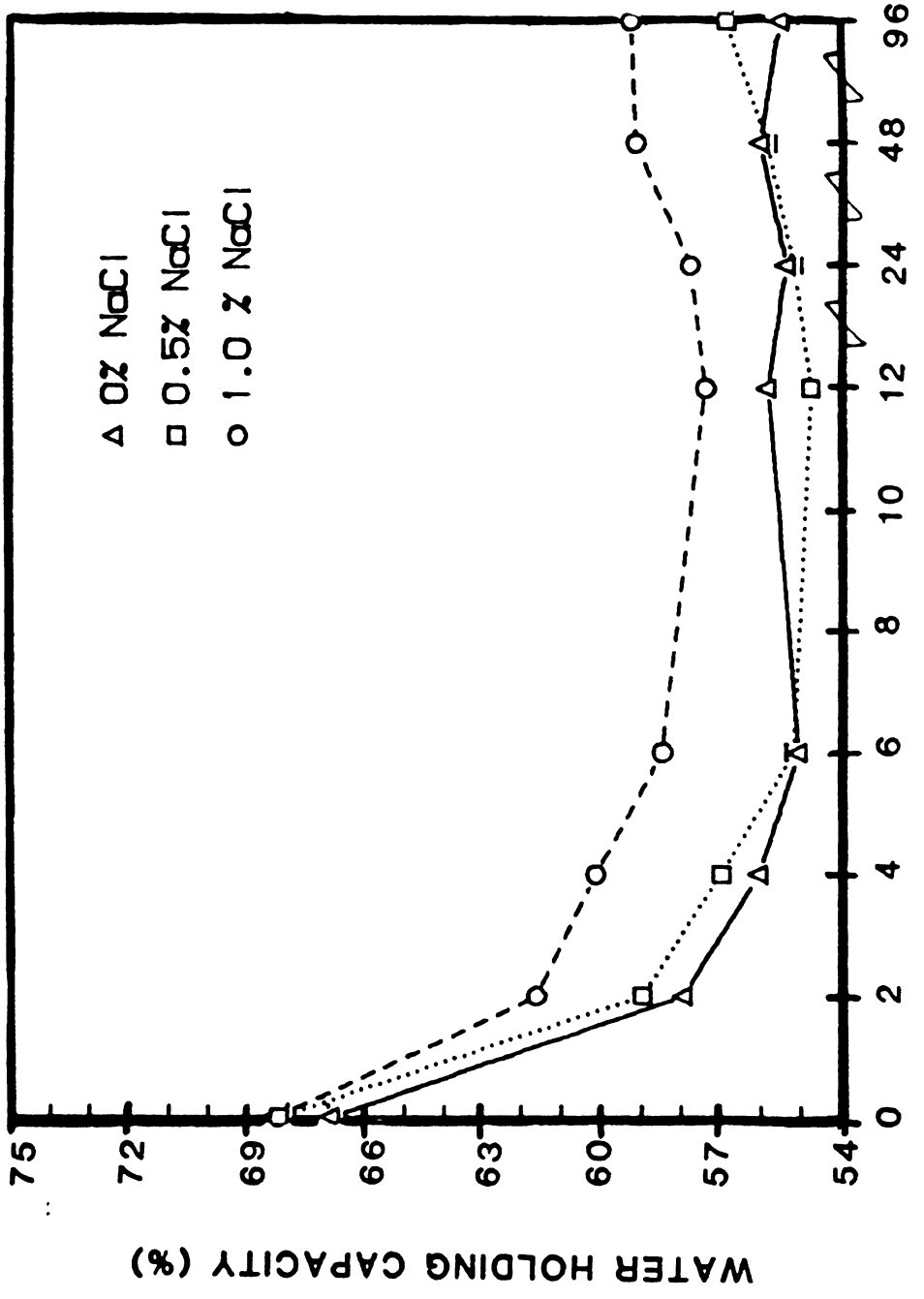


Figure 8. - Prerigor WHC means averaged over phosphate concentration. Each point represents the means of 9 animals with triplicate determinations. MSD within periods and within treatments are 2.85 and 1.92%, respectively, at $\alpha = 0.05$.

