

ABSTRACT

DEAL, HOLLY DARE. Evaluation of Sodium Phosphate and Meat Protein Isolate as Enhancement Methods to Improve Pork Quality. (Under the direction of Dana J. Hanson).

Enhancement is a common processing method used in the pork industry in order to improve sensorial characteristics such as juiciness and tenderness. Initial meat quality can have a large impact on the eating satisfaction of pork and can also impact the effectiveness of an enhancement method. In study 1, the objective was to determine the impact of pH and intramuscular fat has on the flavor characteristics of pork. The results of study 1 were utilized to determine a selection criterion for study 2, which evaluated sodium phosphate and salt (P) as well as meat protein isolate (I) as enhancement methods compared to a control (C). Study 3 evaluated both (P) and (I) for cooking yield and Warner Braztler Shear Force values (WBS).

In study 1, pork chops were assigned to treatment groups above (\geq) and below (\leq) pH 5.6 , crude fat 4%, National Pork Producers Council color score 2.5, and Iodine Values (IV) 65 (n=16) to determine how a trained panel would perceive flavor attributes of each treatment with an established pork flavor lexicon. Principal component analysis (PCA) biplots were constructed to visualize the impact of treatments on flavor properties. Cooked pork flavor, cooked pork aroma, and sweet were significantly different ($P < 0.05$) among treatments, and were characterized by significant three way interactions between variables. Crude fat % and IV value do not play a significant role when determining the flavor of pork.

In study 2, pork loins were classified as above (\geq) pH 5.5 or below (\leq) 5.5 pH, and further assigned a treatment of P or I enhancement at 0%, 15%, or 20% pump rate to determine how a trained panel perceived flavor and texture attributes of each treatment with

an established pork flavor lexicon. Principal component analysis (PCA) biplots were constructed to visualize the impact of treatments on flavor properties. In flavor study, I may negate astringent mouthfeel and metallic flavors in control C with reported higher sweet flavor scores. In the texture study, loins with P and I enhancement were associated with higher intensities of moisture and juiciness compared to control.

In study 3, C (n=25), P (n=25), and I (n=25) were injected at a 20% pickup. Overall cook yields at 24h were different ($P < 0.05$) among all three treatments with P yielding 95.93%, I yielding 92.65% and C yielding 79.61%. WBS values were different ($P < 0.05$) among all three treatments with I (1.48 kg), P (1.64 kg), and C (2.15 kg). Processors may determine which area of study provides the greatest value, whether in processing yields or sensorial effectiveness.

Evaluation of Sodium Phosphate and Meat Protein Isolate as Enhancement Methods to
Improve Pork Quality

by
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DEDICATION

This work is dedicated to my parents, who instilled in me the values of holding strong in tough times and working hard to achieve goals. Thank you for your continuous love and support!

BIOGRAPHY

Holly Dare Deal was born to Oscho and Martha Deal on March 5, 1984. She grew up on the family farm in China Grove, NC with her older siblings Mandy and Eric. An active member of 4-H and FFA, she entered into NC State as a freshman wishing to contribute to agriculture, but in a non-traditional way. She found her home in the Food, Bioprocessing and Nutrition Science Department, where she received her BS, and continued under the direction of one of her favorite undergraduate instructors, Dr. Dana Hanson, for her MS. Her time at NC State was marked by dedicated and fun work in the Food Science Club, where she was voted by her peers as Most Outstanding Undergraduate Student in 2006 and Most Outstanding Graduate Student in 2008.

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CHAPTER 1

REVIEW OF LITERATURE

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1.1 Condition of Pale, Soft, and Exudative

1.1.1 History of PSE

Pale, Soft, and Exudative is a meat quality problem most often associated with hogs because this species normally has higher rates of post mortem glycolysis than other meat animals (Offer, 1991). Typically, PSE is a result of a rapid fall in pH while the body temperature is still high (Pearson and Young, 1989). Typically, the muscle temperature is above 38°C and ultimate pH of below 6 (Offer, 1991). The physical characteristics of PSE meat include a pale color, soft structure, and poor water holding abilities (Pearson and Young, 1989) resulting from the denaturation of proteins (Warner et al., 1997). Avian muscle has been found to have similar PSE like characteristics (McKee and Sams, 1998).

In the consumer's desire for lean meats, the meat industry has responded by focusing on genetics which produce heavily muscled animals that are more susceptible to stress (Gerrard, 1997), therefore impacting post mortem metabolism and the stimulation of this condition. The impact of PSE on consumer's eating satisfaction includes lack of tenderness (McKee and Sams, 1998) and a loss of product yield and quality upon the processor (Woelfel et al. 2002). As research on PSE has continued in both the pork and poultry industry, pre and post mortem practices have been identified to reduce the occurrence of this condition.

1.1.2 Implications of PSE

In 2002, A National Pork Quality Audit found that the cost of PSE to the pork industry was near \$.90 a head and that 15.5% of pork had characteristics of PSE. In a similar study occurring in 2005, the incidence of meat exhibiting "classic" pale, soft, and

watery characteristics was found to be 3.34% (National Pork Board, 2006). A general estimate of the range of occurrence of PSE pork is 10-40% (Marriott and Schilling, 1998). This reduction in PSE can be attributed to greater awareness of PSE in plants and an industry effort to alter influences pre and post mortem which contribute to PSE development.

As the turkey industry moves away from whole bird product into further processed products, the impact on PSE in turkeys greatly impacts the quality of these processed products. In one particular study, the incidence of PSE in a poultry plant was approximately 47% of the test product which exhibited poor water holding capabilities (Woelfel et al., 2002). This percentage could correspond to a \$2-4 million per year loss due to lost yields (Southern Association of Agricultural Experiment Stations Directors, 2006). These losses are associated with increased purge in cook in bags, resulting in decreased cook yields, an unacceptable dry product with poor texture, and overall a poor eating experience.

For the meat industry as a whole, processors look to new ways to improve functionality of PSE protein in fresh products or to utilize lower quality meats in further processed products, ultimately to preserve the profitability of the industry by providing consumers with superior eating satisfaction. The use of non meat ingredients to improve functionality of proteins is commonly used to improve overall quality of muscle. Young et al., (1987) suggested that salt and phosphate in combination provide a synergistic effect to improving water holding capacity and cook yield of chicken breast patties. Marination injection techniques such as this are employed to negate the effects of poor color, water holding ability, and soft texture of PSE meat.

1.1.3 Environmental Factors

Events leading up to animal slaughter have an impact on end meat quality. Under normal circumstances, a gradual decline in pH occurs until rigor mortis onset occurs 6 to 8 hours after slaughter (D'Souza et al., 1998). However, short term stress results in the acceleration of glycolysis due to a higher consumption of glycogen energy stores in the body when the body temperature is still high (Pearson and Young, 1989). The breakdown of glycogen results in the production of lactic acid while the body temperature is still high, therefore impacting the rate of pH loss in the body. Stress and struggle increase the body temperature and speeds up the rate of glycolysis (Pearson and Young, 1989) and environmental stresses such as transportation, extreme fluctuations in ambient temperature, feed withdrawal, and human handling can impact glycolysis (D'Souza, 1998, McKee and Sams, 1998, Owens and Sams, 2000, Leheska et al., 2002).

