ASSESSMENT OF ULTRASONIC CAVITATION, BRINE TEMPERATURES, AND TUMBLING ON THE QUALITY ATTRIBUTES OF PORK MUSCLE IN SECTIONED AND FORMED HAMS

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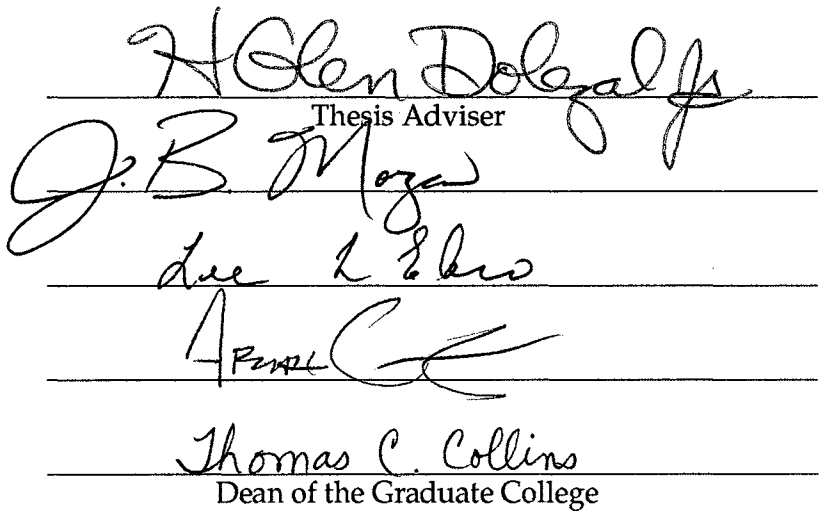

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DEDICATION

This dissertation is dedicated to Mr. Lindsey Weatherspoon, my mentor and friend who enlightened me about a career in agriculture. Thank you for your leadership and high standards toward the pursuit of excellence. Your contributions as an educator and researcher were most rewarding in my life.

May God Bless You Always.

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INTRODUCTION

Major economic considerations have been made by the livestock and meat industries to limit the amount of fat deposition in cattle and pigs, and the total fat content in case-ready (fresh) and value-added (processed) meats. The livestock industry is focusing on producing animals by selection (breeding) and nutrition (feeding management) to accommodate the purveyor and consumer needs for leaner meat. Adipose tissue is an essential component to cattle and pigs because it serves to store energy, protects against heat loss, gives mechanical support, and aids to responsive hormonal and nervous stimulation. Allen et al. (1976) suggested about 10 to 15% of the live weight of a domestic animal is important for biological functions and acceptability to consumers. Reducing the fat content in animals, especially pigs, has partly contributed in part to the increased percentage of porcine stress syndrome (PSS). A trait that is directly linked to muscle development in pigs that are susceptible to stress which results in pigs developing pale, soft, and exudative (PSE) meat after slaughter (Kauffman et al., 1992; Goodwin, 1994).

Sayre et al. (1964) and McLoughlin (1963) reported that PSE reduces protein solubility in pork muscle. Meat protein solubility has a direct relationship to the rate of pH decline in muscle prior to rigor mortis. When muscle is PSE, protein solubility levels are marginal and will adversely affect water-holding capacity (WHC), color, yield, and texture in meat products. In addition, the binding characteristics of salt-soluble proteins through physical and ionic extraction are lowered, thus decreasing the moistness and texture of sausages and ham products.

Research using hot brine and ultrasonic cavitation to enhance protein extraction in meat systems is limited and descriptions of the physicochemical properties at different pH levels have not been fully investigated. The objectives of this study were: (1) to assess the influence of hot brine on PSE and normal pork muscle in sectioned and formed hams; and (2) to determine the effects of ultrasonic cavitation on the binding strength of pork muscle in cooked hams.

CHAPTER II

REVIEW OF LITERATURE

Muscle Quality Attributes

Fat

The relationship between diet and health has received considerable attention in recent years. Part of the reason for this has been the growing concern over diet's role in health and disease, fostered by the hypothesis originating in the 1950's that animal fats in the diet (as cholesterol and/or saturated fats) might contribute to heart disease and cancer (Thomas, 1983). The question arises whether saturated fat intake, total fat intake and cholesterol intake, or a combination of these three causes an increase of atherosclerosia and must be answered to ensure that the health significance of such consumption is known by an informed public. Atherosclerosia is a thickening and loss of elasticity of the inner walls of arteries where the accumulation of cholesterol develops plaque and blocks the blood flow from or beyond the heart. This blockage can lead to a myocardial infarction that often leads to death.

The American public has indicated that they have health concerns about consuming fat and cholesterol from animal tissue. However, information is not always conveyed properly about the facts concerning the linkage between diet and health. Yankelovich et al. (1985) reported that as many as nine out of ten consumers indicated concern about fat consumption. Changes in life styles and health concerns have resulted in decreased red meat consumption in America because of concern over animal fat and cholesterol in the diet and their

contribution to coronary heart disease. The average daily U.S. consumption on a per capita basis is about 4 oz of cooked red meat, supplying 93 mg of cholesterol, which is less than one-third of the 300 mg standard (Williams, 1987). Total fat from red meat supplies 225 calories in the diet, or about 11% of calories of a 2000 calorie diet.

The demand for lower animal fats in the diet has caused the meat industry to reduce the fat content (deposition) in livestock with more muscle development and leaner carcass characteristics. Consequently, the pork industry is focusing on producing animals by selection (breeding) and nutrition to satisfy the purveyor and consumer needs for leaner meat. Since approximately 65% of the carcass weight for pork in the U.S. is converted into processed meats compared to 12% of the beef and 15% of lamb (The National Livestock and Meat Board, 1982), it is important for the pork industry to lower the fat content in pigs. In the 1960's, the lean:fat ratio in hogs was about 1:1 and currently a minimum of 1:0.5 is being obtained. However, this reduction in fat content of hogs may cause the animals to be more susceptible to stress, which results in hogs developing pale, soft and exudative (PSE) meat after slaughter.

Pale, Soft, and Exudative (PSE)

Muscle quality as well as quantity of muscle is an important prerequisite for improved consumer acceptance of pork. Currently, the pork industry is producing fresh pork that varies in quality with respect to visual appearance (color), shrinkage (drip loss), and protein functionality when converting muscle into processed meats. Kauffman et al. (1994) conducted a survey that indicated at least one-quarter of all pork produce was undesirable (16% PSE and 10% DFD), and only 16% was classified as ideal by the National Pork Producers Council (NPPC) standards. Some of these adverse quality characteristics in pork can be attributed to the genetic penetrance of the halothane or HAL locus and ryanodine receptor gene, which have been localized to pig chromosome 6 p11-9 21 (Fujii et al., 1991). The HAL

gene reduces backfat and muscle quality, while increasing loin muscle size and percent lean of the carcass. In addition, this gene is associated with malignant hyperthermia (MH) which triggers a reaction in the pig by inhalation of anesthetics or stress that causes an uncontrolled increase in glycogenolysis and heat production (Denborough and Lovell, 1960; Berman et al., 1970; Hall et al., 1980; Lister, 1987). In MH, the pig develops metabolic acidosis which is associated with porcine stress syndrome (PSS) that causes sudden death and the production of PSE pork (Briskey, 1964; Cassens et al., 1975; Goodwin, 1994).

The HAL gene can produce the same effects on muscle quality in all breeds. There are three possible HAL genotypes in pigs that determine carcass attributes relative to heritability, measurements of the proportion of the traits' variation due to genetics. Pigs will receive one halothane gene from each parent, thereby producing a progeny of NN (normal), Nn (heterozygotes), and nn (mutant) pigs. According to Goodwin (1994), heterozygote pigs produce more loin muscle area (0.38 sq in.) and higher dressing percentage (0.5%) along with paler color, increased toughness and increased cooking loss compared to the normal HAL genotype pigs. However, mutant (nn) pigs are more sensitive to sudden death or PSS and up to 90% will produce a PSE carcass. Halothane gene heterozygotus and mutant pigs will consistently produce lower quality pork than pigs that are free of the halothane gene (NN).

Ante-mortem treatment prior to slaughter also contributes to the variation in pork muscle quality. Pigs can be stressed during transportation, temperature fluctuations, and inappropriate handling prior to slaughter. These environmental stressors before death can alter the change in the pig's metabolic rate after death. Once the animal is exsanguinated(the removal of blood), ATP is still used for energy-consuming sequences but all respiration is eliminated by anaerobic processes and the neutral and hormonal controls are diminished. Bendall (1964) reported that ATP and creatine phosphate concentration are maintained in slow

working muscles by oxidative phosphorylation, but in fast glycolysing muscles where oxygen supply is restricted, ATP is synthesized by the transfer of high energy phosphate from creatine phosphate (CP) to ADP. When CP is limited, attempts to maintain ATP level occur anaerobically via the glycolytic pathway, breakdown of muscle glycogen to lactate and hydrogen ions (Lawrie, 1966; Bendall, 1960). The fast onset of rigor mortis and an extremely rapid rate of glycolysis with the development of low pH values (<5.8) at temperatures above 35°C are associated with the production of PSE pork. Conversely, a slow glycolytic rate or when rigor mortis occurs at a high pH (>6.4) and/or a low temperature, results in muscle that has a tendency to be dark, firm, and dry (DFD). Therefore, glycogen concentration level in muscle immediately prior to death will determine post-mortem chemical and physical properties of the muscle under certain conditions: (1) that glycogen is available for degradation (Lawrie, 1966) which may involve the type of chain length structure; and (2) when the enzyme function is inhibited through the accumulation of lactate by a rapid decline in pH (Bate-Smith and Bendall, 1949).

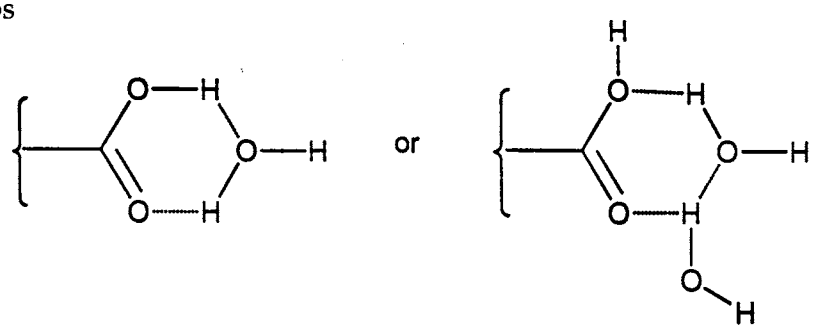
Water-Holding Capacity (WHC)

The rate of glycolysis in muscle will determine the overall quality characteristics of meat systems. Muscle pH, the negative logarithm of $[H^+]$, if above 6.4 and lower than 5.8, changes the WHC for meat systems by altering the positive and negative charges on myofibrillar proteins. Forrest et al. (1975) defined WHC as the ability of meat to retain water during the application of external pressure involved in further processing. Many of the physical properties of meat, including color, texture, and juiciness, are partially dependent on WHC. Water plays an important role in fresh and cooked meat products because of its interaction with proteins, fat, nonmeat ingredients, and heat processing factors. WHC strongly influences the yield of the finished product (Randall et al., 1976). To achieve product stability, whether the muscle will be used for case ready display (fresh meat) or converted into value-added products, the production aspect for

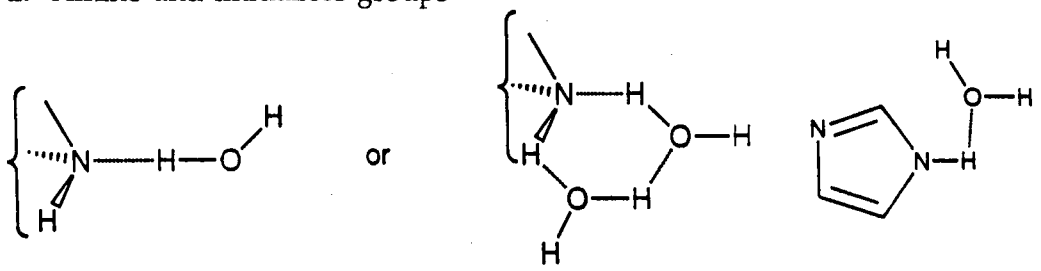
water content and yield requires good water binding capacity (WBC) of free and immobilized water.

According to Hamm (1960) the chemical basis of WHC involves three types of water binding in muscle: type I (bound), type II (immobilized), and type III (free). Bound water is that directly associated with the reactive groups on myofibrillar proteins creating a strong molecular bond. Polar, hydrophilic side chains and undissociate carboxyl and amino groups of peptide bonds are responsible for holding this water tightly. Water in this fraction represents approximately 5% of the total water and can be removed only under extreme dry conditions. The remaining water in muscle can be either immobilized or free depending on the conditioning and handling of the tissue. Molecular immobilized water is a form of structure that has no specific orientation toward reactive charged groups. This type II water is influenced by the spatial structure of the muscle tissue (Hamm, 1960). Free water is not bound in mono- and multi-molecular layers like type I, but exists in a meat system as immobilized water held by surface tension or capillary forces. It can be removed easily by drip loss, drying, or mechanical pressure. Hamm (1960) believed that immobilized and free water share a continuous transition between types II and III in muscle tissue influenced by pH, ionic strength, and rigor factors, which will alter the structural integrity of myofibrillar proteins. Ninety-five percent of free muscle water is immobilized by electrostatic forces and capillary action (Offer and Trinich, 1983; Offer et al., 1984). Sponsler et al. (1940) described several chemical working models of hydrophilic reactive protein groups in muscle that are used in binding water:

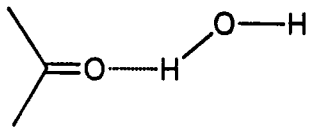
1. Carboxyl groups



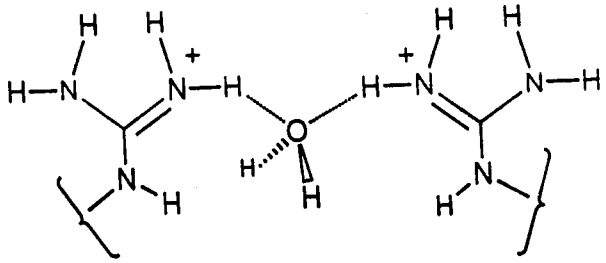
2. Amino and imidazole groups



3. Carbonyl groups



4. Guanidine groups



These side chain chemical structures of muscle molecules will bind water—usually with hydrogen bonding involving polar groups—due to the dipolar character of water because the arrangement of the oxygen (negative charge) and hydrogen (positive charge) are not coinciding. Therefore, electrons are drawn toward the element with the greatest electronegativity (O) which causes a net polarity of the bonds in a molecule. Polar groups contribute to the binding of water in meat systems that involving muscle hydration, where the amount of water binding proteins are by mono- and multi-molecular adsorption (Hamm, 1960).

pH--Influence on WHC

The pH affects meat hydration by influencing the number of reactive groups on proteins and their availability to bind water. Post-mortem changes in muscle

involving the production of lactic acid, loss of ATP, and pH decline prior to rigor mortis along with cell structure changes will determine the level of water binding capacity in meat. The formation of lactic acid with subsequent pH decline prior to rigor mortis will cause the protein reactive groups to decrease. This change causes protein denaturation and loss of solubility in meat (Miller et al., 1968; Forrest et al., 1975). Protein reactive groups that are reduced will have the tendency to approach the isoelectric point (pI). The pI is the pH at which the positive and negative charge groups on proteins are equal to zero which is known as the net charge effect. Net charges on proteins at zero result in the formation of a maximum number of salt bridges between protein chains. Once the pH approaches the pI (5.0) in a meat system, the reactive groups begin to attract each other and the remaining protein groups are available for binding water. Consequently, WHC will be decreased in meat systems.