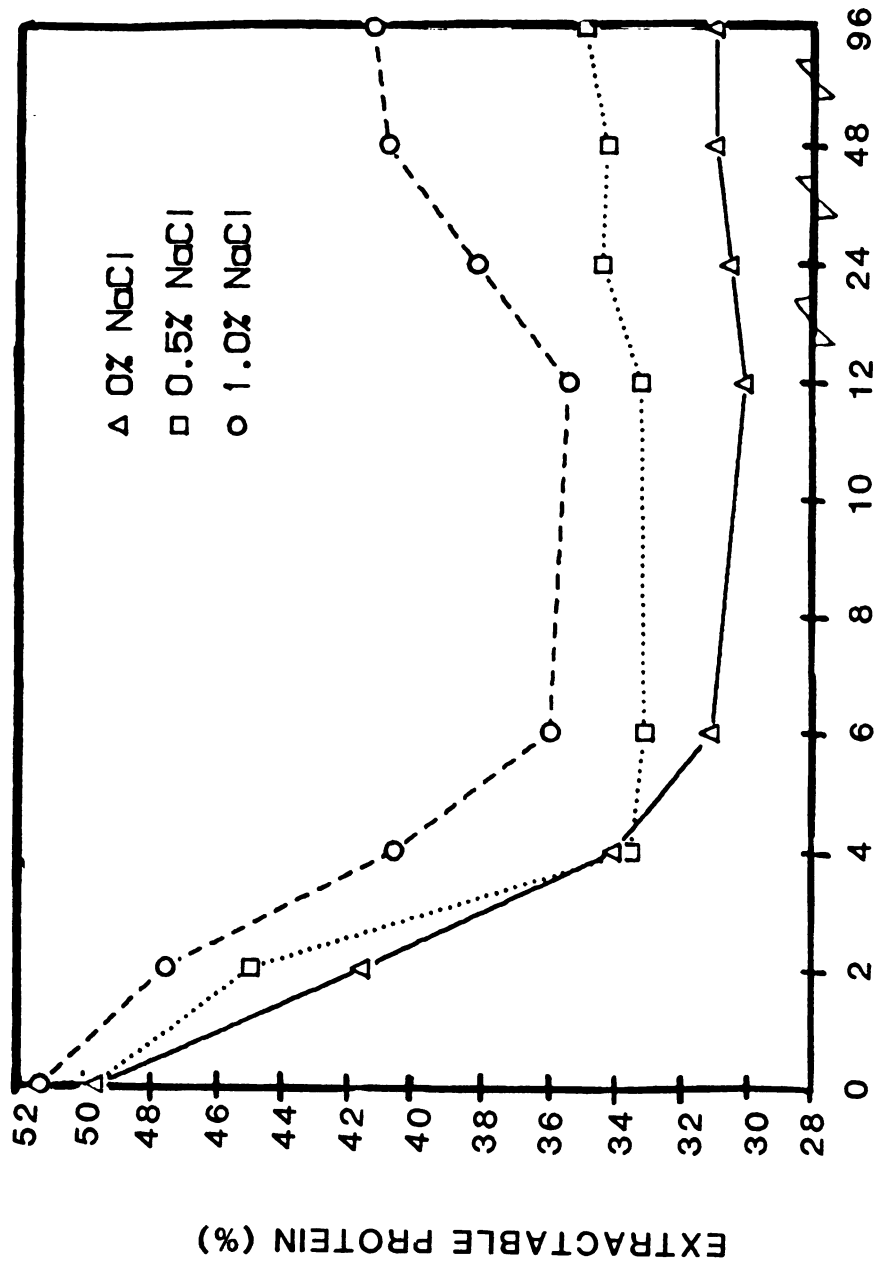


Figure 9.- Prerigor EP means averaged over phosphate concentration. Each point represents the means of 9 animals with duplicate determinations. MSD within periods and within treatments are 3.75 and 3.6%, respectively, at $\alpha = 0.05$.

phosphate treated homogenates had lower ($P<0.05$) pH values than the treatments containing 0% phosphate. Likewise, EP and WHC values were higher ($P<0.05$) for the 0.5% phosphate homogenates at 2 and 4 hours, but after 6 hours there was no advantage when compared to other treatments (Figures 5 and 6).

When evaluating homogenates averaged over phosphate concentration, no differences ($P>0.01$) in pH were noted within periods. However, 1% NaCl-treated homogenates appeared to have higher values after 2 hours (Figure 7). Similarly, no WHC differences were noted between the 0 and 0.5% NaCl-treated homogenates, but after 2 hours the treatments containing 1% NaCl had the highest WHC values (Figure 8). In the same manner, EP values were higher ($P<0.01$) for 1.0% NaCl-treated homogenates then 0% NaCl-treated homogenates at all time periods after 2 hours (Figure 9).

Because both prerigor and postrigor values change over time, it is difficult to make direct statistical comparisons of prerigor and postrigor response variables. However a general comparison of Figures 1 and 4 indicates that prerigor homogenates containing 0% phosphate have a higher pH than similarly treated postrigor homgenates and postrigor homogenates containing 0.5% phosphate and to a lesser extent 0.25% phosphate have higher ultimate pH values than similarly treated prerigor homogenates. When comparing WHC values, Figure 2 vs Figure 5, it is apparent that 0.5%

phosphate-treated postrigor homogenates have as high or higher values than any prerigor treatment after 6 hours postmortem. Finally, when analyzing EP in postrigor (Figure 3) and prerigor (Figure 6) homogenates it is evident that 0% phosphate postrigor homogenates have lower EP values than similarly treated prerigor homogenates.

DISCUSSION

EP and WHC values in prerigor and postrigor meat homogenates are affected by pH, IS and the changing functionality of TSPP over postmortem time. In prerigor samples the initial effect of TSPP was to act as a buffer and raise pH values. However, it appears that TSPP also stimulated glycolysis as pH values were lower for phosphate-treated samples. Newbold and Scopes (1971) reported that 50 mM and 100 mM potassium phosphate stimulated phosphorylase and phosphofructokinase activity in prerigor sternomandibularis minces. This caused accelerated lactate production and higher ultimate lactate concentrations when compared to undiluted minces. Furthermore, they also found that increasing phosphate concentration prolonged the time required to reach ultimate pH and hypothesized that the lower rate of pH decline was due to an increased buffering capacity of the phosphates. It has been established that the buffering capacity of potassium dihydrogen phosphate decreases sharply as the pH declines from 6.8 to 5.8, where

the buffering capacity is minimal (Datta and Grzybowski, 1961). Thus, the lower pH values found in prerigor homogenates containing phosphate (Figure 4), when compared to postrigor homogenates containing phosphates (Figure 1), might be due to the increased lactate formed in prerigor homogenates causing pH values to move out of the effective buffering range of phosphate. Although this appears to be a reasonable explanation for data presented in Figure 4, it does not preclude the possibility of TSPP acting as an ATP analog and binding to the myosin molecule. Naus et al. (1969) showed that myosin bound 2 moles of pyrophosphate per mole of myosin but actomyosin bound only one mole. If TSPP binding occurred at the actin-myosin-ATP interaction site, the glycolytic rate could be slower because of a decreased ATP requirement. This hypothesis, however, does not explain the lower pH values found in meat homogenates containing phosphate.