D'Souza et al., (1998) found that using an electric goad on pigs prior to slaughter had lower muscle glycogen concentrations therefore exhibiting higher incidence of PSE compared to minimally handled pigs. Removal of feed 48 hours prior to slaughter produced meat that had higher ultimate pH, improved water holding capacity, and darker color over pigs that had feed up until death, suggesting that the reduction of glycogen stores will improve muscle quality through the reduction of glycolytic metabolism (Leheska et al., 2002). A 48 hour preharvest fast was shown to be approximately the same effect as a 2 hour transportation time to slaughter (Leheska et al., 2002). During transport there are increases in body temperature, heart rate, and beta-endorphin (Geers et al., 1994), suggesting the negative effect of handling on an animal from induced stresses. Heat stressed turkeys have

lower initial and ultimate pH with higher rates of postmortem pH decline when compared to non-heat stressed group (McKee and Sams, 1998). As a result of increased glycogen breakdown and higher muscle temperatures, negative handling and stress induced situations influence metabolism and ultimately meat quality.

1.1.4 Genetic Factors

Genetic factors determine if an animal is susceptible to stress and how that animal will adapt to those stressors, and both hogs and turkeys have been identified to have genetic factors which promote PSE like meat (Strasburg and Chiang, 2003). To meet the consumer demand for lean hogs, genetic improvements to increase muscle and reduce fat has occurred, and inadvertently increased the frequency of PSE cases, since more time is needed postmortem to reduce internal body temperature on heavier muscled animals (Doumit et al., 2003).

While there is not a perfect correlation, there is some degree of relation between PSE meat and Porcine Stress Syndrome (PSS), which is genetically inherited as an autosomal recessive gene (Harri, 1995). PSS is associated with fast growth and a lean, muscular body type in hogs (Harri, 1995). Halothane screening can be used to identify animals which are stress susceptible and thus could develop PSE meat upon exposure to halothane gas (Owens et al., 2000). The response to halothane increases body temperature, increases lactic acid production and promotes muscle rigidity (Owens et al., 2000). Halothane positive turkeys were found to have a greater percentage of pale meat compared to halothane negative turkeys (Owens et al., 2000). A genetic dysfunction occurs in PSS hogs to allow excess calcium

from the sarcoplasmic reticulum through the ryanodine receptor, a calcium release channel protein (Wang et al., 1999). Calcium concentration is responsible for the initiation, time course, and force of contraction; through the excitation-contraction coupling of chemical and electrical signals from calcium release (Stratsburg and Chiang, 2003). Calcium release in excess through defective ryanodine receptors increases the rate of anaerobic glycolysis (Doumit et al., 2003). Altered calcium channel regulation may be associated with the incidence of PSE meat from turkeys selected for high growth (Wang et al., 1999).

1.1.5 Postmortem Glycolysis

The conversion of muscle to meat is determined by the supply of glycogen, high energy phosphates, and their metabolites in muscle at the moment of death; these quantities in reaction will impact meat quality. After exsanguination, the lack of aerobic ATP synthesis from ADP in mitochondria from oxidative phosphorylation results in a switch to anaerobic metabolism utilizing glycogen (Shen, 2006). Glycogen, as a source of glucose, can drive the reaction to produce ATP. The rate of ATP breakdown determines the rate of postmortem glycolysis (Hamm, 1977). As glycogen is utilized, lactic acid is also produced, which eventually prevents other products from being re-synthesized back to glycogen (Pearson and Young, 1989). Living muscle pH is around 7.6 and will have a final ultimate pH of about 5.4-5.7 (Pearson and Young, 1989). A pH decline from lactic acid build up is capable of denaturing muscle proteins and enzymes necessary to continue driving glycolysis. As pH falls, the amount of ATP produced begins to disappear when the products of glycolysis can no longer be re-synthesized. The onset of rigor mortis begins as about half of the ATP has

disappeared, since ATP provides the energy for muscle contraction (Bendall, 1963). With this normal occurrence of glycolysis, the loss of extensibility occurs and remains tough until an enzymatic meat aging process proceeds in the resolution of rigor mortis.

Rapid glycolysis particularly at high body temperatures, results in water and salt soluble protein denaturation, thus resulting in PSE characteristics (Fernandez et al., 1994). High post mortem temperature accelerates glycolysis, and low temperatures retard the rate thus lessen the number of hours needed to achieve ultimate pH 5.8 (Pearson and Young, 1989). Delaying post mortem chilling of carcasses keeps the carcass hot while lactic acid continues to build, further stimulating PSE characteristics. An increase in temperature of 10°C (in the region of 30°C) increased protein denaturation 20 fold in poultry meat (Dransfield and Sosnicki, 1999). Thus, the temperature greatly impacts the rate of pH decline and ultimately protein denaturation.

1.1.6 Color

Meat color is the first quality taken into account when a consumer purchases meat at the retail level and for final acceptance of meat at the time of consumption (Ouali et al., 2006). Color can be attributed to the myoglobin concentration found in meat. Greater amounts of myoglobin in different types of muscle fibers are associated with increased oxidative metabolism and a greater supply of blood to the muscle fiber (Pearson and Young, 1989). A strong positive correlation exists between total heme pigment concentration, such as myoglobin, iron, and a* value (redness) while there is a negative correlation between heme pigment concentration, iron, and L* value (lightness) (Boulianne and King, 1998). The

visual perception of pork color is influenced by the degree of lightness (L^*) and by the balance between red and yellow, with the intensity of red (a^*) also being important (Zhu and Brewer, 1998). Visual redness of pork is inversely correlated with L^* value (Zhu and Brewer, 1998).

As light enters meat, most of it is scattered, but some of the scattered light reflects back, and is seen with the human eye. If a high degree of scattering shortens the light path, selective absorbance is decreased and the meat appears less pink than normal (Swatland, 1995). Potentially the increased separation of myofibrils in PSE meat increases the chances of light scattering on the surface of myofibrils (Swatland, 1995).

Several researchers have determined that there is a negative correlation between pH and color lightness (Allen et al., 1998, Fletcher et al., 2000, Fletcher, 1999, Bendall and Swatland, 1988). Subjective and objective color measurements account for up to 43 and 75% of variations found in ultimate pH values (Jeremiah et al., 1991) when used as a method to segregate beef carcasses for tenderness. Light colored breast meat which has low pH have higher percentages of drip loss and cook loss, thus water binding properties of meat are comprised, ultimately impacting cooked meat tenderness (Allen et al., 1998).

1.1.7 Water Holding Capacity

Skeletal muscle is approximately 75% water (Pearson and Young, 1989). Binding of water to proteins is related to polar hydrophilic groups, and the capacity of proteins to retain moisture is dependent on the type and number of polar groups that are the primary site of protein-water interactions (Wierbicki and Deatherage, 1958). Myofibrils occupy over 80%

of the volume of muscle fibers and they hold most of the immobilized water between the thick and thin filaments of the myofibrillar lattice (Tarrant, 1995). Most of the water in muscle tissue is immobilized, and is held in the lattice spacing between myofibrils (Zayas, 1997). Water holding capacity (WHC) is defined as the ability of food to hold its own and added water during application of forces, pressing, centrifugation, or heating (Zayas, 1997). Gains or losses of water are due to the shrinking and swelling of the myofibrils caused by expansion or shrinkage of the filament lattice (Offer and Trinick, 1983). Longitudinal muscle contraction together with lateral myofibrillar shrinkage drives water out from the myofibrillar space (Bertram, 2004). Also during rigor, the pH has declined which decreases solubility of proteins from their denaturation, thus impacting water binding (Zayas, 1997). In PSE meat, denaturation of the myosin head may cause additional shrinkage and more fluid to be lost from the lattice, thus increasing the amount of drip formation (Offer, 1991). Water retention is at a minimum at the isoelectric point of actomyosin, pH 5-5.4, and rises on either side, where the net negative charge of proteins increases, thereby increasing protein-water interaction and improving WHC (Thompson and Zeuthen, 1988). Low WHC and low pH results in high cooking losses in pork (Aaslyng et al., 2003) while increasing WHC above the isoelectric point of meat positively influences cooked beef tenderness (Gault, 1985).