All changes in meat WHC are not necessarily attributed to the net charge of proteins (Miller et al., 1968). Steric conditions that are associated with the breakdown of ATP, protein interactions and rigor mortis involve the attachment of Ca^{2+} and Mg^{2+} ions to protein reactive groups which limits the availability of other reactive sites from binding water. These divalent cations tend to pull protein charge groups together, causing steric hinderances—a lack of space for free water to be converted into immobilized water. Swift and Berman (1959) revealed that an increase in WHC involves the transition of free water to immobilized water and is directly related to pH values. Raw material pH values usually range from 5.8 to 6.4 within 48 hr post-mortem for optimal utilization in cooked ham products. Meats with a pH below 5.8 or higher than 6.4 are not to be recommended for the following reasons (Kreibig, 1991):

High pH-value (Over 6.4)	=	good water absorption, low curing salt absorption, mild in salt flavor, less stable cured color, and reduced shelf-life, especially when packed.

Low pH-value (Under 5.8)	=	poor water absorption, intensive curing salt absorption, strong salt flavor, good curing color, and better shelf-life (meat and flavor).

Protein-protein interactions for WHC and swelling of myofibrillar proteins are affected by the pH level. The addition of acid (negative) groups or base (positive) groups can cause alteration in protein net charge by increasing the water binding through enlargement of interfilament spacing (April et al., 1972; Hamm, 1986). For the alkaline pH range, the hydrating effect of the anion is more pronounced than the hydrating effect of the cation because of the ion bound strength to the protein structure.

pH--Influence on Solubility

Pre- and post-rigor processes (Bate-Smith and Bendall, 1947; Cassens et al., 1963; Sayre et al., 1963) and the rate of post-mortem pH decline (Marsh, 1954; Bendall, 1960; Scopes, 1963) are all associated with changing (increasing or lowering) the characteristics of myofibrillar and sarcoplasmic proteins in muscle. The physiological and chemical condition of myofibrillar proteins in muscle prior to further processing alters the level of protein functionality (Camou and Sebranek, 1991). When the glycolytic rate in porcine tissue is accelerated with rapid pH fall prior to rigor mortis, this causes a high temperature in the muscle which leads to substantial protein denaturation. Therefore, the protein solubility is decreased significantly (Sayre and Briskey, 1963). McLoughlin (1963) indicated that as pH at 45 min post-mortem decreased, solubility also decreased. In contrast, a slow glycolytic rate of high pH causes the muscle to be DFD with increased protein solubility traits (Hamm, 1986). Muscle protein solubility and protein extractability in raw muscle can be augmented by the conditions of both temperature and pH, and that protein solubility is an essential factor affecting WHC.

Protein functionality is the ability of solubilized myofibrillar protein to emulsify fat, bind water and stabilize protein-protein interactions during thermal

processing. Saffle (1968) and Hansen (1960) explained that the fundamental structure of a meat emulsion was a mixture of finely chopped particles involving a dispersed (fat-in-water) phase into a continuous phase of solubilized proteins. The salt-soluble, heat-coagulated proteins create an elaborate protein matrix around the fat globules along with the entrapment of water, causing the reactive groups to attach to the negative and positive charged groups' interface (Becher, 1965; Schmidt, 1986). Heating this protein-fat matrix system stabilizes the emulsion and thus gives the product its overall characteristic juiciness and texture.

Schmidt (1986) suggested that emulsification is similar to the binding of meat chunks in luncheon hams. He believed that the major difference is that the emulsion sausages have no large chunks of meat. The binding between chunks involves the structural rearrangement of solubilized myofibrillar proteins, mainly myosin that binds meat pieces. Siegel and Schimidt (1979) reported that the interaction of myofibrillar proteins between the super thick synthetic filaments and heavy myofilaments extracted from the intact muscle causes meat pieces to bind in sectioned and formed products.

pH--Influence on Color

A low pH value in porcine tissue causes the muscle fibrils to open and scatter light which results in a paler appearance in color (Walters, 1975). Lister (1987) reported that in stressed pork the soluble proteins which are precipitated onto the structural proteins interfere with the optical properties of the surface layers and cause a decrease in the translucency of meat. Pork contains the lowest concentration of myogloblin compared to lamb and beef. Once the rapid pH decline occurs in pork muscle, myoglobin becomes easily oxidized into metamyoglobin which reflects a low color intensity (Walters, 1975).

Tumbling--Protein Extraction

Prior to tumbling, meat is usually injected with a liquid brine consisting of water, salt, phosphate, nitrite, and ascorbate. The water mainly acts in three different ways during the tumbling process: (1) as a medium to dissolve the ingredients; (2) as a distribution carrier for additives and actomyosin after extraction; and (3) as the compensator for cooking loss. Salt and phosphates are ingredients used to solubilize the myofibrillar proteins by increasing the ionic strength of the meat system. This enhances electrostatic repulsions between similar chemical charged groups on the filaments, causing swelling in the muscle (Wierbicki et al., 1957; Hamm, 1960). Actomyosin, the main structural component of muscle, becomes dissociated into actin and myosin for the purpose of binding water, fat and meat pieces. Nitrite is used for color development and as cure bacteriostat. Cure accelerators, sodium ascorbate or erythorbate, are used for speeding up and stabilizing the color development for ham products.

Tumbling is a process in which the meat, injected with pickle is placed in a container and intermittently or continuously struck by paddles or baffles through rotation. The result is a transfer of kinetic energy into the muscle that alters, splits, or bursts the fibril muscle, the actomyosin of meat. This disruption of the actomyosin causes a release of salt-soluble proteins, which in turn coats the meat chunks and is then heat coagulated by cooking. Tumbling muscle pieces enhances the extraction of myofibrillar proteins to adequately bind particles in chunked and formed products (Ockerman et al., 1978; Kreibig, 1991; Schmidt, 1986). The effects of tumbling and sodium tripolyphosphate on salt and nitrite distribution in porcine muscle was investigated by Krause et al. (1978a). Their results revealed that both sodium tripolyphosphate and tumbling significantly increased the migration of salt and nitrite, and increased cured color development. Krause et al. (1978b) showed that tumbled hams were rated higher by a sensory panel than nontumbled hams for external appearance, internal color, sliceability, taste, and aroma.

Ultrasonic Cavitation

In 1894, John I. Thornycroft and Sydney W. Barnaby noticed a severe vibration on the British destroyer's propeller. These vibrations of propeller blades caused serious erosion over time. They discovered that large bubbles, gas filled cavities, formed by the spinning propeller will implode by water pressure. This was the source of the vibrations. Thornycroft and Barnaby redesigned the ship's propeller to reduce vibration from what came to be known as cavitation (Suslick, 1989). Cavitation is the term used to describe the formation and collapse of bubbles or cavities in liquids. Cavitation tends to occur in liquids by the passage of ultrasonic waves. The ultrasonic waves are propagated through a liquid medium and the particles of the medium oscillate back and forth. These oscillations cause regions of compression and rarefaction to form, which correspond to positive and negative pressures (Reynolds, 1977). Ultrasound waves consist of cycles of compression and expansion. Depending on the pressure and forces holding the liquid together, air or vapor bubbles and cavities can be formed at sites of negative pressure (Reynolds et al., 1978). The reduced pressure makes the gas in the crevice expand, resulting in implosion in the liquid.

Presently, ultrasound is applied in the agricultural and medical fields for visual imaging and in industry for welding and cleaning materials. Ultrasonic cavitation has received limited research in the meat processing area. Ultrasound has been shown to influence protein extraction and cellular disruption. Wang (1975) revealed that autoclaved soybean flakes produced a 90% protein yield by ultrasound extraction of protein compared to the 70% protein yield using a conventional stir method (control treatment). Reynolds et al. (1978) reported an increase in binding strength in cured ham rolls using ultrasonic treatment. His research demonstrated changes in the micro-structure of muscle using an ultrasonic instrument cleaner. Zayas (1985), using a sonic hydrodynamic unit and mechanical homogenizer, demonstrated that ultrasonic waves can increase the

WHC and yield for sausage emulsions. Some studies have shown that ultrasonic cavitation is useful for bacteriocidal effects when organisms were treated while suspended in a culture medium (Stumpf et al., 1946; Davies, 1959). Most production failures are attributed to low pH meats and marginal protein extraction in raw material for manufacturing sausages and chunk and formed ham products. Ultrasonic cavitation under certain conditions offers the potential to increase yield and binding strength for meat products.

CHAPTER III

INFLUENCE OF BRINE TEMPERATURES AND TUMBLING ON PSE AND NORMAL PORK MUSCLE IN SECTIONED AND FORMED HAMS

Introduction

Consumer diet and health concerns have caused the meat industry to seek changes at every phase of livestock production. The pork industry has focused on producing animals by selection (breeding) and nutrition to satisfy consumer demands for leaner carcasses. The lean:fat ratio in hogs was 1:1 approximately 35 years ago; currently a minimum of 1:0.5 is being obtained. However, this reduction in fat content of hogs may cause the animals to be more susceptible to stress, which results in hogs developing pale, soft , and exudative (PSE) meat after slaughter. Another contribution to the cause of PSE characteristics in muscle is the animal's genetic susceptibility of the halothane (or HAL) gene which was identified by Fujii et al. (1991). This particular gene is responsible for the animal becoming easily stressed by extrinsic factors involving environmental temperature fluctuations and ante-mortem handling of pigs prior to slaughter. If a pig has the HAL gene, it is more prone to having fast glycolysing muscle (accelerated breakdown of glycogen and a simultaneous accumulation of hexose monophosphates) prior to slaughter, which causes a swift decline in muscle pH. A rapid pH drop below 5.8 results in PSE which causes the proteins to become partially denatured and unacceptable for cooked hams. This form of muscle degeneration will affect protein solubility of pork muscle in the conversion to processed meats. Protein solubility is the

essential component in the manufacturing of sectioned and formed meats. Consequently, some processors believe it is important to know the color and pH values of pork meat.

According to Kreibig (1991), pH value is the standard measurement for establishing the selection of raw material for cooked ham products. Hoogenkamp (1989) reported that raw hams at pH 5.7 to 5.8 (borderline hams) can cause problems similar to PSE traits and that 30 to 40% of all raw hams processed have some PSE properties. Few meat processors are capable of establishing pH selection criteria for raw materials for cooked ham processing. The majority of processors generally accepts what is available to them from the open market. The pH value determines the water binding, color properties and microbial shelf life of the end product. Normally, primal sections (hams and loins) are not entirely saturated by PSE properties, but the protein functionality may be lowered. Kauffman et al. (1992) revealed that reddish, soft. exudative (RSE) pork may be of questionable quality because of the wide variation in appearance, shrinkage, and protein functionality from market hogs. The extended quality variation in pork is not always relegated to pale color but involves texture and water-holding capacity (WHC). Offer and Knight (1989) reported that WHC of meat is determined by pH, protein denaturation, intra- and interfascicular spacing and sarcomere length. To insure consistent final products, the meat processor needs alternative procedures to maximize quality attributes of meat sources with pH values ranging from 5.4 to 6.0 without separating acidic meat based on color and pH before or during production .

Historically, research efforts with PSE pork have focused on three general areas: (1) the reversal of protein denaturation by enzymatic reactions (chemical modifica-tion), (2) the incorporation of time and temperature relationships after slaughter, chilling carcasses at extremely cold temperatures to diminish the PSE properties, and (3) the process of breeding, feeding, and preslaughter treatments to control final pH values of carcasses. These procedures are beneficial to the meat

industry, but more research is needed in solving the PSE condition at the point of product manufacture. Research using hot brine (curing) to enhance the extraction of salt soluble proteins from pork meat systems is limited and descriptions of the physicochemical properties at different temperature levels have not been fully investigated. Johnson and Bull (1952) compared a conventional method of processing cured bacon at 6°C for 21 days to a 55° pickle at 45°C for 24 hr. They demonstrated that the bacon treated with hot brine (45°C) was no different in cured color and palatability from the dry cured treatment. Also, an injection of brine into prerigor carcasses will improve color, juiciness and tenderness in hams (Mullins, 1957). In processing pork, there is an interrelationship between pH, WHC, temperature, and protein functionality in muscle systems that influences the quality characteristics of ham products. The purpose of this study was to determine the influence of hot brine temperatures on PSE and normal pork muscles in manufacturing luncheon hams.

Materials and Methods

Carcass Measurements

Twenty-eight (95 to 107 kg) market gilts were slaughtered at the Oklahoma State University Meat Science Laboratory. All right sides of carcasses (pre-rigor) were subjected to thermal processing for 4 hr in an Alkar-DEC oven to an internal temperature of 37.7°C at 95% RH to induce pale, soft, and exudative (PSE) pork (McKeith, 1995). After processing, temperature and pH vlaues were measured with a Sentron® model 2001 electrode probe (Sentron® Integrated and Sensor Technology Co., Federal Way, WA) on ham and loin sections of pork sides. These sides were subsequenlty chilled for 24 hr at 4°C along with the left side (control) carcasses. Colormetric values (L, a, and b) were taken on the longissimus dorsi muscle between the tenth and eleventh rib interface using a Minolta Chroma Meter CR-300 (Minolta Camera Co., Ltd., Ramsey, NJ). Water holding capacity (WHC) values were determined according to a modified procedure of Hamm

(1986). Duplicate 30 g portions of each raw sample were sealed in plastic bags under atmospheric pressure and stored at 4°C for 24 hrs. Then samples were weighed to calculate drip loss.