As the pH declined in prerigor homogenates, EP and WHC values decreased to levels as low or lower than similarly treated postrigor homogenates (Figures 4 and 5). Hamm (1981) reported that a NaCl concentration of at least 1.8% is required in order to maintain a high WHC for several days. These experimental results suggest that lower concentrations of NaCl, with or without phosphate, will not produce a similar prerigor salting effect. However, higher WHC and EP values were observed in all prerigor homogenates at 0 hours and for up to 4 hours for homogenates containing

0.5% phosphate. These time-responce variable curves (Figures 4, 5 and 6) could perhaps be modified using different storage temperatures. Hamm (1982) reported that lowering the tissue temperature from 37° C (immediately after death) to 6-8° C results in a continuous decrease in postmortem glycolysis rate. Thus, immediate cooler storage of prerigor phosphate treated homogenates might extend the period of enhanced WHC and EP.

Contrary to the transient pH advantages observed in phosphate-treated homogenates, homogenates containing a 1% NaCl concentration had slightly higher ultimate pH values than 0 or 0.5% NaCl-treated homogenates (Figure 7). In earlier experiments, we found that with increasing NaCl concentration there was a linear pH increase in ultimate pH of prerigor meat homogenates (Chapter 1). This phenomenon was not noted in this experiment when NaCl concentrations were evaluated averaged over phosphate concentration. However, similar to our earlier observations, there was an increase in WHC (Figure 8) and EP (Figure 9) at 24, 48 and 96 hours. Offer and Trinick (1983) hypothesized that ultimate WHC of myofibrils is partially dictated by the structural constraints of the M- and Z-line. From our results in this experiment and previous experiments, it appears that prerigor homogenates are more susceptible to aging, which causes progressive weakening of the linkages between the myofibril fragments (Davey and Gilbert, 1968), than postrigor homogenates.

In postrigor homogenates, EP increased with increasing phosphate concentration (Figure 3). A major portion of this increase can be attributed to higher pH values observed with higher alkaline phosphate concentration (Figure 1). Previous researchers (Saffle and Galbreath, 1964; Honikel et al., 1981) have reported that as pH values are increased away from the isoelectric pH of proteins, there is an increased WHC and protein solubility. Trout and Schmidt (1984) found that between 90 and 96% of the variation in binding of restructured beef rolls could be explained by changes in IS and pH. The IS of protein extraction solutions in this experiment were only increased by 3 and 7%, when 0.25 and 0.50% phosphate were added respectively (Table 1). It is doubtful whether this small increase in IS would have a significant influence on EP. However, Bendall's theory (Bendall, 1954) of increased solubility of muscle proteins as a result of pyrophosphate-induced dissociation of actomyosin cannot be overlooked. Although pyrophosphate is a less powerful dissociating agent than ATP, its properties as an actomyosin-dissociating agent are well documented (Nanninga, 1964; Granicher and Portzehl, 1964).

The pH and EP values of postrigor homogenates containing phosphate declined from 0 to 24 hours. This decline might have been due to hydrolysis of TSPP. In the presence of water, TSPP is hydrolyzed to 2 moles of dibasic sodium phosphate (Bell, 1947). The pH of a 1% aqueous

solution of dibasic sodium phosphate and TSPP is 9.1 and 10.2, respectively (Anonymous, 1983). Morita et al. (1983a) reported that TSPP is rapidly hydrolyzed to orthophosphate in emulsion sausages. Furthermore, Sutton (1973) showed that the more commonly used STPP is rapidly hydrolyzed to TSPP which then undergoes a slower hydrolysis to orthophosphate. Thus, the decrease in pH over time in postrigor homogenates containing phosphate, due to hydrolysis, could account for the lower EP values.

WHC in postrigor homogenates increased only when 0.5% phosphate was added to homogenates (Figure 2). Morita et al. (1983b) reported that WHC increased with increasing pyrophosphate content and reached a maximum level at 0.2% pyrophosphate, when 2.0% NaCl was included in the homogenates. Apparently, in our experiments, with lower concentrations of NaCl, the IS of treatments containing 0.25% phosphate was not high enough to cause increased WHC. This also may explain the lack of salt x phosphate interaction. However, when analyzing the WHC data of the 9 treatments, the 3 treatments containing 0.5% phosphate had the highest WHC values. Therefore, the postrigor WHC data cannot be explained by IS alone.

CONCLUSIONS

Addition of 0.25 or 0.50% TSPP to prerigor meat homogenates, in the presence or absence of NaCl at low concentrations, will not enhance WHC or EP for extended

periods beyond 6 hour of holding at 21° C. Apparently, TSPP in prerigor samples stimulates anaerobic glycolysis. Therefore, in prerigor preblends the phosphate portion of the ingredient mix should not be added until postrigor conditions exist. This study further verified the advantage of TSPP and low concentrations of NaCl in postrigor and prerigor meat systems, respectively.

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CHAPTER III

Partitioning of Added Tetrasodium [32P] Pyrophosphate In Prerigor and Postrigor Muscle Homogenates Subjected to Low Ionic Strength Aqueous Extraction.

ABSTRACT

Paired sternomandibularis from 5 beef cattle were removed immediately following bleeding, trimmed of visible fat and connective tissue, randomly labelled as prerigor or postrigor and restrained to prevent shortening. Prerigor and postrigor muscles were ground, combined with a solution containing tetrasodium [32] pyrophosphate and subjected to a series of low ionic strength aqueous extractions. Radioactivity (DPM) was monitored in the centrifugation supernatants (S1 - S3) and pellets (P1 - P3). The total DPM in prerigor and postrigor fractions counted were not different ($P>0.10$). Prerigor P1 ($P<0.05$) and P2 ($P<0.01$) fractions had higher DPM values than similar postrigor preparations, however P3 prerigor and P3 postrigor DPM values could not be differentiated ($P>0.10$). Results of this study indicate that a large portion of the phosphate added to prerigor meat is apparently physically entrapped and after 2 washings there are only small differences in the pyrophosphate retained in prerigor and postrigor pellets.