1.1.8 Muscle pH

Following exsanguination, the carcass pH declines as the amount of lactic acid increases from the breakdown of glycogen during postmortem glycolysis. When glycogen is depleted from the muscle, the ultimate pH of the muscle is reached, and is critical for fresh

meat properties including color, texture, WHC, and tenderness.

The pH is a primary factor which determines the severity of the PSE condition (Swatland, 1995). The time taken to reach an ultimate pH of 5.5 in pork will be dependent on the carcass temperature (Bendall, 1963). Muscle with low pH and high temperature at the onset of rigor mortis has a reduction of sarcoplasmic protein solubility by 55% and myofibrillar proteins by 75% (Sayre and Briskey, 1963). This reduction of solubility greatly impacts a proteins ability to function in fresh and processed meats and decreases overall quality.

A relationship has been determined between cooked meat tenderness and its ultimate pH (Purchas and Aungsupakorn, 1993); as ultimate pH increase from 5.5 to 6.0 tenderness decreases, but when ultimate pH increases above 6.0, tenderness increases. Two reasons for this curvilinear relationship have been suggested. First, there is less proteolytic activity at intermediate pH (5.8-6.3) because it lies outside of the pH optima for two separate enzyme systems (Yu and Lee, 1986). Increasing tenderness from 6 to 7 is attributed to greater calpain activity, while increasing tenderness below 6 is attributed to greater acidic protease activity (Yu and Lee, 1986). Secondly, non-enzymatic reasons have been suggested, including the lengthening of sarcomeres as ultimate pH decreases below 6.2 (Purchas, 1990). Sacromere length was highly correlated to shear force (Yu and Lee, 1986) and thus could be used as a tenderness indicator, but only for muscles with the same ultimate pH.

1.1.9 Protein Functionality

There are three categories of muscle protein based on solubility and function

including sarcoplasmic, myofibrillar, and stromal (Pearson and Young, 1989). These proteins are located in the myofibril and extend the length of the myofibril, surrounded by sarcoplasm (Pearson and Young, 1989). Myofibrillar proteins (salt soluble) make up 50-60% of the extractable muscle proteins, with myosin making up 50% and actin 20% of the myofibrillar structure (Pearson and Young, 1989). Actin and myosin are contractile proteins responsible for muscle contraction, other myofibrillar proteins fulfill the role of regulation of contraction, and some myofibrillar proteins provide the structure of the myofibril (Pearson and Young, 1989).

The stiffening of muscle during rigor mortis occurs from permanent cross bridges formed between actin and myosin, resulting in an actomyosin complex (Pearson and Young, 1989). The denaturation of myosin impacts the soft texture and elevated purge loss from PSE meat (Offer, 1991). As actin and myosin join, the myosin head shrinks, and this added shrinkage occurs when the body temperature remains high, creating a higher degree of protein denaturation (Offer, 1991). The thick and thin filaments are joined closer together in PSE meat, and more water is released than from normal condition filament spacing (Honikel et al., 1968). Camou and Sebranek (1991) found that the impact of myofibrillar denaturation impacts protein functionality, including 13% water loss, and 11% loss in protein solubility. Reduced solubility of myofibrillar proteins is caused by the decreased extractability of the myosin heavy chain (Warner et al., 1997).

Sarcoplasmic proteins (water soluble) are approximately 30-35% of extractable muscle proteins and are found in the sarcoplasm (Pearson and Young, 1989). Joo et al. (1999) found that lightness (L^*) value decreased with increasing sarcoplasmic protein

solubility and that sarcoplasmic protein denaturation may also effect WHC to a small degree. Sarcoplasmic solubility increased with increasing ultimate pH (Joo et al., 1999), further supporting the relationship of pH and muscle protein denaturation. At low pH and high temperature, phosphorylase was specifically found to be one sarcoplasmic protein which experienced decreased solubility and reduced activity from its denaturation (Fischer et al., 1979).

1.2 Traditional Enhancement Methods to Improve Meat Quality

1.2.1 Enhancement

Solution enhancement technology has been utilized for decades to provide consumers more tender and juicy meat products (Sheard et al., 1999). Enhancement involves the use of a non-meat ingredient to interact with meat proteins, impacting properties including protein extraction, solubilization, and water retention (Zayas, 1997). Protein solubility is dependent upon the physicochemical state of the protein molecule, and is the determining factor of the effectiveness of protein extraction from raw materials (Zayas, 1997). Protein solubility is measured by the concentration of protein in aqueous solution (Zayas, 1997). Mechanical treatments including mixing, tumbling, massaging, and mechanical tenderization all contribute to cell disruption and breakage with an ultimate release of myofibrillar and other proteins, allowing for more cohesive bonds to be formed within the protein matrix (Zayas, 1997). Water retention is dependent on the volume of the myofibril, as well as pH, which reaches a minimum at the isoelectric point and rising on either side of it (Zayas, 1997). Denaturation of the muscle proteins at low pH will cause a reduction in protein solubility and

will ultimately impact water binding (Zayas, 1997). Enhancement methods manipulate the processing environment to alter these conditions which impact protein functionality. By controlling the physiochemical environment of the protein, a positive impact can be made upon flavor, palatability, and color (Baublits et al., 2006b).

1.2.2 *Water*

The reduction of reactive groups on proteins available for water binding occurs when the pH approaches the isoelectric point (Alberle et al., 2001). At this point, reactive groups are only attracted to each other and any proteins above or beyond this level are available to attract water, therefore only charged hydrophilic groups on proteins attract water, called the net charge effect (Alberle et al., 2001). An increase in repulsion between negatively charged groups causes an opening of the proteins and increased interaction with water (Zayas, 1997). Thus, water retention is affected by the ionic strength, with an increase in water holding occurring as the concentration of neutral electrolytes goes down (Zayas, 1997). Besides improving water holding, and therefore impacting sensorial quality of a cooked meat product, water is also important in enhanced meats to serve as a carrier to solublize enhancement ingredients, and to replace moisture lost during processing, especially thermally (Alberle et al., 2001). Expressible moisture, often associated with WHC, may be partially responsible for many other physical and structural properties, including color, texture, firmness, and tenderness (Alberle et al., 2001).

1.2.3 Sodium Salt

Salts function in enhanced meats to develop flavor, increase moisture retention, increase ionic strength, and solublize myofibrillar proteins, thus enhancing protein binding (Alberle et al., 2001). Boles and Swan (1997) reported that a NaCl solution increased cooking yields, decreased post mortem pH decline, and increased water binding. Salt solublizes myofibrillar proteins by increasing the electrostatic repulsion between the filaments, thus alleviating some of the structural constraints and improving binding (Babdjji et al., 1982). Sodium chloride functions to open the protein matrix, allowing for an increase in the number of side chains available to bind to water, thus increasing WHC (Killefer et al., 2004). The exposure of the protein matrix occurs with increasing pH and ionic strength to retain water (Alvarado and Sams, 2004). More water can be retained by proteins if chloride ions are bound to proteins at pH's above the isoelectric point causing an increase in negative charge (Zayas, 1997). Offer and Trinick (1983) reported that salts and phosphates have a synergistic effect in increasing water holding from the disassociation of actomyosin to expose more water binding sites. They found that the presence of 10mM tetrasodium pyrophosphate halved the concentration of NaCl needed to elicit swelling and water uptake in isolated myofibrils. Detienne and Wicker (1999) found a salt-phosphate interaction for weight gain, purge, cook loss, and expressible moisture. Offer and Trinick (1983) found that .08-1.0 M, 4.6-5.8% salt is needed to achieve maximum water holding, but good functionality can be achieved at .4-.6 M (Trout and Schmidt, 1983).