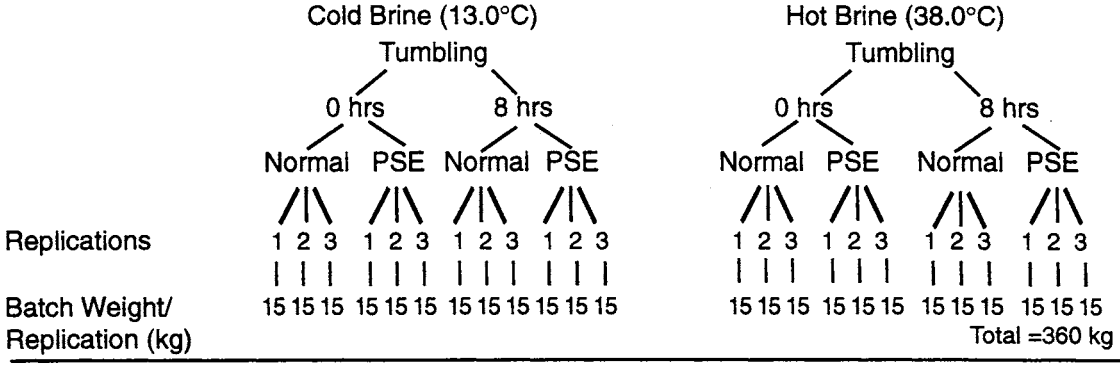
Nuclear Magnetic Resonance (NMR)

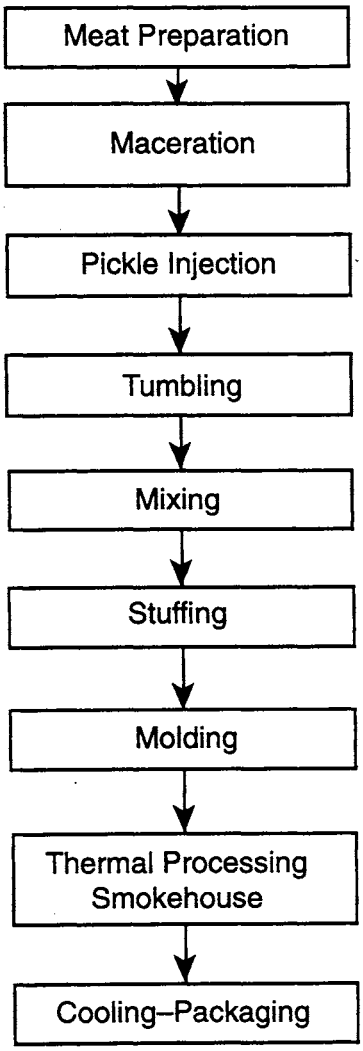
This quantitative method was used to assess the possibility of following the metabolic events connected to normal and thermal induced PSE pork and to categorize the quality muscle type. Four pigs were stunned, exsanguinated, and the M. longissmus dorsi (LD) muscle from the control and treated PSE carcasses was removed at 0 and 4 hr , respectively. The muscle extraction procedures for nucleotide determination were conducted according to Calkins et al. (1982). Extracts prepared for biochemical analysis were diluted with H_2O to an average pH of 8.5. Phosphorus -31 NMR spectra of muscle extraction was recorded at 161.9 MHz on a Varian XL-400 NMR Spectrometer (Palo Alto, CA). Chemical shifts for ^{31}P-NMR were reported in ppm from 85% H_3PO_4 (0 ppm) as a reference with upfield shifts given as negative signs. A 5 mm probe was used with spectra width 100,000, scans 10,000, and delay 0.5 s to a recycle time of 0.66 s, and all spectra were acquired at -15°C with a total time of 110 min per spectrum. Energy metabolites were determined by peak enhancement of each component with muscle extraction.

Manufacturing Procedures

Pork carcasses were segmented into lean primal cuts. Loins and hams were selected and were subsequently trimmed to remove all skin, subcutaneous fat, and connective tissue. Manufacturing procedures (Figure 1) involved triplicate batches for each treatment. Boneless hams and loins were macerated and injected (Fomaco®) at 20% with a cold (13°C) or hot (38°C) brine formulation. Processing consisted of sectioning loin and ham (top and bottom) muscle pieces into 4.5 x 4.5 cm chunks. These lean pork chunks were subjected to a tumbling time of 0 and 8 hr, continuous 4 hr run and 4 hr rest interval, respectively, and further processed

Meat Sources	Thermal induced PSE pork carcasses (28 right sides) pH (5.4 – 5.7)	Control / Normal pork carcasses (28 left sides) pH (5.8 –6.0)





Boneless Sectioned and Formed Hams

Brine formulation for processed hams.

Ingredients	Percent of Brine	Kilograms of Brine
Water	15.2001%	40.8233kg
Nitrite	0.0152%	.0408kg
Erythorbate	0.0557%	.1497kg
Phosphates	.3378%	.9072kg
Salt (NaCl)	2.1956%	5.8967kg
Corn Syrup Solids(CSS)	2.1956%	5.8967kg

Kilograms of brine prepared: 53.7144kg
Percent of brine injection: 20.0%

Processing schedule - Steam cooked cycle

Time	Temperature Dry	Temperature Wet	%R.H.	Smoke
1 hr	49.0°C	49.0°C	100%	Off
1 hr	60.0°C	60.0°C	100%	Off
Hold until the internal temp. reaches 67°C	77.0°C	77.0°C	100%	Off

Product was showered with cold water for 60 min. Hams were chilled and stored at 4.0°C.

Figure 1. Processing Procedures for Sectioned and Formed Hams.

for 10 min using a Leland mixer (Leland Detroit Mfg. Co., Detroit, MI). After mixing, the meat was placed in a Vemag® stuffer (Reiser Inc., Canton, MS) and stuffed into polylined stainless steel molds (Charles Abram, Inc., Philadelphia, PA) with rectangular dimensions of 8.5 x 8.5 x 68 cm. A conventional cooking cycle of 4 hrs was used to achieve an internal temperature of 67.0°C. These molds were then showered for 60 min and chilled for approximately 12 to 14 hr in a 4°C cooler. Hams were removed from molds and one-half of each batch was sliced (8 mm thick) and vacuum packaged, while the remainder was used for electron microscopy analysis and binding evaluation.

Chemical Analyses and Protein Extraction

Proximate analysis (moisture, fat, and protein) was determined on raw and cooked product samples according to procedures outlined by the AOAC (1992). The protein extractions were measured by the Biuret method (Gornall et al., 1949). Meat samples were frozen in liquid nitrogen and blended to form a powdered consistency. Duplicate 2 g samples of meat were placed in 50 ml centrifuge tubes containing 5 mL of KPO_4 50 mM buffer, pH 7.5 containing 100 mM of KCl. Samples were diluted to 1:15 (w/v) and homogenized for 30 sec using a biohomogenizer model 133 (Biospec Products Inc., Bartlesville, OK). Protein concentration of the samples was recorded as percent mg of soluble protein per ml of solution.

Sodium Chloride

Salt (NaCl) content of the meat samples was determined using an Orion model 90-02 chloride ion electrode. Meat samples were frozen in liquid nitrogen and pulverized. Duplicate 5 g samples of meat were weighed in a 250 ml beaker with 0.6 M HNO_3 extraction solution and brought to a boil. Samples were removed and cooled to room temperature. Chloride ion concentration in meat extract was measured to determine the percent salt content.

Sodium Nitrite

Nitrite ($NaNO_2$) analysis was determined on cooked (67°C) luncheon hams according to procedures outlined in AOAC (1992). Triplicate 2.5 g portions of each sample were placed in a 250 ml beaker with 50 ml distilled water and heated in a Blue M constant temperature water bath (Blue M Electric Company, Blue Island, IL) for 2 hr at 80°C. Then samples were transferred into 250 ml flasks and brought to volume with distilled H_2O. Filtration samples were pipetted into a 50 ml flask and reacted with 1 ml of Greiss reagent containing 0.5 sulfanilamide and 0.1 N-(1-naphtyl) ethylenediamine dihydrochloride with 15% acetic acid (150 ml) for color development of aqueous solution. The samples were read on a spectrophotometer at absorbance 540 nm for comparison of residual nitrite content to a standard curve.

Hydrogen Ion Concentration (pH)

Duplicate 5 g portion cooked meat samples were placed in a 250 ml beaker of distilled water and homogenized for 30 sec using a biohomogenizer. Meat samples at 4°C were measured for the negative logarithm of [H+], the hydrogen ion concentration, with a digital Corning 130 pH meter.

Cooked Loss and Smokehouse Yields

Cooking loss (WHC) values for total fluid released were calculated according to the procedures of Lee et al. (1981) and Honikel et al. (1981). Each 5 g sample of processed muscle was weighed into a preweighed 50 ml centrifuge tube and placed in a boiling water bath for 20 min. Duplicate tube contents were cooled and drained off. Meat samples were blotted with filter paper and subsequently placed back into tubes for reweighing. The percentage of moisture loss during cooking was determined. Smokehouse yields were determined by preweighing stainless steel molds without and with sectioned and formed meat prior to thermal processing. Processing yields were calculated on a percentage basis: (final cooked weight/uncooked weight) x 100 = % yield.

Color Evaluation

Color observations were taken on the sliced surface, at four diffferent sites, for each cooked ham within each treatment using a Minolta (Minolta camera Co., Ltd., Los Angeles, CA.) L*, a*, and b* values measuring in CIELAB for L* = lightness, a* = bluish-green/red-purple hue component, b* = yellow/blue hue component, C* $[(a^{*2} + b^{*2})^{1/2}]$ = Chroma, and h° (from arctangent b*/a*) = hue angle (0° = red-purple, 90° = yellow, 180° = bluish-green, 270° = blue).

Muscle Ultrastructure (Electron Microscopy)

Tissue Preparation. After thermal processing, samples were taken from the 8 hr tumbling, brine treated hams at 13 and 38°C. The product was sliced into 1 mm squares and saturated with fixative (8% glutaraldehyde in 0.27 M cacodylate buffer, pH 7.2) and fixed for 2 hr at room temperature. The tissues were washed in 0.2 M phosphate buffer three times for 20 min each. The samples were dehydrated in a graded ethanol series (50, 70, 90, 95, 100, 100, and 100%) for 20 min each at room temperature and washed in 100% propylene oxide three times for 20 min each. The tissues were infiltrated in 1:1 propylene oxide/polybed 812 (21 ml polybed, 13 ml dodecenylsuccinic anhydride, 11 ml nadic methyl anhydride, 0.7 ml 2,4,6-tri dimethylaminomethyl phenol -30°) in capped vials overnight at room temperature. Vials were uncapped in a vacuum desiccator for approximately 7 hr. The 1 mm strips of cooked ham were flat-mold embedded in fresh polybed for 48 hr at 60 to 70°C.

Sectioning. Blocks were thick (0.5 um) and thin (0.07 um) sectioned on a Sorvall MT-6000 microtome (Research and Mfg. Co., Tuscon, AZ). Thick sections of eight cooked hams were placed on glass slides, stained with Mallory's Azure II methylene blue (1% Azure II, 2% methylene blue, and 2% Borax), and examined with an Olympus BH2 light microscope (Hitschfel Instruments, Inc., St. Louis, MO). Light microscopy was used to visually determine the selection of blocks for thin sectioning. Thin sections were placed on 200 mesh nickel grids, post-stained with

5% VA for 4 min and lead citrate (0.03 gm in 10 ml water, 2 drops 50% NaOH) for 5 min, and examined with a JEOL 100-CX STEM at 80 kv.

Binding Evaluation

Binding strength analysis as described by Suter et al. (1976) was performed using the Instron Universal Testing Machine Model #4500. The cooked hams were allowed to equilibrate at room temperature and a sliced section was placed in a tensile testing device which held the sample in place by metal pins. Dimensions of the cooked ham slice were 8.7 cm in length, 5.6 cm in width, and 8 mm thick with a grip distance of 2.8 cm. Sample rate and crosshead speed were 10 pts/sec and 100 mm/min, respec-tively. Binding variables from force and area measurements were: breaking strength (MPa), toughness (MPa), and energy at break (Joules).

Statistical Analysis

Treatments were arranged in a 2^3 (2x2x2) factorial design and randomized during manufacture. Replications of each treatment were performed in triplicate. The three factors were brine temperatures (13 and 38°C), tumbling times (0 and 8 hr) and quality muscle types (normal and PSE). Data were analyzed using general linear model procedures of the Statistical Analysis System (SAS, 1985). Means, where significant, were separated using the least square means (LSM) procedure. The model included effects of brine temperature, tumbling time, brine temperature x tumbling time, quality muscle type, brine temperature x quality muscle type, tumbling time x quality muscle type, brine temperature x tumbling time x quality muscle type, and the replications x brine temperature x tumbling time x quality muscle type = appropriate error term for each F-test. Once interactions for a measurement were detected at $P < 0.05$, all means will be presented to distinguish between the simple effects from all possible treatment combinations. When interactions for a measurement were not significant, main effects were tested and least squares treatment means are presented. For the test procedure when means are discussed as not different ($P > 0.05$), actual probability values are greater than .20.

Results and Discussion

A statistical t-test comparisons for each quality muscle type classification was performed. Identification of parameters to differentiate muscle quality types (PSE and normal) is categorized (Tables 1 and 2) by pH, WHC, and color measurements. The pH value of PSE muscle was lower ($P < 0.05$) than the normal muscle type in Table 1 which subsequently corresponds to the drip loss percentage being greater in PSE muscle compared to normal muscle contents. Loss in weight (shrinkage) for PSE samples was 3.4% more than for normal muscle. These data are in agreement with Sayre et al. (1964) who showed that PSE muscle may lose 6 to 10% of its weight as drip loss. Chemical analyses for percentage moisture, fat, and protein were not different ($P > 0.05$) between PSE and normal muscle type (Table 1). Similarly, Briskey et al. (1959) and Wismer-Pedersen (1959) observed no consistent difference in protein, fat, and moisture content between PSE and normal musculature.

Minolta color values are shown in Table 2. The PSE muscle type was greater ($P < 0.05$) in color intensity for L*, a*, b*, C*, and h° values than normal musculature. Differences in the pH and WHC between the two muscle types (PSE and normal) appear to influence the descriptive values of color intensity because of the wet surface on the PSE muscle. Once an abundance of water is liberated to the surface of the meat, it will begin to reflect more light. Lister (1987) believed that these soluble proteins precipitate onto the structural proteins, thus causing interference with the optical properties of the meat surface.

Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) spectroscopy is a technique which can be used to evaluate cellular and tissue metabolic changes via a noninvasive approach. This method detects the interaction of radiation with matter. The technique depends upon the fact that certain atomic nuclei such as phosphorus (^{31}P) have intrinsic magnetic properties, i.e. spin. When a sample (tissue extraction) contains such muscle and is placed in an NMR magnetic field, the nuclei begin to align with

Table 1. Analytical and chemical analysis of muscle classification of pork sides

	Muscle Type		
	PSE (Right Side)	Normal (Left Side)	SEM
Analytical Analyses			
pH	5.58[a]	5.86[b]	.04
WHC (drip loss %)	7.25[a]	3.83[b]	.30
Chemical Analyses (%)			
Moisture	73.28[a]	73.26[a]	.16
Fat	2.00[a]	1.98[a]	.17
Protein	24.74[a]	24.57[a]	.12

[a,b]Means in rows and followed by the same superscript letter are not different ($P > 0.05$).

Table 2. Minolta color mean values of muscle classification of raw pork sides

	Muscle Type		
	PSE (Right Side)	Normal (Left Side)	SEM
L* Values	58.59[a]	51.65[b]	.55
a* Values	7.22[a]	5.82[b]	.27
b* Values	9.37[a]	6.23[b]	.37
C* Values	11.87[a]	8.60[b]	.43
h° Values	51.92[a]	45.17[b]	1.05

[a,b]Means in rows and followed by the same superscript letter are not different ($P > 0.05$).

respect to the field. Introducing a second field perpendicular to the main field causes a realignment of the nuclei. Decay of the second field allows the original alignment to be regenerated. This magnetic interaction is detected in the radiofrequency range due to the emission of radiation releasing decay of the second field. The composition of the molecular structure of samples which contain the nuclei will determine the type of frequencies that are being emitted. The frequencies are expressed as signals or resonances which are dimensionless parameters known as chemical shifts (ppm). Analysis of the chemical shift allows an interpretation of the structure of molecules in a liquid. The purpose of the phosphorus (P) 31 NMR experiment was to classify normal and PSE quality muscle types according to the metabolic changes or the presence of certain phosphorus compounds in pork tissues.