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INTRODUCTION

Alkaline phosphates are commonly used in the meat industry to enhance water-holding capacity (WHC). Hamm (1970) postulated that this may be due to electrostatic protein-protein repulsion caused by phosphate binding to protein molecules. This binding could occur at actomyosin or myosin specific binding sites or at nonspecific positively-charged protein side groups.

Tonomura and Morita (1959) reported that 2.3×10^5 g of myosin A can bind one mole of pyrophosphate (PP). Furthermore, they suggested that the number of binding sites available to PP is two per myosin A molecule. However, when analyzing myosin B (actomyosin) they stated that one mole of PP was bound by 5.6×10^5 g protein. Similarly, Naus et al. (1968) showed that 5×10^5 g of myosin (1 mole) binds approximately 2 moles of pyrophosphate, but natural actomyosin binds only 1 mole of pyrophosphate per 5×10^5 g of myosin. Although these studies were done using purified myosin or actomyosin preparations, they indicated that the ability of meat to bind pyrophosphate may be different for prerigor and postrigor meat homogenates.

Recently, we reported that with increasing tetrasodium pyrophosphate (TSPP) concentration, there was an increased time required to reach ultimate pH in prerigor meat homogenates (Chapter 2). It was thought that TSPP could be acting as an ATP analogue by binding to the ATP-myosin

interaction site. This could cause a decreased glycolysis rate because of a lower ATP requirement. This hypothesis supported the work of Gallop et al. (1957) as cited by Naus et al. (1968) who found that PP inhibits myosin ATPase.

Prerigor meat could also bind nonspecific positively-charged protein side groups differently than postrigor meat. Vandegrift and Evans (1981) reported that the degree of polyphosphate binding to bovine serum albumen was influenced by pH and the chain length of polyphosphates used. In similar experiments, Melachouris (1972) found that B-lactoglobulin binding of polyphosphate increased at lower pH. They attributed this to more interaction groups (amino acid residues) being available because of protein unfolding caused by polyphosphate binding. Theoretically, maximum binding of phosphates can be acheived when each positive group binds one polyphosphate molecule (Klotz, 1953; as cited by Vadegrift and Evans, 1981). Arginine, lysine and, to a lesser extent, histidine have positively-charged side groups in the pH range 7.0 to 5.6 (Segel, 1968). Therefore, proteins containing these amino acid residues could bind phosphates in prerigor or postrigor homogenates.

In the aforementioned binding studies, purified proteins were diluted with water and binding was assessed after addition of low concentrations of phosphates. The objective of this experiment was to evaluate TSPP binding to prerigor and postrigor muscle proteins indirectly by following tetrasodium [P32] pyrophosphates disappearance

from homogenates subjected to low ionic strength aqueous extraction.

MATERIALS AND METHODS

Five beef cattle were conventionally slaughtered at a local abattoir. Paired sternomandibularis were removed immediately following bleeding, trimmed of visible fat and connective tissue, randomly labelled as prerigor or postrigor, and restrained to prevent shortening. Muscles were held in styrofoam coolers while in transit to the laboratory. Prerigor muscles were weighed and ground (within 30 min postmortem) through a 4 mm plate (Kitchen Aid Food Grinder attachment, Model FG-A, Hobart Co., Troy, OH). Four g of ground tissue was added to a 50 mL disposable centrifuge tube containing 2 mL of 1.5 percent (w/w) tetrasodium pyrophosphate solution and 100 uL, containing 500,000 CPM (Counts Per Minute), of tetrasodium [32P] pyrophosphate (Amersham, Arlington Heights, IL) solution. After storage for 2 hours, with mixing every 30 minutes, the samples were centrifuged at 5000 x G for 20 minutes. The supernatant (S1) was decanted into 25 mL glass scintillation vials and 8.5 mL of Safety-Solve Cocktail (SSC) (Research Products International Co., Mount Prospect, IL) were added. The pellet (P1) was homogenized with a Brinkman Polytron (Model PCU-2, Westbury, NY) for 3 seconds after addition of 20 mL of 50 mM Tris, pH 7.5, buffer. Two 1 mL aliquots were

transferred to scintillation vials containing 1 mL NCS Tissue Solubolizer (Amersham, Arlington Heights, IL), labelled P1, and digested at 50° C for 4 hours in a Precision Scientific (Model 83, Chicago, IL) water bath. Eight mL of SSC were added to the digested samples. The remainder of the P1 slurry was centrifuged at 2000 x G for 15 minutes. The supernatant (S2) was decanted and two 1 mL aliquots were transferred to scintillation vials containing 9 mL SSC. The pellet (P2) was homogenized for 3 seconds after addition of 20 mL of 50 mM Tris, pH 7.5, 100 mM KCl. Two 1 mL aliquots were labelled P2 and handled similarly to P1. The remainder of the P2 slurry was centrifuged at 2000 x G for 15 minutes. The supernatent (S3) was decanted and sampled similarly to S2. The pellet (P3) was processed similar to P2 to yield P3 samples for counting. Postrigor muscles were held 12 hours at 21° C, held 36 hours at 21° C and processed similar to prerigor samples (P1 - P3 and S1 - S3).