1.2.4 Calcium

The addition of exogenous calcium to early postmortem muscle improves tenderness (Lawrence et al., 2003). The calcium activated calpain enzyme system that degrades Z disk proteins provides the degradation necessary to promote tenderization (Koohmaraie, 1994). Most calcium enhancement research has utilized calcium chloride as its tenderization source. Wheeler et al., (1993) found that the optimum injection rate of calcium chloride was 200mM at 5% into 24h postmortem beef. However, there have been problems associated with the use of calcium chloride. Adverse discoloration occurs (Milligan et al., 1997), and is likely due to calcium chloride's oxidative action as a salt (Lawrence et al., 2004). Additionally, off flavors which are metallic and bitter (Wheeler et al., 1996) and increased purge (Pringle et al., 1999) are all associated with calcium chloride use. Therefore, other calcium salts have been studied. Lawrence et al., (2003) compared the use of calcium ascorbate, calcium chloride, and calcium lactate. They found that an injection of 0.1 M calcium lactate provided greater tenderness and palatability traits over calcium chloride and lactate. The incidence of off flavors and color deterioration occurred in 0.1 M calcium ascorbate and calcium chlorides injected at 11%. The use of calcium in poultry increases tenderness, but cooking losses increase as the calcium concentration increase (Young and Lyon, 1997). Phosphates chelate calcium in solution, preventing their combined use in marinades (Lawrence et al., 2004), but the use of a solution of calcium lactate first and then an injection with phosphate could successfully improve palatability. When compared side by side, phosphates and salts solutions provided higher yields, increased water binding ability, and have higher sensory panel scores for tenderness than calcium lactate solutions, however, calcium lactate provides

better initial color and color stability throughout retail display (Lawrence et al., 2004).

1.2.5 Phosphate Mode of Action

In the past 20 years, an awareness of the negative health effects of high sodium products as occurred and the meat industry as reacted by replacing sodium salts with phosphates (Trout and Schmidt, 1983). Phosphates can be added to an enhancement solution at a maximum of .5% by USDA law in the final product to increase sensory attributes of the product (Killefer et al., 2004). Phosphates work similarly to salts, and as previously mentioned, work synergistically with phosphates. Optimizing the combination of phosphate type, phosphate concentration, and salt concentration in an enhancement system will maximize the impact to fresh meat sensorial quality. Phosphates were shown not to increase functional properties when the sodium chloride was greater than 2.0% and between 1.25-1.5% (Trout and Schmidt, 1984). Phosphates do not increase functionality at intermediate and high sodium chloride levels because sodium chloride's high pH and ionic strength increases protein functionality to a maximum (Trout and Schmidt, 1984). Trout and Schmidt (1983) cited (Hamm, 1960, 1970) to summarize the effects of phosphates for their ability to increase pH, increase ionic strength, bind proteins, and dissociate actomyosin.

Increasing the pH of meat from 4.0 to 7.0 results in a progressive increase in water binding capacity (Hamm, 1970). The small pH increase produced specifically by phosphates, by approximately .1-.3 pH units, produces only a small increase in water binding (Hamm, 1970). Phosphates that can increase pH of a protein product to the upper limits by moving the isoelectric curve to a more alkaline environment will be most effective in

increasing water binding (Trout and Schmidt, 1983). Water binding is lowest at the isoelectric point, 5.4, and increases as muscle pH increases (Zheng et al., 2000). Increasing the pH of meat increases its functional properties that are associated with water binding, including moisture, tenderness, and texture (Torley et al., 2000).

Increasing the ionic strength of a meat product increases its water binding, but ionic strength is difficult to measure since phosphates do not completely dissociate in solution (Trout and Schmidt, 1986a). These authors also determined the degree of dissociation of six different phosphates whose chain length varied from 1 to 20.8. They found that as chain length increased, there was a reduction in the degree of dissociation. Therefore, the lower ionic strength of a long chain length phosphate will be less effective at increasing water binding than a high ionic strength short chain phosphate. The increase in water binding (both with and without phosphates) begins when the total ionic strength is approximately 0.4 and continues until the ionic strength is approximately 0.6 (Trout and Schmidt, 1983). None of the phosphates can increase ionic strength to this extent, but when used in conjunction with 1-2% salt, the ionic strength can be maximized (Trout and Schmidt, 1984). Polyphosphates mainly affect the non-denatured muscle proteins, thus changing the ionic condition does not return the denatured proteins to a functional form (Torley et al., 2000). However, a high ionic strength along with the presence of polyphosphates can reduce the differences in functional properties in PSE pork and normal pork, but with a smaller response by PSE pork to these conditions (Torley et al., 2000). Trout and Schmidt (1986b) found that the most important effect of ionic strength was to increase the phosphates' ability to increase cook yield and tensile strength, where tensile strength is calculated as a force required to reach a

certain point of a cross sectional area.

The binding of phosphate anions to proteins has been theorized as a method of increasing water binding (Trout and Schmidt, 1983). Phosphate binding results in an increase in the net negative charge of a protein, causing greater repulsion among the proteins, and therefore an increase in water binding (Hamm, 1970). The extent of non-site specific binding may determine the degree to which the water binding is increased (Trout and Schmidt, 1983).

According to Trout and Schmidt (1983) Bendall (1954) first hypothesized that increased water binding capacity was due to an increase in solubility of muscle as a result of the pyrophosphate induced dissociation of actomyosin. Phosphates other than pyrophosphate have been shown to be effective in increasing water binding as well; however, Trout and Schmidt (1983) found no differences in effectiveness of pyrophosphate, tripolyphosphate, tetrapolyphosphate, and hexametaphosphate, when both ionic strength and pH were taken into consideration.

Of these properties, the ability to bind water is most impacted by pH and ionic strength. The effect of pH and ionic strength is associated with the alpha-helix random coil transition of the rod portion of myosin (Trout and Schmidt, 1983). Increasing the ionic strength tends to depress the pH of the system, and the buffering effect of salt also alters pH (Young et al., 1992). Thus, all factors, including pH change, phosphate concentration, and ionic strength change must be considered when assessing the effects of phosphates on meat protein systems. Water binding increases with increasing ionic strength and pH until the total ionic strength is greater than .6 and the pH of the uncooked product is greater than 6.0

(Trout and Schmidt, 1983). Other measurements of binding quality, including cook yield and tensile strength increase linearly with increasing ionic strength and pH until a plateau is reached. Approximately 80% of the increase in binding is due to the combined efforts of an increase in ionic strength and pH (Trout and Schmidt, 1986b). However, at higher ionic strengths, the general effect of pH is not as apparent on the rate of increasing cook yield and tensile strength (Trout and Schmidt, 1986b).