Figure 2 represents a typical P-31 NMR spectrum obtained approximately 20 min post-mortem from a normal muscle. Nine resonances were assigned, from left to right field (ppm), to the following metabolities: (1) glucose 6-phosphate (G6P); (2) α-glycerol phosphate (α-GP); (3) inorganic phosphate (Pi); (4) phosphocreatine (PCr); (5) γ-adenosine triphosphate (γ-ATP); (6) α–adenosine triphosphate (α–ATP); (7,8) nicotineamide adenine dinucleotide (NAD and NADH); and (9) β-adenosine triphosphate (β-ATP). In Figure 3, PSE characteristics were induced on the right sides of pork carcasses at a thermal processing temperature of 37.7°C with 95% RH. The P-31 NMR analysis revealed only three intracellular concentrations of phosphorylated compounds, namely (1) G6-P, (2) α-GP, and (3) Pi in pig muscle extraction. Thermal-induced samples did not reveal observed changes in P-31 signals for PSE conditioned muscle because of the 4 hr time duration prior to tissue extraction. This is not in agreement with Miri et al., (1992) who showed that 31-P NMR spectrum for PSE muscle had a low pH, low to medium ATP content, low PC, and high phosphomonoester (PME) content. Figure 3 demonstrates when creatine phosphate declines to zero within 4 hr

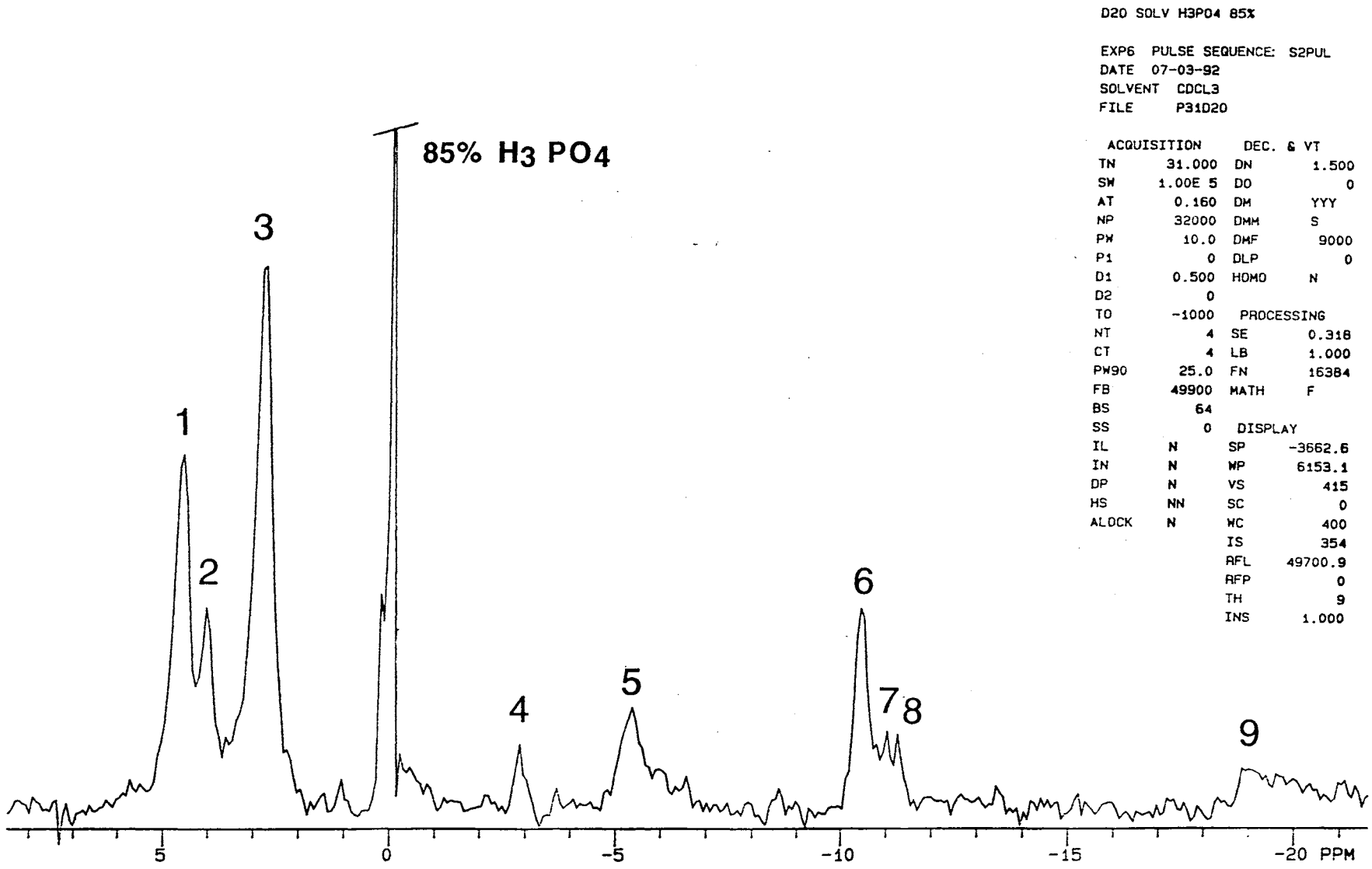


Figure 2. Phosphorus—31 NMR spectrum of normal pig muscle at 0 hr after slaughter

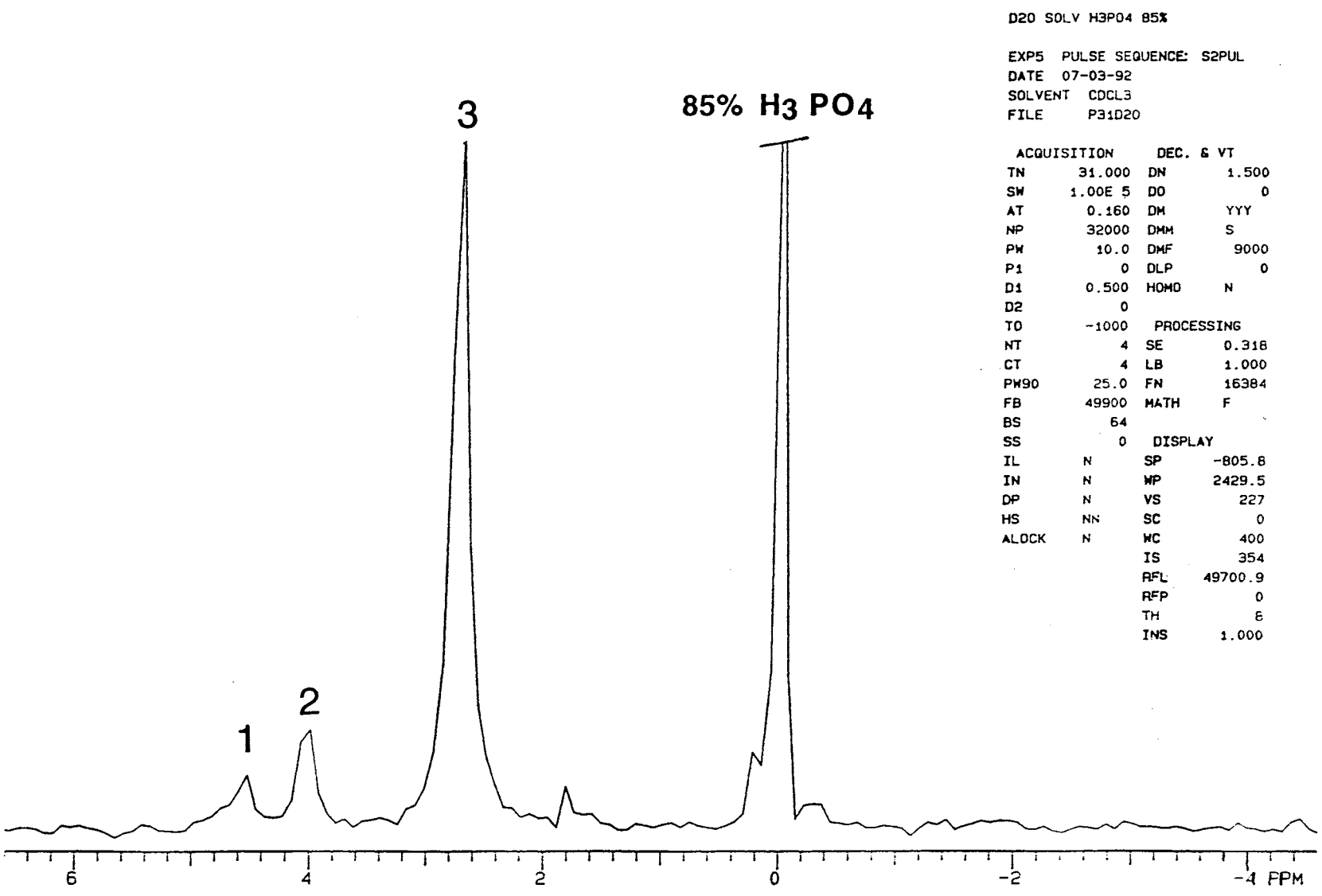


Figure 3. Phosphorus—31 NMR spectrum of thermal induced PSE pig muscle at 4 hrs after slaughter

post-mortem, the ATP level is not maintained through rephosphorylation of ADP by creatine phosphate. Therefore, the ATP level is low causing rigor to be established without the generation of P-31 signals.

Chemical Analyses (Raw Products)

Mean main effect values for chemical analysis of raw meat are given in Tables 3, 4, and 5 for brine temperature, tumbling time, and quality muscle type factors. Moisture and fat percentages in Table 3 were not different between brine treatment levels (13 and 38°C), but the protein content was influenced ($P < 0.05$) by the elevated brine temperature of 38°C. Table 4 shows that when comparing 0 and 8 hr tumbling moisture and protein percentages were similar, but the fat content was different ($P < 0.05$) between tumbling times. Based on these data, the increase in brine temperature from 13 to 38°C causes slight modifications in moisture and fat levels, but the utilization of tumbling alters the fat content which may be attributed to the increased rate of brine absorption into muscle pieces. Mean values for quality muscle types are given in Table 5. The influence of normal and PSE muscles did not affect ($P > 0.05$) the proximate composition (moisture, fat, and protein) of processed hams. As expected, the brine injection levels for muscle types (normal and PSE) contained the same chemical contents regardless of brine temperature levels used in pumping muscle portions for further processing.

Table 3. Main effects for chemical analyses of raw sectioned and formed pork meat according to brine temperatures

	Cold Brine (13°C)	Hot Brine (38°C)	SEM
Chemical Analyses (%)			
Raw			
Moisture	74.64[a]	74.94[a]	.30
Fat	1.33[a]	1.12[a]	.21
Protein	19.31[a]	17.56[b]	1.75

[a,b] Means in rows followed by the same superscript letter are not different ($P > 0.05$).

Table 4. Main effects for chemical analyses of raw sectioned and formed pork meat according to tumbling times

	0 Hr Tumbling	8 Hr Tumbling	SEM
Chemical Analyses (%)			
Raw			
Moisture	74.79[a]	74.79[a]	.01
Fat	1.00[a]	1.45[b]	.46
Protein	18.93[a]	17.94[a]	.99

[a,b] Means in rows followed by the same superscript letter are not different ($P > 0.05$).

Table 5. Main effects for chemical analyses of raw sectioned and formed pork meat according to quality muscle type

	Normal Muscle[a]	PSE Muscle[a]	SEM
Chemical Analyses (%)			
Raw			
Moisture	74.97	74.59	.38
Fat	1.27	1.19	.07
Protein	18.19	18.69	.49

[a] Means in rows followed by the same superscript letter are not different ($P > 0.05$).

Chemical Analyses (Cooked Products)

There was a significant ($P < 0.05$) three-way interaction involving brine temperature x tumbling time x quality muscle type for percentage moisture of cooked hams (Table 6). These data suggested that the level of factors had a proportional effect at different levels on at least two of the factors in cooked ham treatments. Moisture percentages were affected between 0 and 8 hr levels of tumbling for normal and PSE hams within 13°C brine temperature. Normal tumbled hams were different across the 13°C treatments. Both PSE treatments regardless of tumbling time were different from the normal treated hams injected with a cold brine(13°C). Treatments within the hot brine temperature (38°C) were

Table 6. Chemical analyses of cooked hams according to brine temperatures arrangement.

	Cold Brine (13°C)				Hot Brine (38°C)			
	Tumbling				Tumbling			
	0 Hours		8 Hours		0 Hours		8 Hours	
	Normal	PSE	Normal	PSE	Normal	PSE	Normal	PSE
Moisture (%)	72.90^{d}	72.46^{ef}	74.52^{a}	72.17^{f}	73.79^{c}	73.97^{c}	72.76^{de}	74.33^{a}

^{a,b,c,d,e,f} Means in rows followed by the same superscript letter are not different ($P > 0.05$). SEM = .15.

not different between the nontumbled normal and PSE muscle groups. PSE 38°C, was different from the treatments within the hot brine (38°C) treated hams.

The cold (13°C) and hot (38°C) brine levels for fat and protein contents in Table 7 were lower in the hot brine treated hams. There were no differences ($P > 0.05$) among tumbling times (0 and 8 hr) for fat and protein contents (Table 8). As for the quality muscle types in Table 9, only the protein level was higher in PSE treated hams. The PSE sample appears to have loss more solids content in the cooked ham compared to the normal muscle type. Certain chemical properties were slightly altered between brine temperature and muscle type factors within treatments. These subtle changes may be attributed to the homogeneity of protein solubility or inherent variation of PSE muscle.

Meat Protein Extraction

Mean percentage values for soluble protein content are given in Figure 4. The quality muscle type (normal and PSE) affected ($P < 0.05$) the overall solubility levels of ham treatments. As expected, protein solubility was lower in PSE compared to the normal muscle system. This loss of protein functionality is attributed to the denaturation of myofibrillar proteins (Briskey and Sayre, 1964; Penny, 1969) which

Table 7. Main effects for chemical analyses of cooked hams according to brine temperatures

	Cold Brine (13°C)	Hot Brine (38°C)	SEM
Chemical Analyses (%)			
Cooked			
Fat	2.31[a]	1.86[b]	.46
Protein	19.73[a]	18.78[b]	.95

[a,b] Means in rows followed by the same superscript letter are not different different ($P > 0.05$).

Table 8. Main effects for chemical analyses of cooked hams according to tumbling times

	0 Hr Tumbling[a]	8 Hr Tumbling[a]	SEM
Chemical Analyses (%)			
Cooked			
Fat	2.04	2.13	.09
Protein	19.36	19.15	.20

[a] Means in rows followed by the same superscript letter are not different ($P > 0.05$).

Table 9. Main effects for chemical analyses of cooked hams according to quality muscle type

	Normal Muscle	PSE Muscle	SEM
Chemical Analyses (%)			
Cooked			
Fat	2.15[a]	2.02[a]	.13
Protein	18.44[a]	20.07[b]	1.63

[a,b] Means in rows followed by the same superscript letter are not different ($P > 0.05$).