Scintillation vials were equilibrated 2 hours at 2° C and beta-particle emissions were evaluated utilizing a liquid scintillation counter (Isocap, Model 6872, Tm Analytical, Elk Grove Village, IL). The direct internal standard method (Neame and Homewood, 1974) was used to determine efficiency of counting and CPM were converted to DPM (Disintegrations per minute). Efficiencies of vials containing P1, S2, P2, S2, P3 and S3 samples were above 95%. However, the efficiencies of vials containing S1 samples

were low and variable which can cause instrument instability (Neame and Homewood, 1974). Therefore, the difference between 500,000 DPM and P1 DPM was used as an estimate of S1 DPM (Table 1). A paired T-test (Gill, 1978) was used to evaluate prerigor and postrigor binding.

RESULTS AND DISCUSSION

Means, standard deviations and ranges of beta-particle emissions (DPM) found at various processing stages and significance of differences between prerigor and postrigor values are presented in Table 1. The total DPM were not different ($P>0.10$) for prerigor vs postrigor homogenates. However, prerigor homogenates had higher ($P<0.05$) and ($P<0.01$) DPM values in P1 and P2, respectively, than similar postrigor pellets. This was not observed after the final washing with Tris and 100 mM KCl as prerigor P3 and postrigor P3 DPM could not be differentiated ($P>0.10$).

The quantity of pyrophosphate in P1 amounted to 82.8 and 75.2% of the phosphate added in prerigor and postrigor meat homogenates, respectively. Assuming equivalent amounts of radioactive and non-radioactive phosphate were present in P1, the total amount of TSPP retained was approximately 0.024 g or .09 mmoles. Furthermore, if myosin and actomyosin bound 2 and 1 mole of pyrophosphate, respectively, 100% binding would account for only approximately 1.07 and 0.59% of the DPM found in P1 of prerigor and postrigor meat homogenates. This calculation

Table 1. Partitioning of tetrasodium [32P] pyrophosphate in prerigor and postrigor muscle homogenates.

Processing Stage	DPM		
	Prerigor	Postrigor	SED
	[a]		[b]
S1	85,963 ± 9,891 [c] (71,420 - 94,675)	123,715 ± 13,958 (113,970 - 147,758)	23,372 P<0.05 [d]
P1	414,037 ± 9,891 (405,325 - 428,580)	376,285 ± 13,958 (352,243 - 386,030)	23,372 P<0.05
P1 Counted [e]	33,123 ± 791 (32,426 - 34,287)	30,103 ± 1,116 (28,180 - 30,883)	1,870 P<0.05
S2	284,102 ± 7,850 (276,011 - 293,194)	263,175 ± 8,677 (253,862 - 277,347)	6,750 P<0.01
P2	91,686 ± 9,425 (79,537 - 103,383)	78,201 ± 2,886 (75,961 - 83,075)	7,911 P<0.01
P2 Counted	7,335 ± 754 (6,363 - 8,271)	6,256 ± 231 (6,076 - 6,647)	633 P<0.01
S3	60,754 ± 6,680 (51,796 - 69,751)	51,998 ± 3,278 (47,888 - 56,008)	5,021 P<0.01
P3	22,559 ± 3,338 (19,748 - 26,457)	21,171 ± 2,693 (17,813 - 24,234)	2,678 P>0.10
Total Counts [f]	493,836 ± 17,656 (468,714 - 513,634)	496,418 ± 12,295 (484,334 - 514,210)	19,568 P>0.10

[a] Value represents DPM + SE of the mean. N = 5 with duplicate determinations.

[b] Value represents SE of the difference between paired prerigor and postrigor samples.

[c] Value in parenthesis represents the range.

[d] Significance of difference between prerigor and postrigor DPM.

[e] P1 and P2 Counted are the DPM removed when analyzing the representative pellets.

[f] Value represents total DPM, out of 500,000, accounted for (S1 + P1 Counted + S2 + P2 Counted + S3 +P3).

is based on three assumptions: (1) Ground sternomandibularis contains 20% protein, (2) 25% of the ground meat protein is myosin, (3) The molecular weight of myosin is 500,000. Therefore, the differences in DPM found in P1 prerigor and P1 postrigor cannot be accounted for by specific myosin or actomyosin binding. Furthermore, it is doubtful whether other basic amino acids found in meat proteins could bind the large quantity of pyrophosphate found in P1. Therefore, it is likely that a large portion of the phosphate found in P1 was physically entrapped and was not removed with the S1 fraction. A probable cause for the differences in prerigor P1 and postrigor P1 DPM values can be attributed to the varying quantities of liquid found in S1. Volumes of prerigor S1 fractions averaged 0.9 mL, compared to postrigor S1 volumes of 1.2 mL. Centrifugation is a method used to evaluate WHC (Hamm, 1986). Therefore, the difference in volumes of prerigor and postrigor S1 fractions may have been due to an increased WHC in prerigor meat. This could subsequently result in the higher DPM found in S1 postrigor samples.

The homogenization of P1 with 20 mL of Trisma buffer apparently released entrapped phosphate as S2 fractions in prerigor and postrigor preparations contained large quantities of labelled phosphate. Furthermore, prerigor S2 DPM were higher ($P < 0.05$) than postrigor S2 DPM. The addition of extraction solution may have diminished the WHC effect noted in P1. Therefore, the additional phosphates

retained in prerigor P1, due to an increased WHC of prerigor preparations, were released in S2 and hence prerigor S2 had higher DPM values.

In P2 and P3 slurries, no differences were noted in prerigor vs postrigor volumes. Therefore, it is apparent that prerigor preparations retain as much or more added phosphate than postrigor preparations when subjected to low ionic strength aqueous extraction. This could be due to increased binding by myosin vs actomyosin or increased non-specific phosphate binding by other meat proteins. However, the methods used in this experiment could not differentiate between phosphate binding and physical entrapment of phosphates in the various fractions.