1.2.6 Phosphate Type

Commonly used phosphates in meat and poultry include tetrasodium pyrophosphate (TSPP), tetrapotassium pyrophosphate (TKPP), sodium tripolyphosphate (STPP), and hexametaphosphate (SHMP or GLASS) (Zheng et al., 2000). These condensed phosphates are composed of two or more phosphate atoms bonded by a shared oxygen atom (Zheng, et al., 2000). Of these, STPP is the most used in the poultry industry (Zheng et al., 2000), and is often combined with longer chain phosphates for a sustaining effect. The active form of phosphates is thought to be pyrophosphate (Zheng et al., 2000). As a generalization, the different phosphates increase functional properties in the following order: pyrophosphate > tripolyphosphate > tetrapolyphosphate > hexametaphosphate (Trout and Schmidt, 1984). Bernthal et al., (1991) showed an increase in ultimate pH, water binding capacity, and extractable proteins as TSPP concentration increased from 0 to 0.5%. TSPP produce good binding ability because of their high pH (around 11) (Alvarado and Sams, 2003), and these high pH phosphates can improve PSE poultry breast meat. Zheng et al., (2000) studied the phosphate type and its impact on tenderness and yield. They found that TSPP injected

poultry breast had the highest final product yield when compared with GLASS and STPP and had similar effects on purge, net weight increase, and moisture retention as STPP. GLASS injected breast had the lowest retention and moisture content (Zheng et al., 2000). TSPP and STPP have been found to increase pH about .2 units (Zayas, 1997).

1.2.7 Phosphate Level

Purge loss is significantly impacted by pump rate (10%, 6%, 12%, and 18%) and pH (Brashear et al., 2000), such that as pump rate increases, vacuum package purge increases, but higher raw material pH reduced purge. Sheard et al., (1999) reported a higher pH in pork loins injected at 10% compared to untreated loins, but reported no difference in pH between loins with 5% pump rate, suggesting a pump rate greater than 5% for differences in meat quality to begin to occur. Enhancement at an 18% pump rate allowed for improved overall tenderness compared to a 12% pump rate (Baublits, 2005a). More specifically, steaks enhanced at 18% pump level with 0.4% phosphate concentration compared to 0.2% performed better in sensory tenderness ratings (Baublits, 2005b). Baublits et al., (2006b) reported that loins enhanced at a 12% pump rate had a higher pumped yield and cook loss as a 6% pump rate, but there were few differences in sensory characteristics or consumer acceptance, indicating no improvement in palatability with increasing pump rate from 6% to 12%. These data suggest a minimum pump rate is necessary to improve meat quality; however, molar concentration of the phosphate plays a more instrumental role in improving consumer acceptance of product than pump rate alone.

1.2.8 Phosphate Effect on Texture

The presence of phosphate in a brine solution results in more tender beef roasts, with less energy to break the sample and lower peak shear force values, than calcium chloride, salt, sodium lactate, or water alone (Boles and Swan, 1997). Young et al., (1987) found that Texture Profile Analysis measurements including cohesiveness, springiness, and chewiness were increased at the highest concentration of phosphate (.5%) in the absence of NaCl but in the presence of NaCl, the phosphate tended to increase cohesiveness and chewiness at lower phosphate levels. The ability of phosphates to alter textural properties is greater at a pH value near the pKa of the phosphate (Young et al., 1992).

Texture measurements including shear stress at fracture, and cook loss are also impacted by phosphate use. As ionic strength increases there is an increase in gel strength and a decrease in cook loss (Torley et al., 2000). Cook loss can be defined as a percentage of “green weight” or as cooked weight divided by uninjected weight times 100 (Boles and Swan, 1997). Young et al., (1987) reported a cook loss reduction by 22% in chicken patties containing .5% STPP and 1.5% NaCl but by only 6% in patties containing .5% STPP with no salt. Lamkey et al., (1986) found that salt and phosphate (.2% each) decreased cooking loss and increased bind equal to phosphate (.5%) alone. These results show the importance of using salt and phosphate combined for maximum protein functionality. Cook loss is independent of the concentration of functional muscle protein whereas shear stress (which is a measure of gel strength) is dependent on the concentration of functional proteins (Park et al., 1993). In shear stress values, the strength of the heated gel is dependent on the amount of un-denatured muscle protein available to form the protein network structure prior to heating

(Park et al., 1993). Therefore, the lower un-denatured protein concentration in PSE meats results in a weaker gel. Salts reduce cook losses by altering the charge and solubilizing un-denatured proteins, allowing the tissue to bind more water (Offer and Trinick, 1983). Phosphates also reduce cook loss by solubilizing protein and increasing muscle pH, to increase water binding capacity (Hamm, 1960).

1.2.9 Phosphate Effect on Color

Improvements in palatability with phosphates can be offset by poor instrumental color defined in the $L^*A^*B^*$ color space and retail display characteristics (Baublits et al., 2005a). STPP at 0.4% was more effective than SHMP or TSPP in maintaining color of beef biceps femoris similar to untreated steaks which had higher L^* values over phosphate treated, therefore untreated steaks still have better color characteristics than enhanced beef steaks (Baublits et al., 2005b). Sodium pyrophosphate and sodium tripolyphosphate decreased the Hunter L and B values and decreased the A value in raw beef but have no effect on the Hunter color values in cooked beef (Jun Lee et al., 1998). Polyphosphate treatment results in meat that is darker and less red than controls in cooked chicken breast (Young et al., 1996). TSPP/NaCl injection produces darker color meat in beef triceps brachii steaks (Baublits et al., 2006b). Baublits et al., (2006c) found a linear relationship for $L^*A^*B^*$ values and vividness to decline with increasing salt concentration, but steaks enhanced with STPP and .5% NaCl were similar in A^* values and vividness to untreated samples. Detrimental color of meat is a negative impact of phosphate use.

1.3 Meat Protein Isolate Enhancement

1.3.1 Protein Source

There is a growing interest in the use of muscle proteins as ingredients because of their functional and nutritional properties (Hultin and Kelleher, 2001). Protein sources which are not currently used in human foods because of their instability due to lipid oxidation and poor sensorial qualities including dark color, strong flavor, and poor texture can be processed to produce a stable protein concentrate for human consumption. Sources of protein include fish fillet waste, deheaded and gutted fish, krill, chicken, and pork (Hultin and Kelleher, 2001). Particularly, a large amount of deboned chicken meat is produced from the skeletons of birds after removal of retail muscle cuts, and there is very little usage of this deboned chicken; a process involving acid and or alkaline extraction could produce protein rich product from this waste material (Hultin and Kelleher, 2001).

1.3.2 Surimi Processing

“Surimi is a refined fish protein product prepared by washing mechanically deboned fish to remove blood, lipids, enzymes, and sarcoplasmic proteins” (Kataoka et al., 1998). The Japanese found that mechanically deboned, or minced fish, could be transformed into a highly functional protein by water leaching and stabilizing with sugars to endure harsh frozen storage (Lanier, 1985). Pieces of surimi protein can be bound together to simulate the appearance and texture of whole muscle products, or to create new products with unique textures (Foegeding et al., 1996). This protein is often formed to create imitation crab product, so much so that in 1982 and 1983, the United States imported more surimi based

simulated crabmeat from Japan than was actually produced in real king crab meat (Lanier, 1985). Besides being the main component of imitation meats, surimi's heat gelling properties can be useful as a binding ingredient in processed meats (Lanier, 1985). Muscle protein flavor can be blander than soy protein, and is superior in nutritional quality over vegetable proteins (Lanier, 1985).