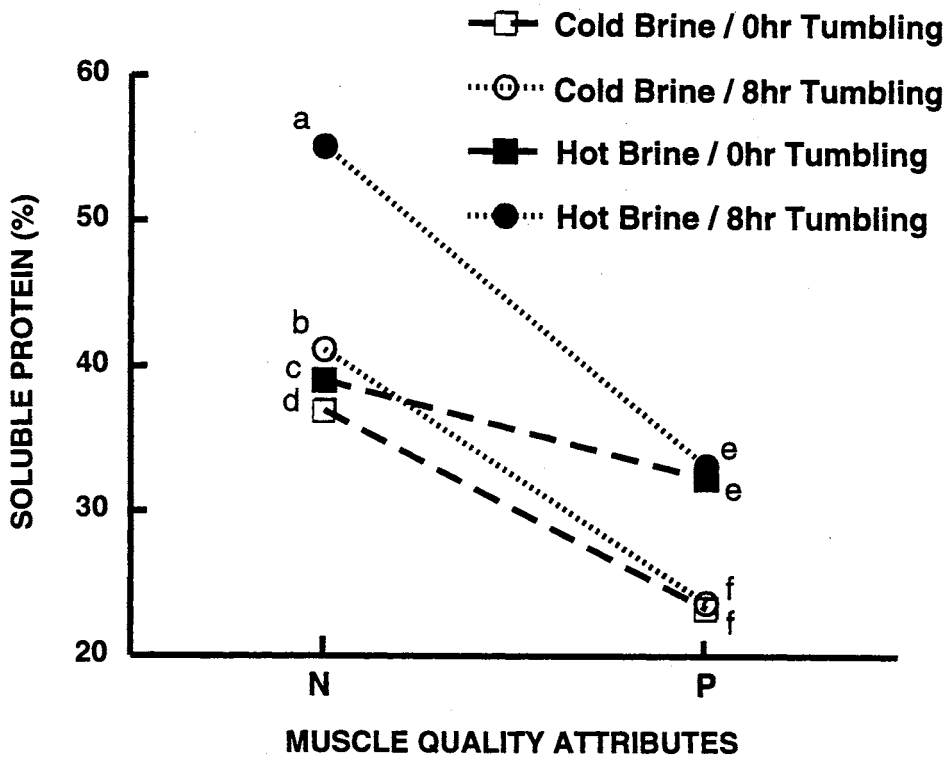


Figure 4. Soluble protein means expressed in percentages (%) for normal (N) and PSE (P) pork muscles in sectioned and formed hams arranged by muscle quality attributes.[a,b,c,d,e,f] Mean values with same superscript are not different ($P > 0.05$).

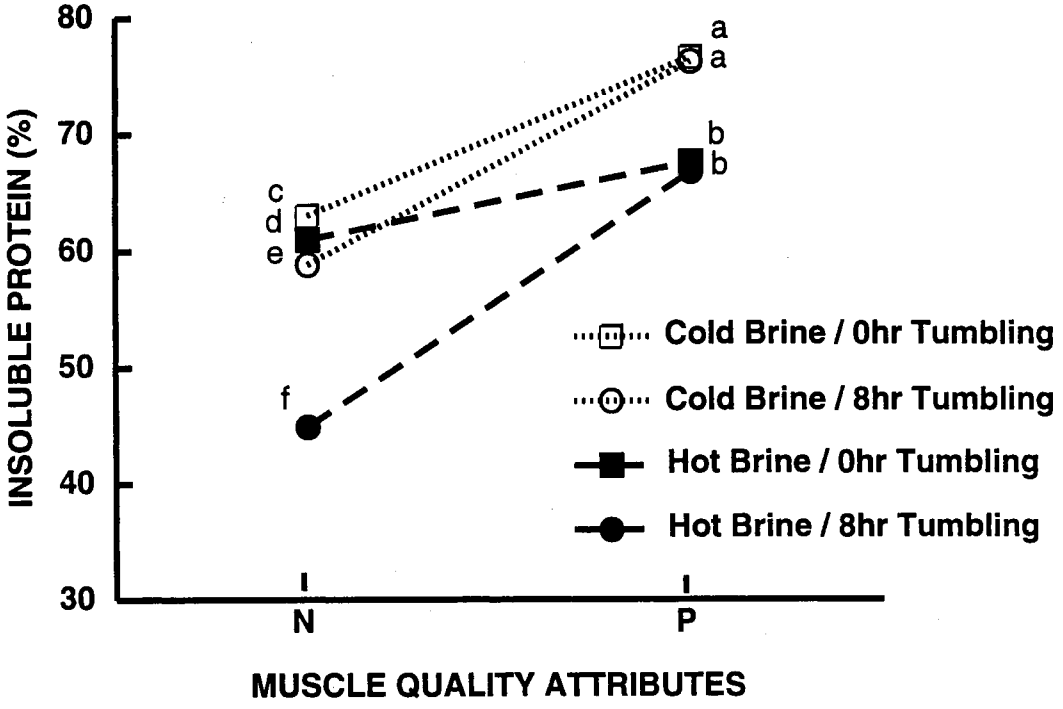


Figure 5. Insoluble protein means expressed in percentages (%) for normal (N) and PSE (P) pork muscles in sectioned and formed hams arranged by muscle quality attributes.[a,b,c,d,e,f] Mean values with same superscript are not different (P > 0.05).

will decrease the amount of protein extraction in meat products. Protein solubility values were higher in normal treated hams than the PSE treated hams, whereas the hot brine (38°C) at 8 hr tumbling for normal muscle contained the highest protein solubility value ($P < 0.05$) across treatments. Hot brine (38°C) treated PSE hams had greater solubility ($P < 0.05$) regardless of tumbling times (0 and 8 hrs) compared to the remaining PSE treatments. These data (Figure 4) suggest that quality muscle type is the single most important factor that determines the degree of protein solubility in meat systems. However, increasing the brine temperature to 38°C prior to injection into the muscle will enhance the protein extraction of salt-soluble proteins in both normal and PSE meats. Wismer-Pedersen (1959) and McLoughlin (1963) reported that the myofibrillar protein salt-solubility is substantially reduced in PSE meat, and Camou and Sebranek (1991) found that the PSE condition causes adverse chemical changes in meat protein functionality. Consequently, a reduction in the protein solubility of muscle will cause a decrease in WHC and texture of meat products. Furthermore, mean percentage values for insoluble protein (Figure 5) are the direct inverse of the aforementioned data in Figure 4. Meat products with a high level of insoluble protein content can directly be associated with increased cooking loss, poor texture, and extended product variability.

Muscle Ultrastructure (Electron Microscopy)

Transmission electron microscope (TEM) micrographs of four product treatments were observed at 3600 magnifications. Figures 6 through 9 show the morphological differences in muscle ultrastructure for normal and PSE, brine injected (13 or 38°C), tumbled products. Figure 6 represents the conventional method of manufacturing cooked hams. TEM observations revealed coagulation and shrinkage of muscle fibers in normal 13°C tumbled samples, resulting in a compressed network structure of disrupted muscle fibers. Conversely, the sample

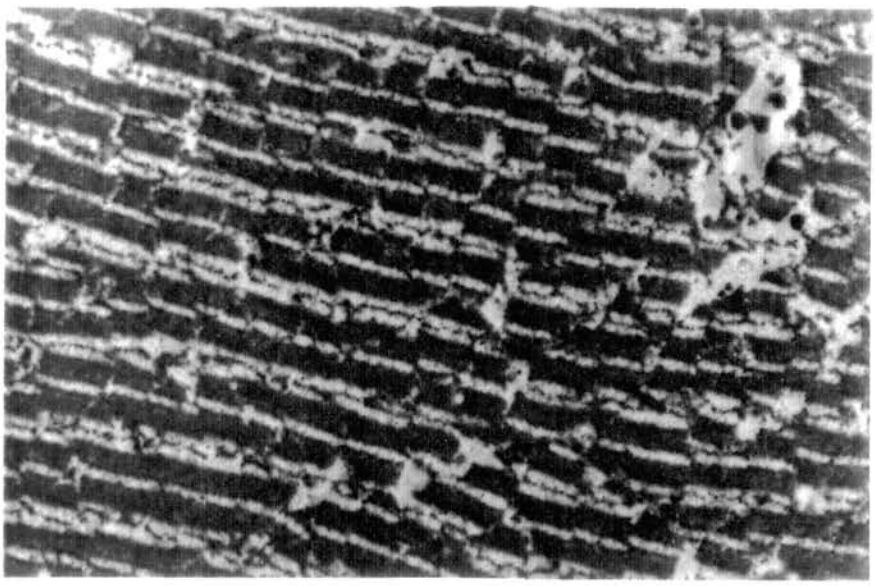


Figure 6. Transmission Electron Microscope Micrograph of Normal Muscle Injected With Cold Brine (13°C), Tumbled (8 hr), Mixed (10 min) and Thermal Processed (67°C) - 3600 magnifications

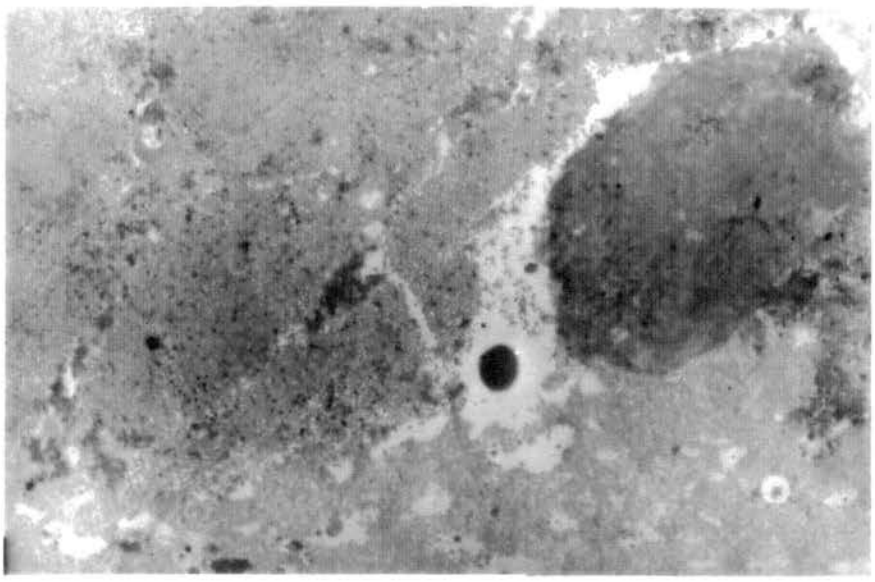


Figure 7. Transmission Electron Microscope Micrograph of Normal Muscle Injected With Hot Brine (38°C), Tumbled (8 hr), Mixed (10 min), and Thermal Processed (67°C)- 3600 magnifications

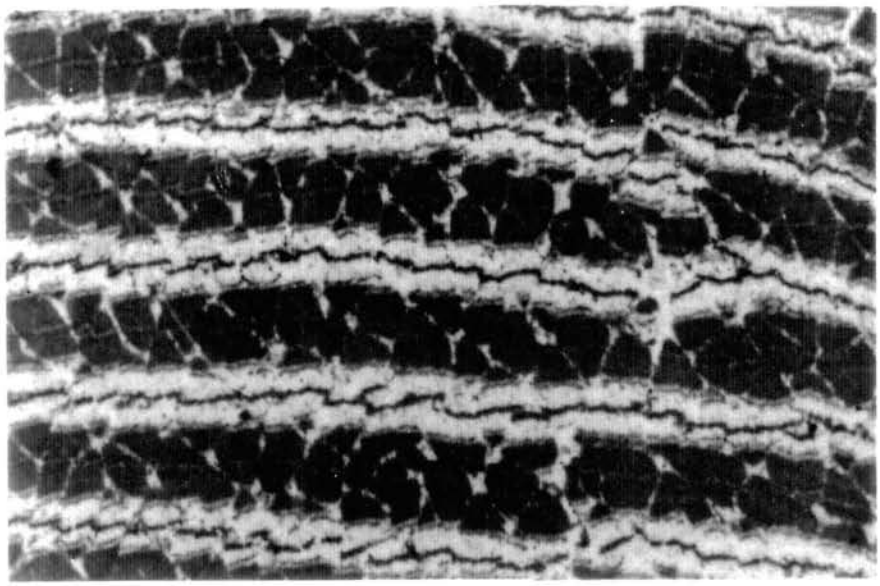


Figure 8. Transmission Electron Microscope Micrograph of PSE Muscle Injected With Cold Brine (13°C), Tumbled (8 hr), Mixed (10 min), and Thermal Processed (67°C)- 3600 magnifications

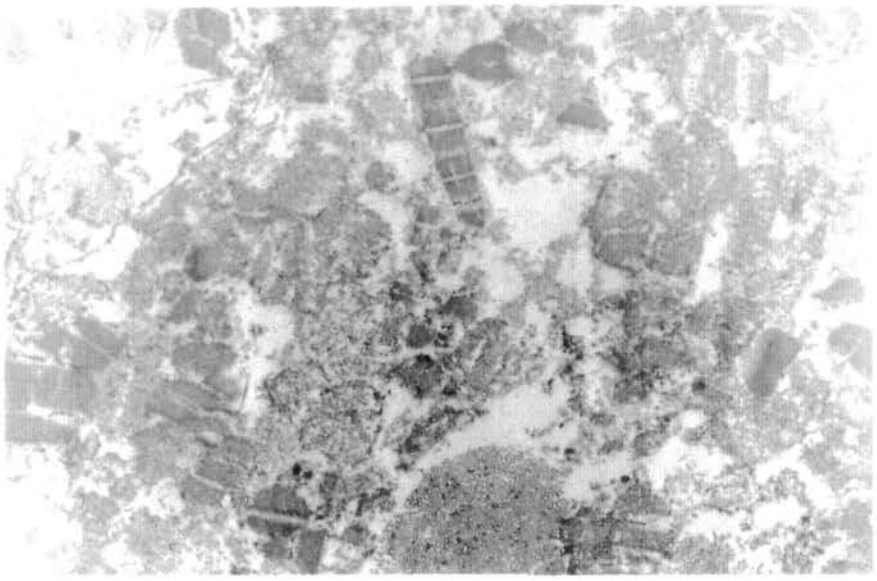


Figure 9. Transmission Electron Microscope Micrograph of PSE Muscle Injected With Hot Brine (38°C), Tumbled (8 hr), Mixed (10 min), and Thermal Processed (67°C)- 3600 magnifications

in Figure 7 possesses a stable protein matrix created by a pronounced dissociation of actomyosin that causes the release of salt-soluble proteins, which in turn coats the meat chunks and is then heat coagulated by cooking. This normal 38°C tumbled treatment contained more molecular interactions, through the solubilization of myofibrillar proteins and water-binding ability, compared to the normal lower temperature (13°C) ham samples. The large oval-shaped material present in Figure 7 is a protein sol (a mass of solubilized proteins).

The PSE 13°C tumbled product in Figure 8 shows that the muscle bundles and epimysium, endomysium, and perimysium collagen structures after thermal processing were intact without being completely solublized. Usually, muscle fibers become distorted and much more tightly packed together during heat denaturation between 40 and 60°C of the sarcoplasmic and myofibrillar proteins (Penny, 1967; Bendall and Restall, 1983). This particular tumbled 13°C treatment contains visible and distinct muscle bundles. The theory is muscle fibers from PSE meat are partially denatured and contain a high level of protein insolubility with salt and phosphate agents and are less pliable during mechanical (tumbling) action. However, after hot brine injection (38°C), the tumbled PSE treatment (Figure 9) revealed a semi-loose protein matrix structure with broken fiber fragments, protein sol, and coagulated fibers.

In summary, cold brine (13°C) treatments showed the usual product structure and orientation of normal and PSE luncheon hams. The incorporation of a 38°C brine into both normal and PSE hams demonstrated the effects of temperature-sensitive myofibrillar protein extraction. Meat pieces became easily pliable once the temperature of muscle was increased from 8 to 21°C by a hot liquid brine injection.