In conclusion, Hamm's (1970) theory of increased WHC due to increased phosphate binding by meat proteins, may not be true for both prerigor and postrigor meat homogenates. Recently, we reported decreased WHC for prerigor vs postrigor homogenates containing phosphate (Chapter 2). The data in Table 1 indicates little or no differences ($P>0.10$) in phosphate concentrations of P3 prerigor and P3 postrigor preparations. After evaluating results of this study and earlier studies, it is evident that WHC is affected more by changes in pH, than by binding of phosphates.

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SUMMARY AND CONCLUSIONS

The functional characteristics of prerigor and postrigor muscle homogenates, as they are influenced by post-blending storage, NaCl and tetrasodium pyrophsophate (TSPP) were evaluated. Further, TSPP binding to homogenate proteins was investigated. The major findings of this study are:

1. At 0 hours (immediately after grinding) prerigor homogenates have higher extractable protein (EP) and water-holding capacity (WHC) than postrigor homogenates.
2. Treatment of prerigor homogenates with 2 or 4% NaCl causes irreversible changes in the myofibrillar structure and WHC and EP remain high for at least 4 days.
3. Ultimate pH increases linearly from 5.88 at 0% NaCl to 6.35 at 4.0% NaCl in prerigor homogenates. When samples were treated with NaCl all prerigor homogenate pH values were higher than postrigor homogenate pH.
4. Prerigor homogenates containing 0.5 and 1.0% NaCl had higher WHC at 12, 24, 48, and 96 hours than similarly treated postrigor homogenates and as high or higher WHC than any postrigor treatment.
5. When adding NaCl concentrations of 0, 0.5 and 1.0% and

TSPP concentrations of 0, 0.25 and 0.5% to prerigor or postrigor homogenates, there is little evidence of a salt x phosphate synergistic effect on pH, WHC and EP.

6. Ultimate pH values of prerigor homogenates containing phosphate were lower than prerigor homogenates containing 0% phosphate and similarly treated postrigor homogenates.
7. After 6 hours no differences were noted in EP or WHC at different phosphate concentrations in prerigor homogenates.
8. With increasing phosphate concentration of postrigor homogenates, there was an increase in pH and EP at 0 hours. However, homogenates containing 0 and 0.25% phosphate WHC values could not be differentiated at any postrigor time period.
9. A large portion of the phosphate added to prerigor meat is physically entrapped and after 2 washings in a low ionic strength aqueous extraction solution there are only small differences in the pyrophosphate retained in prerigor and postrigor pellets.

These results indicate that in prerigor preblending situations 0.5 and 1.0% NaCl could have advantages, due to an accelerated aging effect, when comparing prerigor to postrigor homogenates. Furthermore, addition of TSPP to prerigor homogenates apparently stimulates anaerobic glycolysis and therefore, in prerigor preblends the phosphate portion of the ingredient mix should not be added

until postrigor conditions exist. Finally, although phosphate binding to meat proteins may be important it does not explain the differences in WHC noted in prerigor and postrigor homogenates.

PROPOSALS FOR FUTURE RESEARCH

These studies raise several questions which merit further investigation. Specific areas which should be addressed include (In no order of priority):

1. An investigation into the aging effect noted in prerigor homogenates. Specifically, SDS Gel Electrophoresis and MFI (Myofibrillar Fragmentation Index) analysis could show differences in myofibrillar structure which may be related to enhanced meat functional properties. Furthermore, addition of EGTA to meat homogenates could help in determining whether CAF (Calcium Activated Factor) is the active proteolytic enzyme.
2. Application of knowledge gained in this study to actual production of sausage or restructured meat products. Evaluating the effects of different temperature regimes, amount of added water, and different muscles would be of interest.
3. The possible applicability of utilizing salted prerigor meat in oxidatively susceptible products in an effort to delay or inhibit rancidity development.
4. The effect of the change in lactate production in prerigor and postrigor muscle homogenates containing phosphate. Specifically, what effect does incremental changes in lactate have on muscle pH.

5. The possible applicability of utilizing NaCl and/or phosphates in prerigor whole muscle systems.
6. The effect of TSPP hydrolysis in prerigor and postrigor meat homogenates.
7. The effect of sodium and potassium tripolyphosphate on prerigor and postrigor meat functional properties.

APPENDICES

Appendix 1

Statistical Formulas used in Experiment 1.

A. Test of treatments within periods

Tukey's test

Minimum Significant Difference (MSD)
$= \pm(q_{\alpha, a, \hat{v}_E})(\hat{\sigma}^2/r)^{0.5}$

$\hat{\sigma}^2$ = non-correlated residual error

$= [MS_{D/A} + (b-1)\ MS\ ]/b$

$\hat{v}_E =$

$= (\hat{\sigma}^2)^2 / \{[(MS_{D/A})^2 + (b-1)(MS_E)^2] / ab^2(r-1)\}$

MS_E = residual error

$MS_{D/A}$ = animals/Trt

a = number or Trts b = number of time periods

r = number of animals per treatment

Critical value for Tukey's Test found in Table A.8 (Gill, 1978)

B. Test of periods within treatments

Scheffe's Test

$MSD = \pm\{(b-1)\ f_{\alpha,\ b-1,\ a(r-1)\ b-1}\ [V_{\bar{q}_{i,K}}]\}^{0.5}$

- a, b, r, MS_E as defined in 1.

$V = [2(MS_E)] / r$

Critical value for Scheffe's Test found in Table A.5 (Gill, 1978)

C. Test of prerigor versus postrigor differences.

Student's t test

Confidence Interval (CI) = $(\hat{\sigma}^2/r)^{0.5}(t_{\alpha/2},\ \hat{v}_E)$

If CI includes 0, prerigor and postrigor are not different at $(1 - \alpha)$ 100% confidence.