The bond between meat pieces in processed meats is affected by the heat induced gelation of soluble myofibrillar protein (Foegeding et al., 1996). Myofibrillar proteins become concentrated in surimi manufacturing, and form a strong gel network when solubilized with salt and heat (Kataoka et al., 1998). A gel network formed primarily by denatured myosin entraps other non meat ingredients and contributes to the structure and texture of gelled meat products (Foegeding et al., 1996). The heat induced gelation of soluble myofibrillar protein is dependent on the swelling and dissolution of proteins to provide sites for protein-protein interactions (Foegeding et al., 1996).

Surimi processing takes minced fish and adds from 3 to 5 parts fresh water to every one part meat. The water should be less than 10°C, and a pH of 6.8-7.0 since high pH may result in partial denaturation of proteins and a loss in gelling properties, while low pH can result in loss of desirable proteins (Lanier, 1985). The water pH can be adjusted with sodium bicarbonate, or food grade hydrochloric acid or sodium hypochlorite. The fish meat is commonly washed three times, each time at the same meat and water ratio. 20-30% of the fish muscle is solubilized when the ground muscle is washed with water; these soluble proteins (sacroplasmic proteins) are not recovered from the wash water of the process (Hultin and Kelleher, 2001). This loss is not desirable since sacroplasmic proteins could be useful as

a food. Between washes, the meat is allowed to settle and then decanted to separate the water from the meat. After the final washing step, a high speed centrifuge removes any final water. A high speed centrifuge allows for higher protein contents (thus lower water contents) to be attained, to yield a concentrated surimi which is approximately 24% protein (Lanier, 1985). Cryoprotective substances are then added, and then the meat is shaped into blocks by extrusion and frozen.

Cryoprotectants prevent the denaturation of a protein during freezing, frozen storage, and thawing (Hultin and Kelleher, 2001). An indicator of cryoprotective action of an ingredient against protein denaturation is the maintenance of the extractability of salt soluble proteins during frozen storage (Park et al., 1988). Common cryoprotective substances are 8-9% of a 50:50 sucrose and sorbitol mix (Lanier, 1985). The stress at failure measurements, which relates the strength of a surimi gel, is similar from the use of polydextrose as the more commonly used sucrose and sorbitol mix (Park et al., 1988), therefore, there is positive results from the use of polydextrose as a cryoprotectant that would contribute no sweetness to the surimi and would contain only ¼ the calories of sugar and sorbitol (Lanier and Akahane, 1986). The use of polydextrose may also stabilize proteins from mechanically deboned chicken, beef, and pork, which are all potential starting materials for isolate manufacture (Lanier and Akahane, 1986).

Surimi processing has been unsuccessful on unconventional raw materials, such as pelagic species, due to the abundance of unstable lipids and pro-oxidants (especially heme proteins), which result in poor color and high oxidation (Hutlin and Kelleher, 2002). Using conventional methods to process fish has led to underutilization, since much of the protein

material is lost and not recovered for human use.

1.3.3 Acid and Alkali Aided Processing for Protein Recovery

Acid or alkaline extraction to create muscle protein isolate is based on the concept that muscle protein can be diluted with water and treated at either high or low pH to achieve a low viscosity and then centrifuged to yield a protein free of membranes and lipids (Hutlin and Kelleher, 2000). This particular process was developed to produce protein isolates from fish sources of low value, which helps alleviate some of the problems associated with surimi processing (Kristinsson et al., 2005). This process relies on the pH dependent solubility properties of fish protein for their separation and recovery from other muscle components which are not desirable in a protein isolate product (Kristinsson et al, 2005).

The method for this processing is given in detail in US Patents 6, 451,975, 6,136,959, and 6,288,216. As a generalization, the process involves subjecting homogenized muscle to low pH (2 to 3.5) or to high pH (10.5 to 11.5). These pH values allow the protein to be solubilized and disrupts the cellular membrane holding the myofibrillar proteins. These pH values are far enough way from the isoelectric point (pH 5-6) that the protein side chains gain a charge, which causes solubilization through the repealing of proteins. As the proteins are solubilized, the viscosity decreases, which allows membranes, bones, scales, and fat to be separated from the soluble proteins by high force centrifugation. The resulting membrane and lipid free soluble proteins are then recovered by isoelectric precipitation by raising the pH to 5.5, and the resulting protein isolate can be used as a functional food ingredient (Kristinsson and Hultin, 2003c).

1.3.4 Differences from Surimi Processing

Acid and alkaline processes produce greater protein recoveries in comparison to surimi processing (Kristinsson et al., 2005). Similarly, Choi and Park (2002) obtained 20% higher protein recovery when using an acid aided process over 3 wash surimi processing. Surimi processing, which involves multiple washing steps, loses water soluble sarcoplasmic proteins in the wash water, and therefore, recovery/yields are low (Kristinsson et al., 2005, Kim et al., 2003). The varying levels of connective tissue in the starting material could have an impact on protein recovery, such that if connective tissue is excluded from yield calculations, a higher recovery is obtained (Mireles Dewitt et al., 2002). The presence of connective tissue is undesirable for land animal muscle protein because it adversely effects gel formation and strength (Mireles Dwitt et al., 2002). However, the acid and alkaline aided processes have been shown to be very effective in removing the majority of connective tissue (collagen) found in cattle (Mireles Dewitt et al., 2002). Highly soluble proteins will produce greater processing yields (Kim et al., 2003).

Acid and alkaline processes have greater lipid reduction capabilities in comparison to surimi processing (Kristinsson, 2004). These processes are more effective since at high and low pH, solubilized proteins are separated from storage lipids and membrane phospholipids, and are easily separated by centrifugation on the basis of density and solubility differences (Kristinsson et al., 2005). Lipid removal aids in the stabilization of the isolate (Undeland et al., 2003).

Acid and alkaline aided processing gives myofibrillar proteins, specifically myosin, improved emulsification properties, including surface hydrophobicity and interfacial activity

(Kristinsson and Hutlin, 2003b). Myosin, with the rod portion of the molecule having high charge, is sensitive to changes in pH, and pH changes will impact the solubility properties of the protein (Kristinsson and Hultin, 2003c). Myosin changes including the unfolding of the myosin rod, enhancing gel strength and emulsification (Kristinsson and Hultin, 2003b). Myosin takes on different molecular composition based on its exposure to acid or alkaline, such that at low pH, myosin light chains are completely dissociated, and at high pH, only half of the light chains are dissociated, therefore, manipulation of the processing parameter can control the functionality of the protein (Kristinsson and Hultin, 2003c).

1.3.5 Differences Between Acid and Alkaline Aided Processing

Acid processed isolate have poorer gel forming ability than alkali processed isolate (Theodore et al., 2003, Kim et al., 2003, Kristinsson, 2004, Perez-Mateos et al., 2004). Gelometer stress values are higher for alkali than acid produced gels, which have been proposed to be from the retention of more myosin heavy chains in alkaline processing (Undeland et al., 2002). Gel strength was also found to be stronger in alkali isolates over acid isolates in a study performed by Davenport et al. (2004), and was dependent on the separation of insoluble components, such that insolubles like membranes and connective tissues increases the protein-protein interaction during the cooling phase of gelation at high and low pH. Similarly, Yongsawatdigul et al., (2001) obtained stronger and more elastic gels with alkali produced isolates. Specifically, disulfide linkages appeared to be most predominant in alkaline treated gels, which correspond to high punch force test data (Yongsawatdigul et al., 2001). The development of gel formation in alkali isolates positively

impacts preference scores of texture over acid isolates in sensory analysis of the two processes (Ramsoey et al., 2005).