Salt, Nitrite, and pH

Mean values for salt concentrations are shown in Table 10. Normal and PSE hams injected with a hot brine that were not tumbled (0 hr) were different ($P <$

0.05) in salt concentrations compared to tumbled 13 and 38°C hams. The tumbled treatments containing cold (13°C) or hot (38°C) brine were not different ($P > 0.05$) in salt levels between normal and PSE hams. Cold brine (13°C) hams at 0 hr tumbling were the lowest in salt content among treatments.

Differences in salt concentration for certain treatments may be attributed to the brine level of solubility. Cursory observation revealed that brine ingredients (salt, nitrite, erythorbate, phosphate, and corn syrup solids) tended to be more solubilized at the elevated temperature of 38°C compared to the liquid brine at 13°C.

Residual nitrite values are given in Table 10. Each treatment was different ($P < 0.05$) in residual nitrite concentration (ppm) in hams, but the nontumbled 13°C, PSE treated product contained the highest nitrite level among treatments. All the treatments were inadvertently stored longer than the PSE 13°C nontumbled products prior to residual nitrite analysis which may explain the higher concentration. The pH levels were not different ($P > 0.05$) between treatments except for normal 13°C nontumbled product.

Table 10. Analytical analyses of cooked hams according to brine temperatures

	Cold Brine (13°C)				Hot Brine (38°C)			
	Tumbling				Tumbling			
	0 Hours		8 Hours		0 Hours		8 Hours	
	Normal	PSE	Normal	PSE	Normal	PSE	Normal	PSE
Salt (%)	2.07^{c}	2.07^{c}	2.23^{b}	2.14^{bc}	2.54^{a}	2.48^{a}	2.25^{b}	2.27^{b}
Nitrite (ppm)	20.13^{h}	42.75^{a}	22.15^{g}	24.21^{e}	27.10^{c}	28.21^{b}	25.48^{d}	22.77^{f}
pH	5.91^{b}	6.23^{a}	6.12^{a}	6.12^{a}	6.28^{a}	6.23^{a}	6.30^{a}	6.21^{a}

^{a,b,c,d,e,f,g,h} Means in rows for treatments followed by the same superscript letter are not different ($P > 0.05$).

Color Values for Cooked Hams

The Minolta color evaluation involved a significant ($P < 0.05$) three-way interaction among brine temperature x tumbling time x quality muscle type factors for b* values (Table 11). This factorial experiment provided information on the average effects of each factor and their proportional influence at different levels. The normal and PSE treated hams were different at 0 and 8 hr at 13°C. The normal 13°C, nontumbled treated ham was not different ($P > 0.05$) from the normal 13°C tumbled ham. PSE 13°C tumbled meat was lower ($P > 0.05$) in yellowness (b) intensity compared to the PSE 13°C nontumbled product. Hot brine (38°C) treated hams were different in yellowness intensity between normal tumbled and nontumbled hams. The PSE treated hams were not different between the tumbled and nontumbled levels within the 38°C brine temperature factor.

Table 11. Minolta color mean values for cooked hams arranged by brine temperatures, tumbling times, and quality muscle types

	Cold Brine (13°C)				Hot Brine (38°C)			
	Tumbling				Tumbling			
	0 Hours		8 Hours		0 Hours		8 Hours	
	Normal	PSE	Normal	PSE	Normal	PSE	Normal	PSE
L* Values	62.58	63.06	61.76	64.20	58.81	60.91	60.16	62.26
a* Values	10.51	9.23	11.14	9.88	9.34	9.13	10.14	10.05
b* Values	7.34^{d}	9.92^{a}	7.01^{d}	8.96^{b}	7.75^{abc}	7.50^{bcd}	5.17^{e}	7.18^{cd}

^{a,b,c,d,e} Means in rows for treatments followed by the same superscript letter are not different ($P > 0.05$). SEM = .31.

Water-Holding Capacity and Smokehouse Yields

WHC values measured by cooking loss are presented in Figure 10. Quality muscle type and brine temperature factors contributed significantly to the amount of fluid released from meat systems ($P < 0.05$). Hot brine (38°C) PSE samples that were subjected to 8 hr tumbling were equal to the normal 38°C, nontumbled hams, and subsequently were higher in WHC percentage than all of the remaining cold brine (13°C) treatments. Normal 38°C, tumbled hams retained the most WHC across treatments. As a result of elevating the initial temperature of meat from 8 to 21°C, the brine solution was allowed to enhance pliability and optimize the soluble extraction of proteins. It appears that the 38°C liquid brine extracts more salt-soluble proteins from the dissociation of muscle filaments by allowing greater WHC than a colder temperature brine. The lowest ($P < 0.05$) WHC levels were found in the 13°C PSE samples. Usually PSE pork holds less water than normal meat (Bendall and Wismer-Pedersen, 1962). This occurs because PSE muscle has a low pH, which will alter the protein functionality of meat, causing the WHC to be lowered. Water loss from PSE meat may be attributed to muscle hypertrophy which is associated with decreased capillary density in red fibers within muscle (Koch, 1968 and Weatherspoon, 1969).

The influence of brine temperature x tumbling time x quality muscle type factors contributed to a three-way interaction for smokehouse yield percentages (Figure 11) in luncheon hams. Actual smokehouse yields for normal and PSE treatments were numerically increased ($P < 0.05$) by hot brine (38°C) injection. Hot brine (38°C) treated normal and PSE hams, tumbled for 8 hr were not different in yield percentages, and were the highest among treatments. PSE 38°C, tumbled products were equivalent to nontumbled normal 38°C, hams, even though PSE 38°C, nontumbled hams were not different from the normal 13°C, nontumbled products. All normal 13°C, treatments had higher product yields than PSE 13°C, hams.

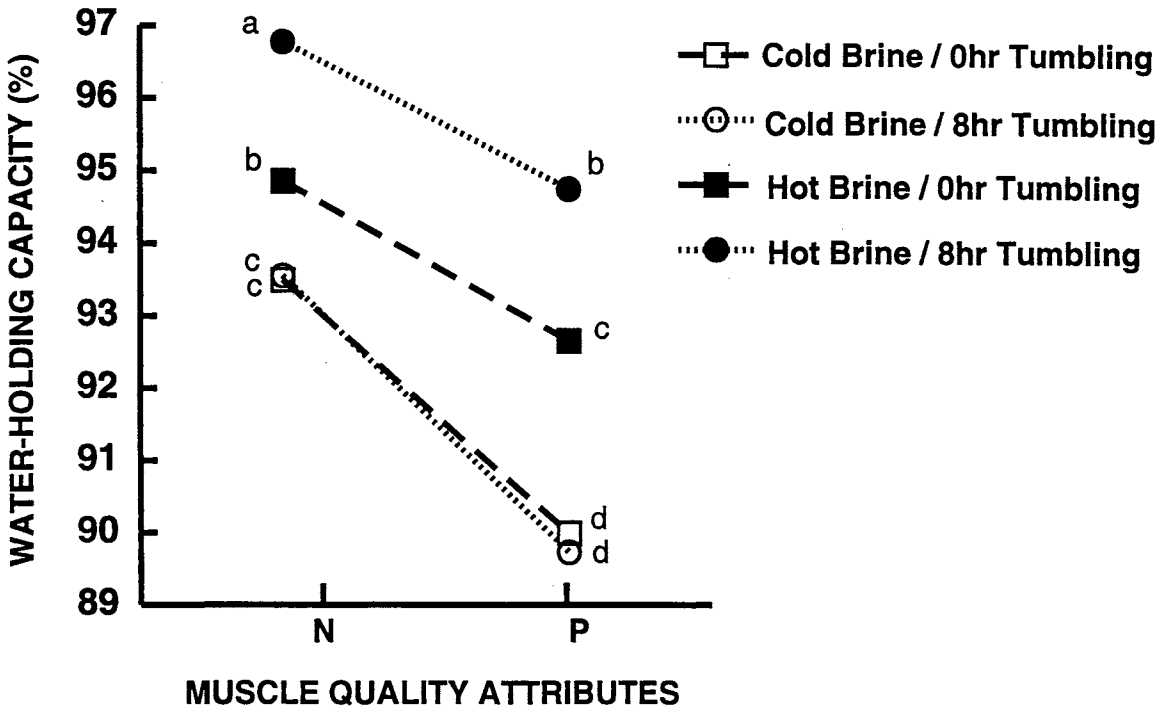


Figure 10. Water-holding capacity percentages, measured as cooking loss, for normal (N) and PSE (P) pork for cooked hams arranged by muscle quality attributes.[a,b,c,d] Mean values having he same superscript are not different ($P > 0.05$).

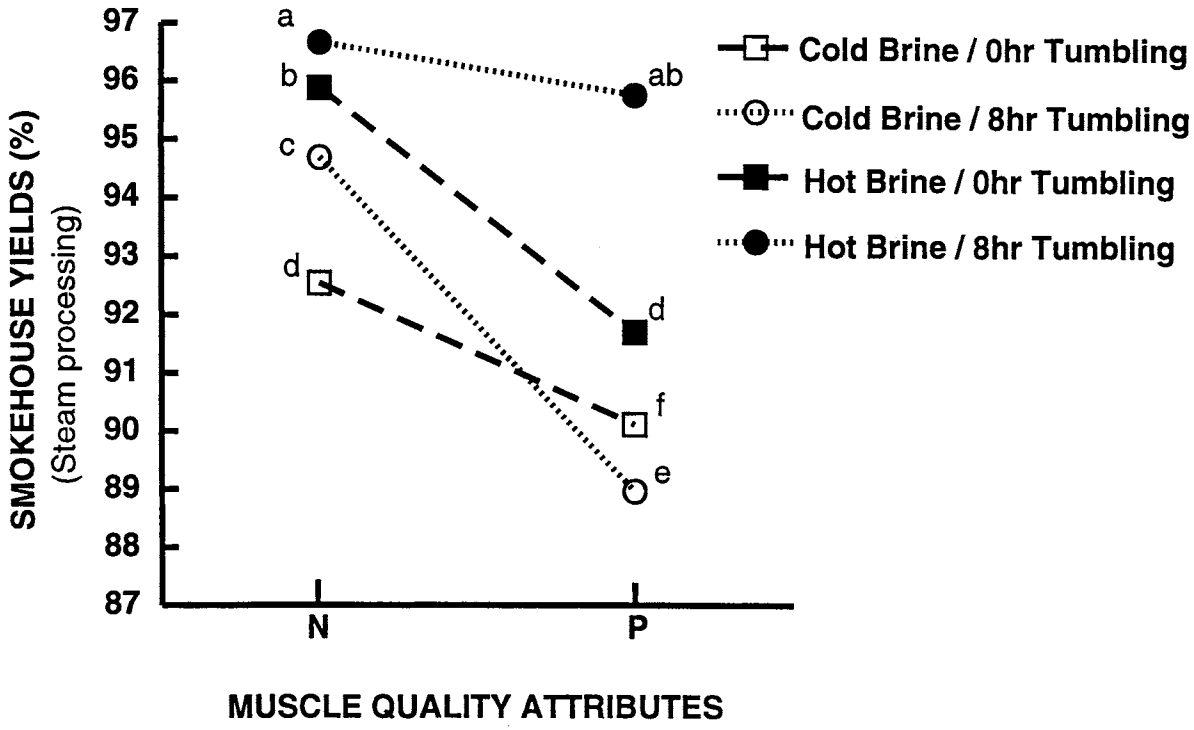


Figure 11. Interaction of smokehouse yields using a steam cycle process for normal (N) and PSE (P) pork for cooked hams arranged by muscle quality attributes.[a,b,c,d,e,f] Mean values having the same superscript are not different ($P > 0.05$).

The product yields for PSE 13°C, treatments were the lowest because of the low pH and partial denaturation of myofibrillar proteins. Wismer-Pedersen (1959) demonstrated that PSE hams normally have decreased water-binding capacity. Similarly, Camou and Sebranek (1991) showed that PSE extracts when thermally induced produce a lower yield (greater water loss) than control treated normal pork. In Figure 10 the WHC values have a similar magnitude in product yields for brine temperature, particularly the 38°C level, and muscle type correlation with lowered temperature (13°C) treated hams. From these data, the analysis of variance indicated that brine temperatures accounted for 40%, and quality muscle type 38% of the variation in cooked yield. The tumbling levels (0 and 8 hr) contributed only 7% to the overall factors in processing hams. The results revealed that by altering the solubilization levels of myofibrillar proteins with an elevated brine temperature and tumbling there is an upward trend of increased yields on normal and PSE meat products.

Binding Quality Evaluation

The analysis of variance indicates a significant ($P < 0.05$) three-way interaction for brine temperature x tumbling time x quality muscle type for breaking strength (Figure 12). The toughness and energy at break followed the same trend of parallelism between simple effects (Figures 13 and 14). In Figure 12, breaking strength was the highest for normal tumbled treated hams at both brine temperatures 38°C, followed by 13°C. The PSE tumbled and normal nontumbled hams containing a hot brine injection (38°C) were similar in binding strength and different from the remaining treatments. PSE 13°C, treated hams were not different from the nontumbled PSE hot brine treated products. With a cold brine injection without tumbling, normal hams were similar to the PSE 13°C, treated samples. As for toughness and energy at break (Figures 13 and 14), product values between normal 13 and 38°C, tumbled hams at different brine temperatures were not different ($P > 0.05$) and were higher in toughness than the other treatments.

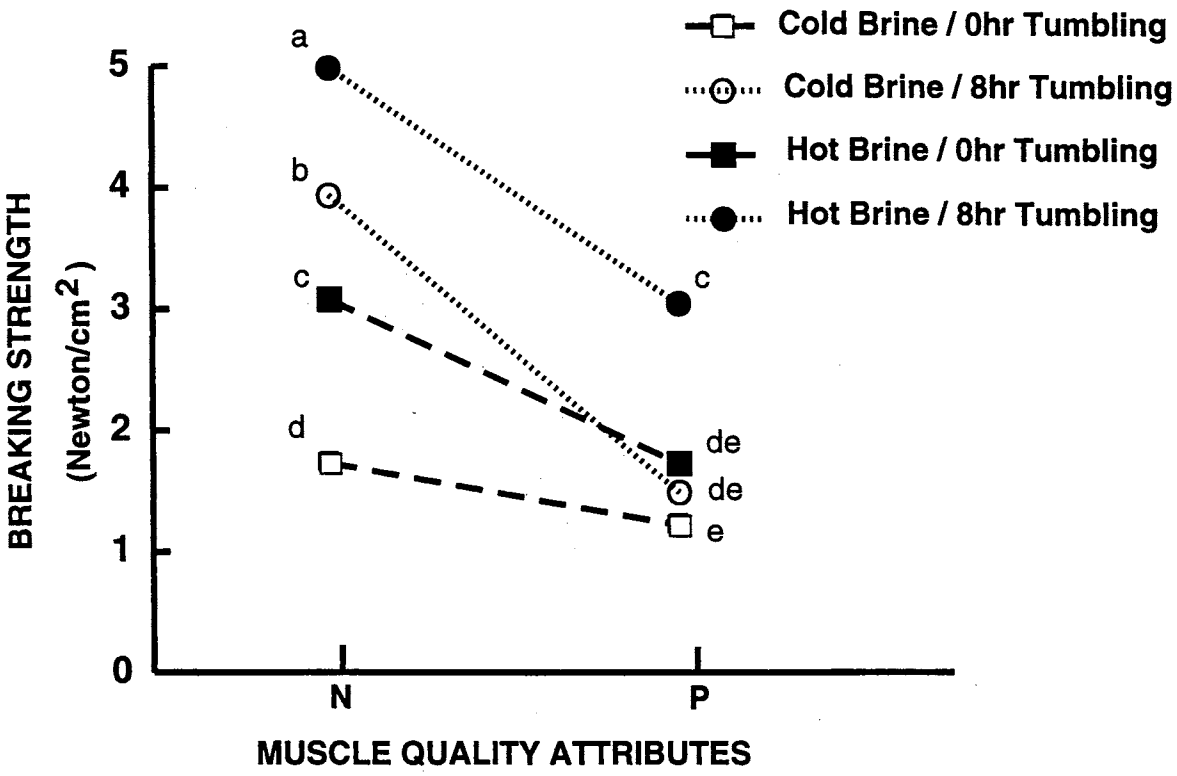


Figure 12. Breaking strength interactions for normal (N) and PSE (P) pork for cooked hams arranged by muscle quality attributes. ^{a,b,c,d,e,f} Mean values with the same superscript are not different ($P > 0.05$).