Critical value for T Test found
in Table A.4 (Gill, 1978).

D. Test of linear, quadratic and cubic responses

Orthogonal Polynomial Contrasts

Test Statistic = $t = \psi_K / \sqrt{(\sum_{i=1}^{t} r_i C_{iK}^2) MS_E}$

Critical Value = $t_{\alpha/2}$, n-T

Critical value for T Test found
in Table A.4 (Gill, 1978)

Where $\psi_k = C_{1K} Y_1 + C_{2k} Y_2 + C_{3k} Y_3 + C_{4k} Y_4 + C_{5k} Y_5$

C_{1k}, C_{2k}, C_{3k}, C_{4k}, C_{5k} = Contrast Coefficients

Y_1, Y_2, Y_3, Y_4, Y_5 = Sum of 3 animals pH values within a specific treatment and period

r = number of replicates per group

Coefficients[a] of treatments totals for linear, quadratic and cubic responses.

Contrast	C_{1k}	C_{2k}	C_{3k}	C_{4k}	C_{5k}
L	-0.474342	-0.316228	-0.158114	0.1581139	0.7905694
Q	0.5459642	0.0352235	-0.334623	-0.651635	0.4050702
C	-0.469987	0.4222581	0.514346	-0.57050	0.10388

L = linear, Q = quadratic, C = cubic

[a] Generated by Michigan State University's Statistic Analysis System.

E. Statistical Model

Response = T + B + TB + P + TP + BP + TBP
T = fixed treatment effect
B = random block effect
TB = Block * treatment interaction (Error 1)
P = fixed time effect
TP = treatment * period interaction
BP = Block * period interaction
TBP = Treatment * block * period interaction

Data were analyzed by Michigan State University's Statistical Analysis System.

Appendix 2

Analysis of Variance tables: Experiment 1.

A. Analysis of Variance: pH postrigor

Source	DF	SS	F Value	Sign.
TRT	4	0.013320	0.22	NS
BLOCK	2	0.009613	0.31	NS
BLOCK*TRT	8	0.123387		
TIME	2	0.001213	2.79	P<0.10
TRT*TIME	8	0.003053	1.75	P<0.16
BLOCK*TIME	4	0.004653	5.35	P<O.01
BLOCK*TRT*TIME	16	0.003480		

B. Analysis of Variance: pH prerigor

Source	DF	SS	F Value	Sign.
TRT	4	2.576234	45.33	P<0.0001
BLOCK	2	0.091486	3.22	P<0.10
BLOCK*TRT	8	0.113654		
TIME	8	8.505241	952.63	P<0.0001
TRT*TIME	32	0.480166	13.45	P<0.0001
BLOCK*TIME	16	0.112234	6.29	P<0.0001
BLOCK*TRT*TIME	16	0.003480		

C. Analysis of Variance: WHC Postrigor

Source	DF	SS	F Value	Sign.
TRT	4	82.396444	3.27	P<0.08
BLOCK	2	39.376444	3.12	P<0.10
BLOCK*TRT	8	50.463555		
TIME	2	2.149778	1.47	NS
TRT*TIME	8	25.890222	4.44	P<0.006
BLOCK*TIME	4	2.264889	0.78	NS
BLOCK*TRT*TIME	16	11.661778		

D. Analysis of Variance: WHC Prerigor

Source	DF	SS	F Value	Sign.
TRT	4	3008.6783	40.82	P<0.0001
BLOCK	2	60.4052	1.64	NS
BLOCK*TRT	8	147.4032		
TIME	7	480.9619	56.13	P<0.0001
TRT*TIME	28	139.6510	4.07	P<0.0001
BLOCK*TIME	14	30.8228	1.80	P<0.07
BLOCK*TRT*TIME	16	68.5555		

E. Analysis of Variance: EP Postrigor

Source	DF	SS	F Value	Sign.
TRT	4	23.244502	1.06	NS
BLOCK	2	18.052884	1.64	NS
BLOCK*TRT	8	43.995671		
TIME	2	1.048964	0.62	NS
TRT*TIME	8	7.930257	1.18	NS
BLOCK*TIME	4	2.751502	0.82	NS
BLOCK*TRT*TIME	16	13.437809		

F. Analysis of Variance: EP Prerigor

Source	DF	SS	F Value	Sign.
TRT	4	7225.9558	21.61	P<0.0002
BLOCK	2	306.0500	1.83	P<0.23
BLOCK*TRT	8	668.8295		
TIME	7	1145.0557	24.44	P<0.0001
TRT*TIME	28	1099.8206	5.87	P<0.0001
BLOCK*TIME	14	165.7421	1.77	P<0.07
BLOCK*TRT*TIME	16	374.8015		

G. Analysis of Variance: pH prerigor - postrigor

Source	DF	SS	F Value	Sign.
TRT	4	2.684884	11.43	P<0.003
BLOCK	2	0.094173	0.80	NS
BLOCK*TRT	8	0.469716		
TIME	8	8.468133	888.21	P<0.0001
TRT*TIME	32	0.476089	12.48	P<0.0001
BLOCK*TIME	16	0.117773	6.18	P<0.0001
BLOCK*TRT*TIME	16	0.076271		

H. Analysis of Variance: WHC prerigor - postrigor

Source	DF	SS	F Value	Sign.
TRT	4	2136.7264	11.90	P<0.002
BLOCK	2	8.9878	0.10	NS
BLOCK*TRT	8	359.0043		
TIME	7	481.7549	67.46	P<0.0001
TRT*TIME	28	154.6180	5.41	P<0.0001
BLOCK*TIME	14	30.6713	2.15	P<0.03
BLOCK*TRT*TIME	16	56.1072		