Strong gel formation is attributed to the increase in electrostatic repulsion between muscle proteins, which opens up the gel matrix structure and allows water to enter (Kristinsson and Hultin, 2003b). Therefore, as pH increases away from the isoelectric point in either direction, an increase in a gel's ability to hold water also increases (Feng and Hutlin, 2001). The ability to increase water uptake and capacity by protein gels will enhance the physical properties of an enhanced meat system.

Studies have been performed to find the optimum conditions for solubilization and precipitation. Undeland et al., (2002), found that 92% of herring light muscle protein was solubilized at pH 2.7 with 96% recovery during precipitation at 5.5, while only 89% of proteins were solubilized at 10.8 with 94% recovery. The theoretical maximal protein recovery in these procedures is determined by the difference between the total amount of proteins that become solubilized at acid or alkali pH and the amount of proteins that remain in solution during precipitation (Undeland et al., 2002). This aggregation of proteins during pH adjustment is thought to be induced by hydrophobic forces (Undeland et al., 2003). Catfish proteins were found to have the highest solubility at pH 2.5 and 11 (Kristinsson et al., 2005). The differences between maximum solubility at different pH values could be attributed to the species used in these studies, which could contain a higher degree of dark muscle over the preferred light muscle source. Dark muscle tends to be more unstable in processing since it has a higher concentration of heme monomers, contributed by hemoglobin and myoglobin (Hultin et al., 2005). Protein solubilization arises from the formation of net

positive (Acid aided) or net negative (alkaline aided) changes. These charges create electrostatic repulsion that forces the molecules apart and allows more binding sites for water, which increases surface hydration, and thus solubility (Undeland et al., 2002).

Acid protein isolate is more susceptible to lipid oxidation than alkali, due to more retention of denatured heme proteins (Kristinsson, 2004). The low pH required in the acid process can lead to oxidation of the hemoglobin, thus muscle tissue exposed to low pH should be kept to a minimum exposure time in the process (Hultin et al., 2005). The alkali process is more successful in lipid reduction compared to the acid process (Kristinsson et al., 2005), however, one study comparing the two processes resulted in the acid isolates having less rancid odor and tastes (Ramsoey et al., 2005).

1.3.6 Advantages of Acid and Alkaline Aided Processing

As outlined in U.S. patent 6,451,975, the acid and alkaline aided processing improves upon traditional surimi processing in several ways. First, aged or frozen muscle can be utilized, which allows for a wider array of starting material to be used. This new process provides for increased yield of protein. Greater than 90% of total muscle protein is typically obtained from light muscle sources; whereas surimi processing provides less than 60% protein recovery. The improved yield of protein means that there is less protein to recover/remove from waste water; therefore is an improvement by a cleaner process in which by-product pollution is decreased. Most importantly, this process solubilizes approximately 98% of the muscle protein, whereas a majority of muscle proteins, especially sarcoplasmic, are insoluble in the surimi process. This almost 100% solubilization allow for the complete

removal and separation of bone or skin fragments from the desirable protein fraction. The removal of nearly all of the lipids stabilizes the product against oxidation, which makes this process especially useful for fatty muscle tissue, typically of lower cost than lean protein, as a starting protein source.

1.3.7 Implications to Meat Enhancement

The addition of protein ingredients in meats is regulated under Title 9 of the United States Code of Federal Regulations. Proteins are utilized as ingredients in a growing number of foods (Swaigood, 1996). All biologically produced proteins can be used as food protein ingredients, but cost and ability to function in food applications must be considered for choosing the correct protein to be incorporated into a product (Damodaran, 1996). Proteins of animal origin, including those from muscle, milk, and egg are used often in fabricated foods for their capability to perform multifunctionally (Damodaran, 1996).

Protein functions such as gelation, water absorption, and solubility are related to the hydration property of a protein molecule, which impacts the water protein interactions in the product (Swaigood, 1996). The hydration capacity of a protein is related to the amino acid composition, such that the greater the number of charged groups on the protein, the greater the protein's hydration capacity (Damodaran, 1996). This hydration property is particularly important for high water systems such as meats, which need to maintain and utilize its natural water for the overall sensorial acceptance of the product. Textural and succulence characteristics of meat products are dependent on muscle proteins (Damodaran, 1996).

The potential for a muscle protein isolate to be used in meat enhancement holds great

promise. As the use of proteins have gained mainstream acceptance in the food industry as functional ingredients, the use of a muscle protein isolate can also find favorable acceptance when this technology proves to provide function and efficiency over currently accepted methods of enhancing meat.

1.4 Sensorial Quality of Enhanced Fresh Meat

1.4.1 Tenderness

Tenderness or toughness is a function of the solid or water insoluble structures of muscle and is directly related to the protein structures of muscle and their denaturation (Deatherage, 1963). The sensation of tenderness is complicated since chewing not only involves cutting and grinding, but also shearing and tearing, which involve vertical and lateral movements of the jaw (Pearson, 1963). Both sensory evaluation as well as mechanical methods such as Warner Bratzler Shear can be used to estimate meat tenderness. In a study conducted by Boleman et al., (1997), consumers were able to tell the difference between three shear force categories of tenderness, and the tenderest steaks had the higher juiciness and flavor ratings, along with greater overall satisfaction. There is an interaction between end point temperature and categories such that Warner Bratzler Shear force increases due to increased end point temperature in less tender steaks than in more tender steaks, thus, temperature abused meats which are inherently less tender will suffer more from poor eating quality (Wheeler et al., 1999).

Sheard et al., (1999) reported that phosphate injected pork roasts were more tender

than non-enhanced fresh cooked pork roasts. Improvements in tenderness scores from precooked, reheated pork chops with enhancement technology has also been observed over freshly cooked controls (Prestat et al., 2002a). However, others have reported no impact on tenderness, specifically shear force values, from phosphate based injection (Baublits et al., 2006b, Lawrence et al., 2003). These conflicting results could be due to the different muscle used as well as the cookery method. In general, tenderness of polyphosphate treated meat samples can be attributed to a weakened muscle structure and a higher water content of the cooked sample (Sheard et al., 1999). Tenderness can be improved in pork chops with the addition of .25 -.5 g of polyphosphate per 100 g of meat (Sheard et al., 1999).

1.4.2 Juiciness

When red meat is tender, juiciness is the next quality of interest for consumers (Ouali et al., 2006). Juiciness can be defined as the amount of perceived juice that is released from the product during chewing (Miller, 1998). The amount and state of immobilized water in the muscle impacts the perceived juices and is often measured through the use of nuclear magnetic resonance (Borisova and Oreshkin, 1992). Juiciness depends on the movement of intracellular water towards the extracellular space, assuming that as the pH becomes more acidic and closer to the isoelectric point of myofibrillar proteins that they will release their bound water (Bertram et al., 2004). Aaslyng et al., (2003) found that juiciness experienced initially in the chewing process depended on the water content of the meat, but juiciness experienced later in chewing was determined by a combination of water and intramuscular fat contents. From nuclear magnetic resonance studies, the reduction in juiciness at higher

temperatures (Bejerholm and Aaslyng, 2003) is theorized to be from the change in pore size confining the myofibrillar water together (Bertram et al., 2005), therefore these alterations to the myofibrillar structure organization is the mechanism for the sensorial perception of juiciness. Several studies have shown that tenderness and juiciness are significantly increased in enhanced meats (Hayes et al., 2006, Vote et al., 2000, Robbins et al., 2002, Wright et al., 2005). However, injection with tripolyphosphate alone does not improve tenderness or juiciness, and tends to impart off flavors characterized by trained panelist as soapy and sour (Vote et al., 2000).