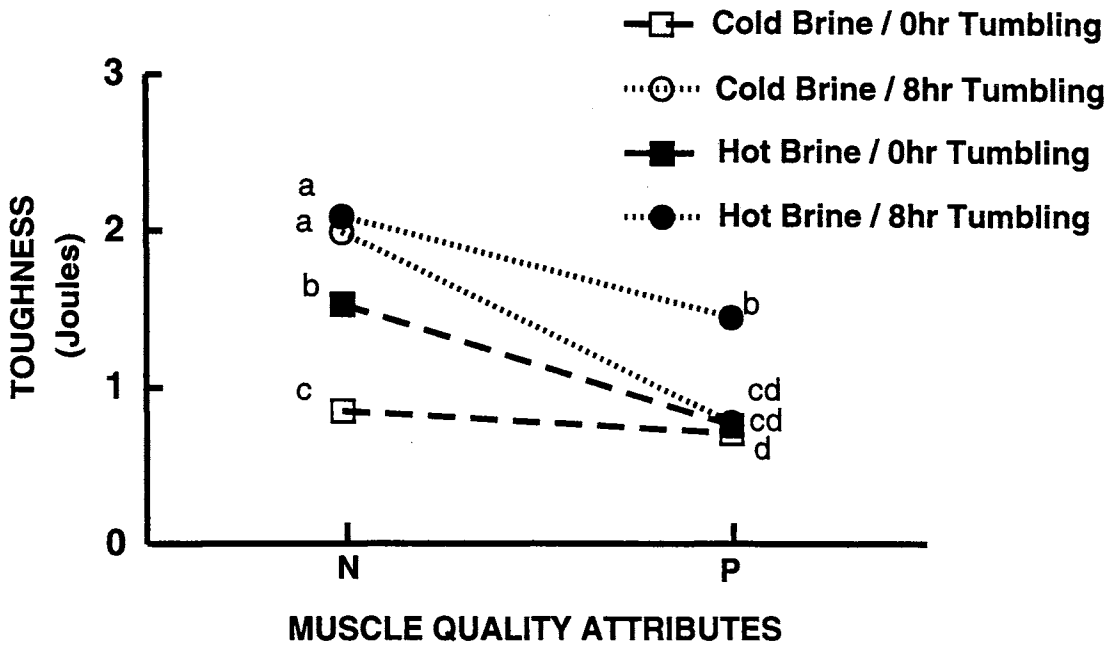


Figure 13. Toughness means for normal (N) and PSE (P) pork for cooked hams arranged by muscle quality attributes. ^{a,b,c,d,e,f} Mean values with the same superscript are not different ($P > 0.05$).

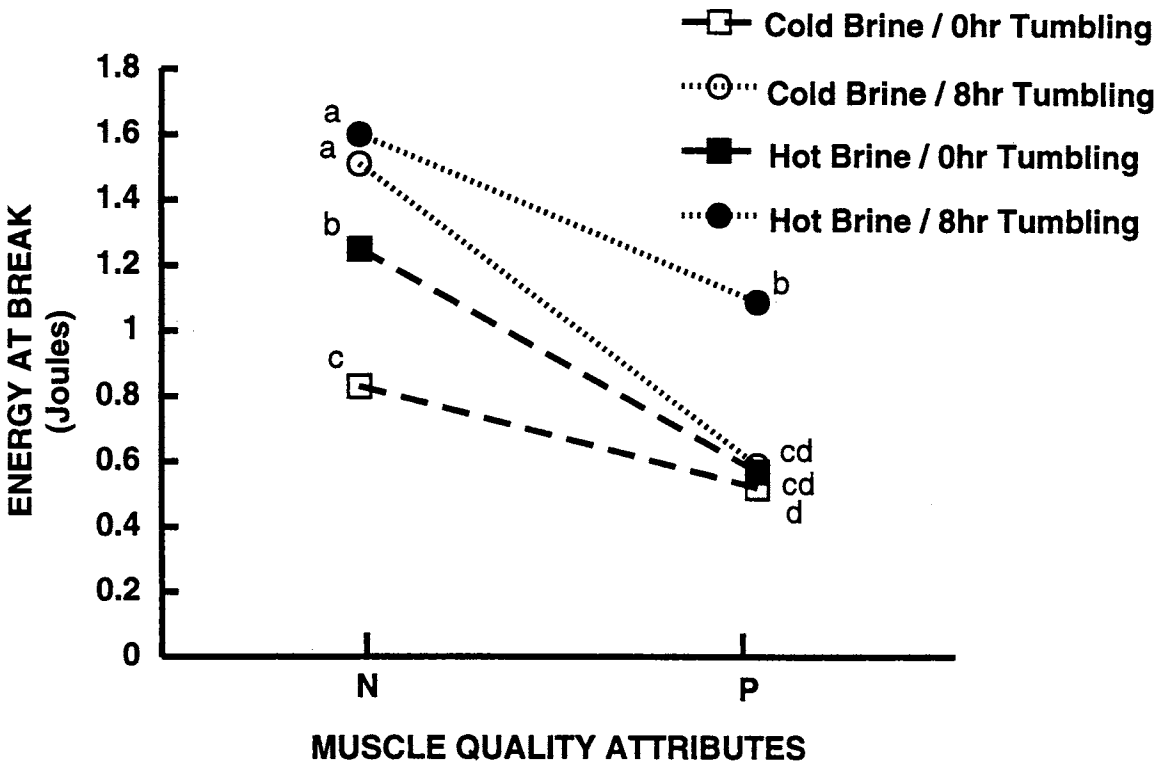


Figure 14. Energy at break means for normal (N) and PSE (P) pork for cooked hams arranged by muscle quality attributes.[a,b,c,d,e,f] Mean values with the same superscript are not different ($P > 0.05$).

Both PSE 38°C, and normal 38°C, tumbled and nontumbled, respectively, were intermediate in toughness and required more energy at break for samples. PSE nontumbled, 38°C and tumbled, 13°C treated hams were similar to the normal 13°C, nontumbled samples but equal to the PSE 13°C, nontumbled products. Furthermore, the analysis of variance indicates a three-way interaction ($P < 0.05$) for the tensile testing parameter of breaking strength. According to the ANOVA data (not shown), muscle quality (normal and PSE) exerted the most influence followed by tumbling and brine temperature factors. This may be attributed to the proportional effects on protein extraction, the amount of soluble proteins present, through mechanical manipulation with cure in binding meat pieces. Moreover, protein extraction enhancement mainly depends on the length of time for mechanical action (tumbling, mixing, and massaging) with curing ingredients to influence the binding quality of meat pieces (Theno et al., 1978; Schmidt, 1986).

In all cases, the conventional manufacturing method using a cold brine in conjunction with tumbling for PSE meat gave the lowest values in binding strength, toughness, and energy to break samples compared to the other tumbled 13°C treated products. From visual and physical observation, PSE 13°C, cooked hams appeared to be soft and crumbly in texture compared to the normal ham products. In contrast, the utilization of a hot brine in comparison to a cold brine for tumbled hams increased the breaking strength by 21% for normal and 51% for PSE treatments. A subsequent numerical increase occurred for PSE treatments for toughness and energy at break parameters. The liquid cure temperatures contribute to the level of binding sectioned and formed meats under certain conditions. This involves the increasing of meat temperature with a 38°C brine that allows greater protein extraction from tumbling and mixing of meat which causes improvement in water binding and protein-protein interactions during cooking.

Conclusions

Hot brine treatments (38°C) increased protein solubility levels compared to the cold brine (13°C) treated hams as determined by protein extraction. WHC, as measured by cooked loss, was higher for normal 38°C, tumbled followed by PSE 38°C, tumbled and normal 38°C, nontumbled meat products. Normal hams treated with a cold brine were different from the lower temperature PSE samples except for the 38°C, nontumbled hams. Smokehouse yields, however, showed that PSE 38°C, tumbled meats were similar to normal, 13°C, tumbled and nontumbled hams. The normal 13°C, tumbled product was intermediate but the PSE and normal 38°C, nontumbled products were equivalent in cooking yields. Brine temperature 13°C treated PSE hams had the lowest yields across treatments. The incorporation of a 38°C brine into both normal and PSE treated hams demonstrated different morphological changes in muscle ultrastructure which resulted in the increase in meat temperature from 8 to 21°C, causing the meat to become easily pliable for enhanced protein extraction of salt-soluble proteins. The hot brine in comparison to the cold brine for tumbled hams increased the binding strength by 21% for normal and 51% for PSE treatments. There was a numerical increase in toughness and energy at break for all 38°C brine treatments.

CHAPTER IV

EVALUATION OF ULTRASONIC CAVITATION AND TUMBLING CHARACTERISTICS ON PORK MUSCLE IN SECTIONED AND FORMED HAMS

Introduction

The manufacture of sectioned and formed meats depends on the formation of the protein matrix to bind meat pieces. Physical processes such as massaging and tumbling are used to extract protein from muscle fibers causing a release of salt-soluble proteins, which in turn coats the meat chunks and is then heat coagulated by cooking. Massaging chunks or meat pieces involves frictional energy resulting from the rubbing together of two meat surfaces, whereas tumbling incorporates kinetic energy into the muscle and a concomitant temperature increase (Addis and Schanna, 1979). Using these conventional methods improved the binding characteristics of meats (Siegel et al., 1978; Krause et al., 1978; Kreibig, 1991). By increasing protein extraction there is a concurrent increase in binding strength (Schmidt and Trout, 1984). Usually pork primal sections (hams and loins) contain variable muscle properties with respect to pH values, WHC, and color. All of these properties will affect the particle-to-particle binding ability (protein gelation) of meat.

Ultrasonic cavitation may be an alternative method to massaging and tumbling meat for protein extraction. Ultrasound technology has been investigated in the homogenization of milk (Newcomer, 1955), the cleaning of eggs (Dawson et al., 1960), and the tenderization of meat (Simjian, 1959; Webb et al.,

1962). Ultrasonication uses low and high frequency shock waves within liquid that causes the formation of minute bubbles to disrupt cellular material. This method can become very detrimental to biological tissues, especially at lower frequencies which create bubbles, gas filled cavities, that enlarge and implode in liquid. Cavitation is the term used to describe the formation and collapse of bubbles or cavities in liquids.

Child and Forté (1976) evaluated the use of ultrasound for the solubilization of protein from cottonseed products. They found that ultrasound increases the protein extraction of heat-treated cottonseed compared to the screw-expressed cottonseed meat method. Wang (1975) reported that ultrasonication increases protein extraction yield by 20% from soybean flakes compared to a conventional stir method. Ultrasonication has received limited research in the meat processing area. Reynolds et al. (1978) demonstrated that ultrasonic cavitation increases the binding strength in cured ham rolls. Their research showed changes in the microstructure of muscle by tank cleaner. Research has indicated that ultrasound has the potential to enhance protein extraction and improve binding strength in meat products. The purpose of this research is to evaluate ultrasonic cavitation on binding and color characteristics in sectioned and formed hams.

Materials and Methods

Manufactured Procedures

A 2x2 factorial arrangement was conducted in this experiment. Prior to cured processing, loins and hams were deboned, and subcutaneous, intermuscular fat and connective tissue were removed. Manufacturing procedures involved triplicate batches for each treatment at 0 and 25 KHz ultrasonication frequencies and subsequently tumbled (0 or 8 hr) respectively. Loins and hams were macerated and injected with a brine formulation (water, salt, corn syrup solids, sodium tripolyphosphate, sodium erythorbate and sodium nitrite) at 20% of green weight. Muscle pieces were sectioned into 4.5 x 4.5 cm chunks and subjected to

ultrasonication at 0 and 25 KHz frequencies, respectively. Meat was submerged in a liquid brine at 1500 watts of power for 15 min. using an ultrasonic tank (Ultrasonic Power Corporation, Freeport, IL) with dimensions of 25 x 20 x 35 cm and three individual 500 watt generators. After ultrasound, tumbling times involved 0 and 8 hr and a rest interval for 4 hr, respectively, and further processing for 10 min using a mixer. The meat was stuffed into polylined stainless steel molds (Charles Abram, Inc., Philadelphia, PA) and steam cooked to an internal temperature of 67°C. After attaining the desired internal temperature, hams were showered with cold water for 60 min and placed in a 4°C cooler. The product was removed from molds and packaged for further analysis.

Chemical Analyses

Percentage of moisture, fat and protein were determined on raw and cooked product samples following AOAC (1992) procedures. The Leco nitrogen determinator (Leco Corporation, St. Joseph, MI) was used to analyze samples for protein content.

Smokehouse Yields

The product yields were determined using the following formula: % yield = (final cooked weight/uncooked weight) x 100.

Binding Strength and Color Evaluation

Binding strength and color evaluation methods were conducted in the same manner as the previous study in Chapter III.

Results and Discussion

This study used ultrasonic cavitation as a technique to extract proteins from pork muscle to enhance the binding characteristics of meat systems. However,

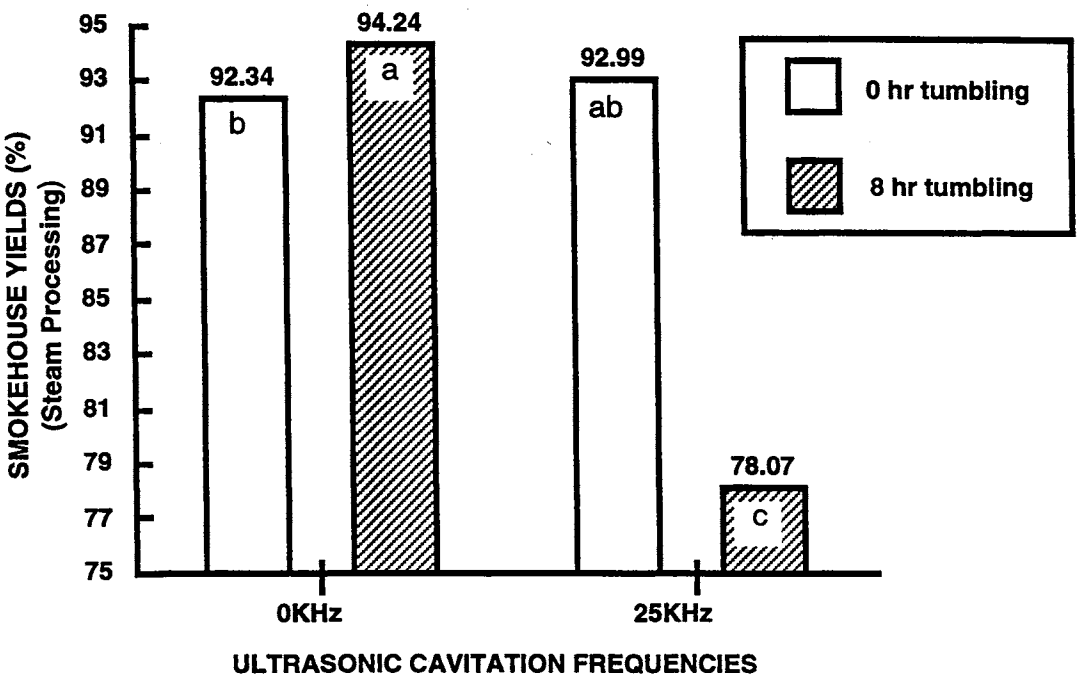


Figure 15. Interaction of smokehouse yields percentages using a steam cycle process for cooked hams arranged by ultrasonic cavitation frequencies.[a,b,c,d] Mean values having the same superscript are not different (P > 0.05).

ultrasonication created a dramatic increase in heat that caused the meat pieces to be scalded. Scalding the meat surface resulted in severe protein denaturation of meat chunks from ultrasonication at a frequency of 25 kHz.