I. Analysis of Variance: EP prerigor - postrigor

Source	DF	SS	F Value	Sign.
TRT	4	6675.3837	14.32	P<0.001
BLOCK	2	115.8874	0.50	NS
BLOCK*TRT	8	932.5846		
TIME	7	1110.6654	24.21	P<0.0001
TRT*TIME	28	1083.3806	5.90	P<0.0001
BLOCK*TIME	14	161.9297	1.77	P<0.07
BLOCK*TRT*TIME	16	366.9709		

Appendix 3

NaCl added to attain 1.0 M NaCl in protein extraction procedure:

Treatment	NaCl in 5 gm of Tissue(g)	NaCl added[a] in 20 ml Extraction slurry(g)	Molarity of added NaCl solution
0% NaCl	0	1.4124	1.2083
0.5% NaCl	0.025	1.3874	1.1869
1.0% NaCl	0.050	1.3624	1.1655
2.0% NaCl	0.100	1.3124	1.1267
4.0% NaCl	0.200	1.2124	1.0372

[a] Calculation based on meat homogenate containing 83.3% water.

Appendix 4

Statistical formulas used in Experiment 2

A. Tests of treatments within periods and periods within treatment are the same as used in experiment 1, formulas can be found in Appendix 1.A and 1.B, respectively.

B. Statistical Model

Responce = P + S + PS + E1 + T + PT + ST + PST + E2

P = Phosphate fixed effect

S = Salt fixed effect

PS = Phosphate x Salt interaction

E1 = Error 1

T = Fixed time effect

PT = Phosphate x Time interaction

ST = Salt x Time interaction

PST = Phosphate x Salt x Time interaction

E2 = Residual error

Appendix 5

Analysis of Variance tables: Experiment 2.

A. Analysis of Variance: pH postrigor

Source	DF	SS	F Value	Sign.
PHOS	2	4.536365	59.38	P<0.0001
SALT	2	0.124980	1.63	P<0.25
PHOS*SALT	4	0.141649	0.93	NS
ERROR 1	18	0.687667		
TIME	2	0.184299	57.13	P<0.0001
PHOS*TIME	4	0.055530	8.61	P<0.0001
SALT*TIME	4	0.004916	0.76	NS
PHOS*SALT*TIME	8	0.010854	0.84	NS
RESIDUAL ERROR	36	0.058067		

B. Analysis of Variance: pH prerigor

Source	DF	SS	F Value	Sign.
PHOS	2	1.027158	10.22	P<0.005
SALT	2	0.217918	2.16	P<0.25
PHOS*SALT	4	0.330983	1.64	P<0.25
ERROR 1	18	0.904770		
TIME	8	35.646356	749.14	P<0.0001
PHOS*TIME	16	2.180583	22.91	P<0.0001
SALT*TIME	16	0.205311	2.16	P<0.01
PHOS*SALT*TIME	32	0.366521	1.93	P<0.01
RESIDUAL ERROR	144	0.856496		

C. Analysis of Variance: WHC postrigor

Source	DF	SS	F Value	Sign.
PHOS	2	319.3736	9.02	P<0.005
SALT	2	13.7770	0.39	NS
PHOS*SALT	4	36.8108	0.52	NS
ERROR 1	18	32.7205		
TIME	2	8.3744	4.61	P<0.02
PHOS*TIME	4	4.9020	1.35	NS
SALT*TIME	4	2.0760	0.57	NS
PHOS*SALT*TIME	8	23.7837	3.27	P<0.01
RESIDUAL ERROR	36	32.7205		

D. Analysis of Variance: WHC prerigor

Source	DF	SS	F Value	Sign.
PHOS	2	691.465	8.79	P<0.005
SALT	2	359.020	4.57	P<0.025
PHOS*SALT	4	160.147	1.02	NS
ERROR 1	18	707.623		
TIME	7	2955.573	141.20	P<0.0001
PHOS*TIME	14	1149.573	27.46	P<0.0001
SALT*TIME	14	61.420	1.47	P<0.014
PHOS*SALT*TIME	28	142.869	1.71	P<0.03
RESIDUAL ERROR	126	376.778		

E. Analysis of Variance: EP postrigor

Source	DF	SS	F Value	Sign.
PHOS	2	1303.250	37.83	P<0.0001
SALT	2	0.017	0.00	NS
PHOS*SALT	4	61.306	0.89	NS
ERROR 1	18	119.899		
TIME	2	334.999	29.58	P<0.0001
PHOS*TIME	4	217.810	9.61	P<0.0001
SALT*TIME	4	16.007	0.71	NS
PHOS*SALT*TIME	8	15.959	0.35	NS
RESIDUAL ERROR	36	203.880		

F. Analysis of Variance: EP prerigor

Source	DF	SS	F Value	Sign.
PHOS	2	563.924	7.91	P<0.005
SALT	2	1557.409	21.86	P<0.0001
PHOS*SALT	4	273.235	1.92	P<0.25
ERROR 1	18	641.317		
TIME	7	7271.786	99.11	P<0.0001
PHOS*TIME	14	1348.192	9.19	P<0.0001
SALT*TIME	14	322.847	2.20	P<0.02
PHOS*SALT*TIME	8	348.220	1.19	NS
RESIDUAL ERROR	126	1320.722		

Appendix 6

Statistical test used in experiment 3

Paired T Test

$T = \bar{Y}_D / (S_D/\sqrt{r})$

$\bar{Y}_D$ = Average difference between prerigor and postrigor DPM

S_D = Standard deviation of the difference values

r = numbers of paired comparisons

Critical value found in Table A.4 (Gill, 1978)