Smokehouse Yields

There was a significant (P <0.05) two-way interaction for ultrasonication x tumbling time on smokehouse yields for sectioned and formed hams (Figure 15).

The ultrasound effects of one factor were not independent of the variations in other factors. Heat denaturation on the surface of meat at 25 kHz may not have been homogeneous between the nontumbled and tumbled treatments in processing luncheon hams. This is evident by the yield increase for the 25 kHz nontumbled product compared to the 25 kHz tumbled hams. Figure 15 shows that the nontumbled treated 25 kHz product was not different from either controls but was different from the 25 kHz tumbled meat product. The interaction between factors may be attributed to the irregular shape of meat sections which interfere with the cavitational effects on the surface of meat.

Minolta Color Values

Minolta color values are shown in Figures 16 through 18. The 25 kHz nontumbled meats were darker in color than either controls and the 25 kHz tumbled products (Figure 16). In Figure 17, both the control treatments were redder ($P > .05$) than the ultrasound treated products regardless of tumbling times (0 and 8 hr). Figure 18 shows no differences between treatments for yellowness (b) values in color intensity. These results suggest that the scorching of meat products treated with ultrasound (25 kHz) developed variations in color intensities.

Binding Characteristics

Binding strength values in Figure 19 were not different ($P < 0.05$) between treatments. In Figure 20, there was a two-way interaction among treatments for toughness in ham products. The analysis of variance data (not shown) indicated that tumbling exerted the most influence on toughness followed by ultrasonication.

Conclusions

The scorching of meat sections prior to further processing into cured hams caused severe protein denaturation. This protein denaturation was not

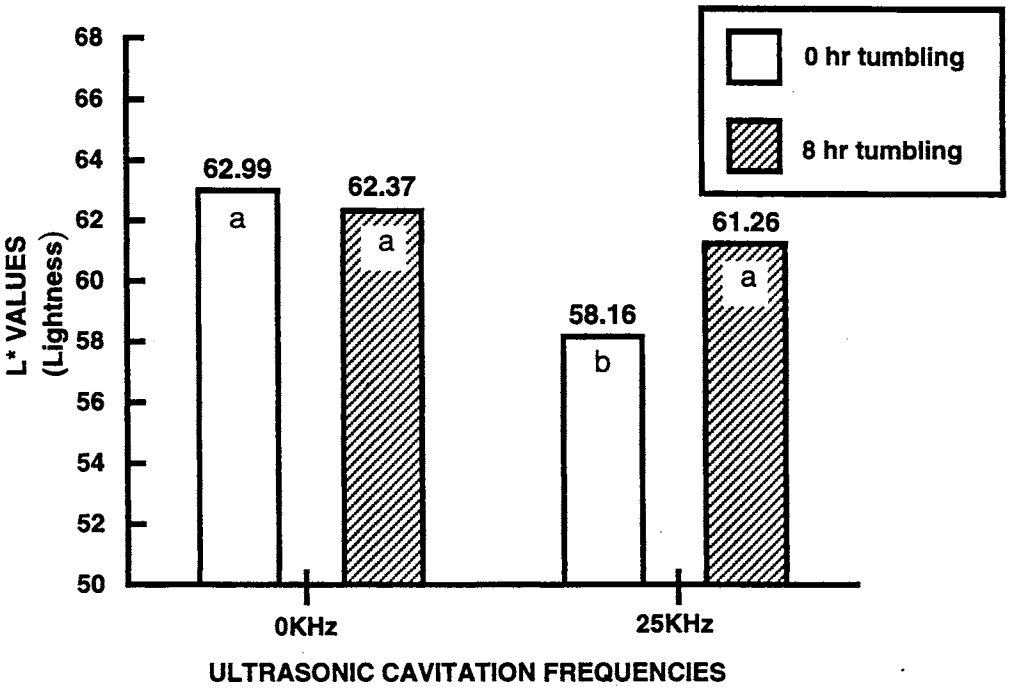


Figure 16. Lightness (L) Minolta color values for cooked hams arranged by ultrasonic cavitation frequencies.[a,b] Mean values having the same superscript are not different ($P > 0.05$).

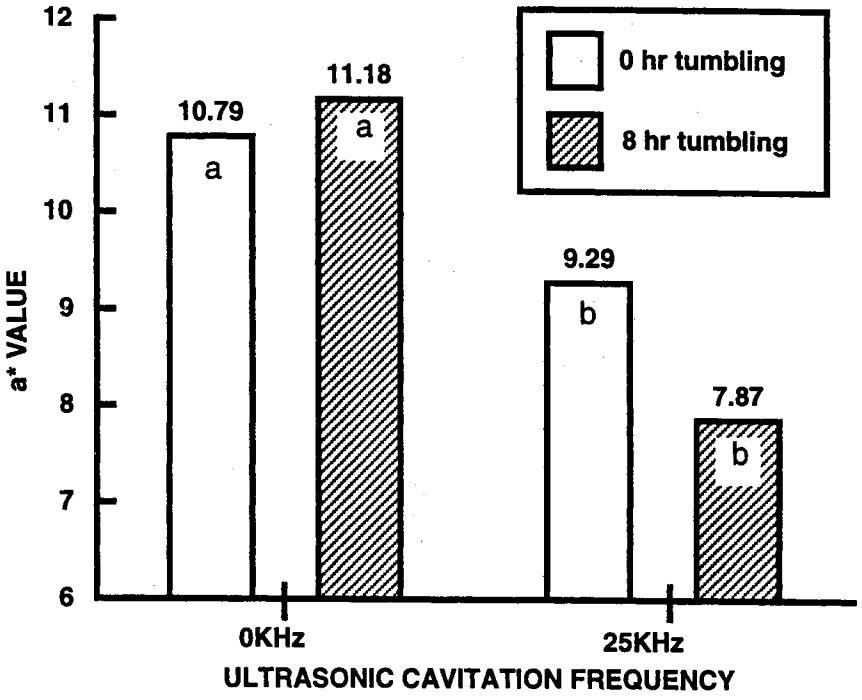


Figure 17. Redness (a) Minolta color values for cooked hams arranged by ultrasonic cavitation frequencies.[a,b] Mean values having the same superscript are not different ($P > 0.05$).

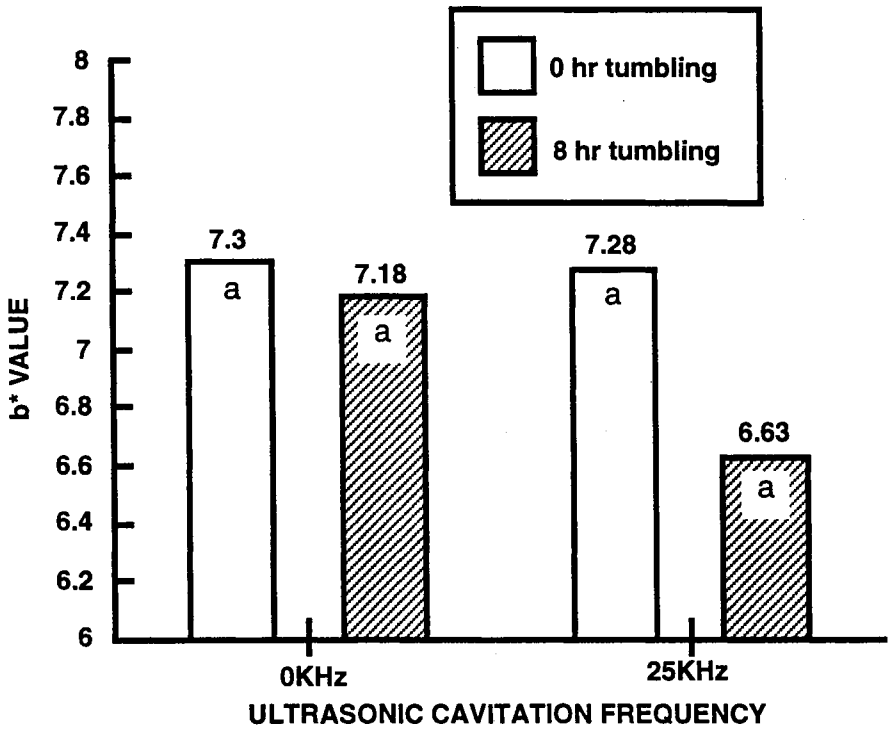


Figure 18. Yellowness (b) Minolta color values for cooked hams arranged by ultrasonic cavitation frequencies.[a,b] Mean values having the same superscript are not different ($P > 0.05$).

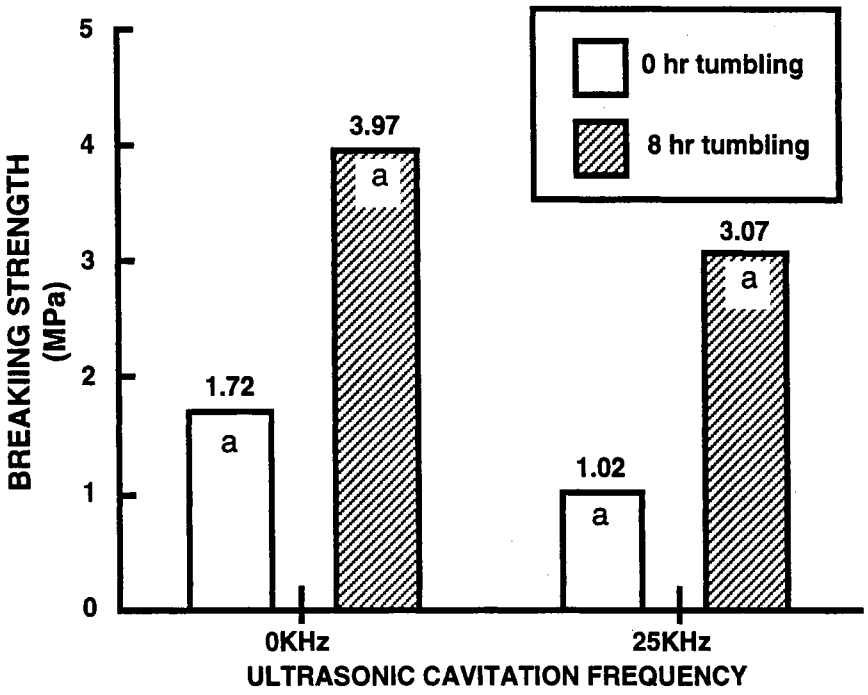


Figure 19. Breaking strength means expressed in mega pascal (MPa) for cooked hams arranged by ultrasonic cavitation frequencies.
[a] Mean values having the same superscript are not different ($P > 0.05$).

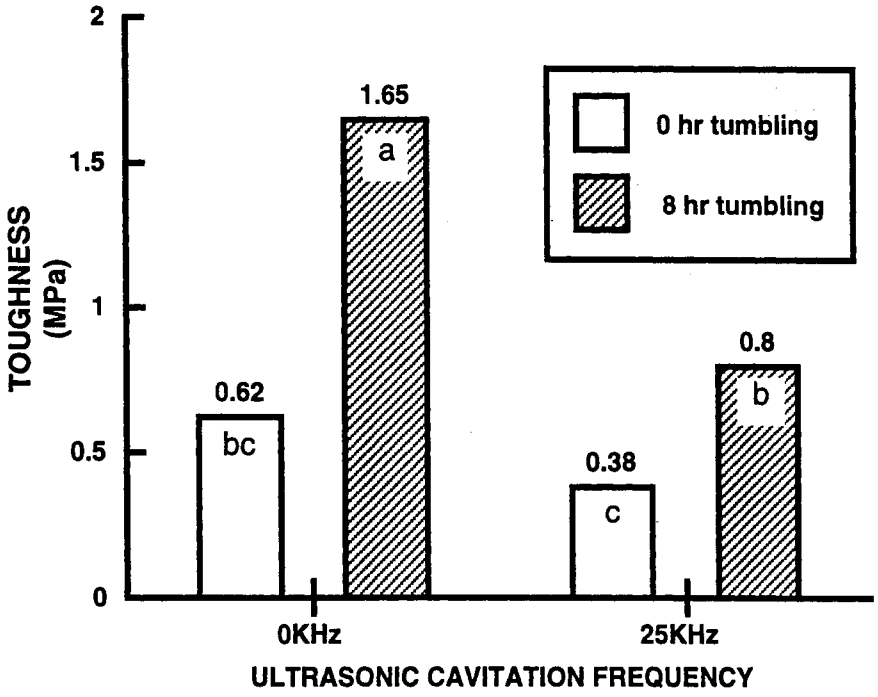


Figure 20. Interaction of toughness means expressed in mega pascal (MPa) for pork muscles in cooked hams arranged by ultrasonic cavitation frequencies.[a,b,c] Mean values having the same superscript are not different ($P > 0.05$).

homogeneous on all meat portions. The variation in binding and color may be attributed to the different degree of scorching between treatments. Therefore, the meat portions will vary in color and binding traits which depends on whether tumbling or nontumbling will be used in altering the product quality attributes of hams.

Recommendations

The ultrasonic tank used for extracting protein from muscle pieces was specially designed for optimal cavitation to occur at higher temperatures (60 to 80°C). This equipment contained a wide temperature bandwidth that accumulates a heating effect at 43°C that causes the scorching of the meat. By designing a narrow temperature band between 15 to 25°C, this will eliminate the cumulative effect of heating that scorches the meat and allow optimal cavitation to a lower temperature. In addition, the lowering of the ultrasonic frequency from 25 to 16 kHz will permit cavitation to be more disruptive to raw material and may improve the extraction of proteins from muscle.

CHAPTER V

CONCLUSIONS

Experiment I, protein extraction, water-holding capacity and binding quality characteristics for cooked hams injected with a hot brine (38°C) were improved in comparison to the conventional processing method of cold brine (13°C) treated hams. The PSE 38°C product cooked yield was equivalent to the normal 38°C and 13°C hams. In experiment II, a 25 kHz frequency using ultrasonication on meat pieces in this study do not appear feasible for manufacturing luncheon hams because the meat surface was scorched and product cooking yields were lowered.

These results demonstrated that hot brine (38°C) injection of hams along with tumbling and/or mixing may numerically increase the water-holding capacity and texture of sectioned and formed hams. In addition, ultrasonication was not effective in improving processing yields and texture to meat systems.

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