1.4.3 Meat Flavor

Meat flavor arises from the combination of lipids peroxidation with amino acids and peptides generated by proteolysis (Campo et al., 2006). The effect of phosphate on pork flavor has been theorized to be related to the reduction of oxidative rancidity (Keeton, 1983). Pork enhanced with salt and phosphates have higher overall flavor ratings in comparison to non-injected controls (Hayes et al., 2006). pH plays a role in this development of meat flavors. Muscles from high pH beef carcasses typically have less beef flavor, less brown roasted flavor, and more rancid flavors than normal pH muscle (Yancey et al., 2005). Polyphosphate use, which typically increases meat pH, decreases pork flavor and increases abnormal flavor intensity (Sheard et al., 1999), with a theory that soluble flavor precursors are carried away with the excess solution which is not held in the meat after needle injection. Oppositely, in phosphate treated pork, abnormal flavors could be more apparent because of the overall lower pork flavor intensity (Wood et al., 1995).

Flavor can be impacted by end point temperature. Prestat et al., (2002b) found that pork flavor increases as endpoint temperature is increased from 70 to 80°C for enhanced samples, but remained constant for unenhanced samples (Prestat et al., 2002b). It has been suggested that as pork flavor intensity increases from higher endpoint temperature, abnormal flavors are masked (Wood et al., 1995). This is supported by the work of Sheard et al., (1999) who showed that as internal temperature increased, pork flavor increased while tenderness, juiciness, and abnormal flavor intensity decreased. A core temperature of 65°C is preferred if the focus is on flavor components, while 75°C is recommended if overall sensory property discrimination is the focus of sensory analysis (Berjerholm and Aaslyng, 2004).

Cookery method also effects meat flavor. Off flavors are lower when chops are fried rather than grilled (Prestat et al., 2002b). Additionally, pork flavor is more intense in fried than in grilled chops from pumped loins, and in pumped chops cooked by either frying or grilling compared to unpumped chops (Prestat et al., 2002b). Prestat et al., (2002b) suggests that frying generates unique flavor compounds which mask potential off flavors, and to observe a significant change in off flavors, a threshold phosphate concentration must be reached. When comparing cookery methods, it was found that odor and flavor are the most effected sensorial traits, and that pan frying provides the best observations on appearance, flavor, and texture over low temperature oven cooking (Bejerholm and Aaslyng, 2004).

1.4.4 Muscle pH

Marinating at high pH (6.0-6.3) improves the sensory perception of tenderness and

juiciness (Cannon et al., 1993). In addition to the improved sensory scores, high pH also improves objective Warner Braztler Shear measurements such that the mechanism of water holding capacity above and below the isoelectric point affects the ultimate tenderness (Cannon et al., 1993). The influence of increased pH on the water holding capacity of meat away from the isoelectric point of the myofibrillar proteins, increases WHC, and therefore cooked meat tenderness (Gault, 1985).

The pump rate has been found to have an effect on pork loin pH. Baublits et al., (2006a) found that loins had a higher pH at a 12% pump rate compared to untreated loins, but that there was no difference in pH between a 6% pump rate and untreated loins. Similarly, Sheard et al., (1999) reported higher pH in pork loins injected at a 10% pump rate compared to untreated, but no difference in pH between untreated and treated loins at 5% pump rate. These reports suggest that at low pump, pH is not affected, but at higher levels of pump, pH is impacted, and therefore, other intrinsic quality measures such as previously discussed are also affected.

1.4.5 Marbling

Marbling, or intramuscular fat, is deposited within the muscle in loose networks of connective tissue (Alberle et al., 2001). A trained panel performed by Hodgson et al., (1991) gave the highest rating of overall palatability to pork chops with high intramuscular fat (9.1%), low protein, and low moisture. In addition, juiciness and flavor are rated higher in more highly marbled beef (Killinger et al., 2004). Consumer overall acceptance scores decrease as marbling is decreased in beef (Savell et al., 1987, Killinger et al., 2004). The

likelihood of consumer acceptance of beef steaks increases approximately 10% for each full marbling score increase between Slight to Slightly Abundant (Platter et al., 2003).

Fernandez et al., (1999b) reported that pork texture and taste are enhanced at intramuscular fat levels up to 3.25%. They also found a trend towards a positive effect of high intramuscular fat on flavor, tenderness, and juiciness in pork; however, intramuscular fat levels had to reach a value above 2% before any noticeable effect on sensory quality could be detected (Fernandez et al., 1999a). An increase in intramuscular fat levels is associated with an increase in the visual perception of fat, and therefore a corresponding decrease in the willingness to purchase and eat the meat (Fernandez et al., 1999b, Brewer et al., 2001). The acceptability of pork may be improved by increasing intramuscular fat but the effect decreases at levels greater than 3.5% which is associated with a high risk of meat rejection due to visible fat, and the positive effect of intramuscular fat also holds true as long as there is not a additional increase in the level in intermuscular fat (Fernandez et al., 1999b).

1.4.6 Final Internal Temperature During Cooking

Cooking induces structural changes which decrease the water holding properties of meat and is impacted by final internal temperature as well as cooking method, cooking time, and the amount of connective tissue in the meat (Bendall and Restall, 1983). Juiciness assessed at 68°C is related to relaxation of intracellular water, whereas the amount of intracellular and extracellular water is a greater determinant at higher temperatures (80°C) (Fjelkner-Modig and Tornberg, 1986). Consumers report more desirable flavor when pork chops are cooked to a higher temperature, which could be due to decreased blood serum

intensity at higher temperatures, as well as a general preference for pork that is cooked to “medium well” or “well done” (Baublits et al., 2006a).

Cooking to a higher final internal temperature reduces juiciness and tenderness of meat (Vote et al., 2000), thereby increasing Warner Braztler Shear force values (Wheeler et al., 1999). When beef steaks are cooked to a higher end point temperature, there is a more pronounced effect on tenderness and juiciness than if the steak had been cooked at a lower temperature (Vote et al., 2000). In pork, there is a decrease in juiciness as temperature is increased from 71 to 82°C (Baublits et al., 2006a). Raising the center temperature of pork from 72.5 to 80°C increases cooking loss, reduces tenderness, juiciness, and abnormal flavors (Sheard et al., 1999). However, pork containing 5% polyphosphate and cooked to 80°C were more tender and juicy than pork without polyphosphate cooked to at 72.5°C (Sheard et al., 1999). Therefore, phosphates preserve water within muscle even when meat has been temperature abused.

Additionally, pH, ionic strength, and ion type affect the optimum end point temperature since these variables influence the denaturation of meat proteins (Trout and Schmidt, 1987). Increasing these variables increases the temperature that cooking loss first occurs and decreases the temperature required for maximum tensile strength measured by Warner Braztler shear (Trout and Schmidt, 1987). Cooking to an internal temperature of 66°C was found to maximize cook yield and tensile strength (Trout and Schmidt, 1987).

1.4.7 Pump Rate of Brine

Increasing injection level from 5% to 10% has similar effects as increasing

polyphosphate concentration on improving sensorial properties of tenderness, juiciness, and flavor (Sheard et al., 1999). Several studies show the trend of increased tenderness with increased pump rate (Baublits et al., 2005a, Baublits et al., 2006b). However, when comparing pump rates of 6% and 12%, Baublits et al., (2006a) found that both descriptive sensory panels and consumer acceptance panels found no difference in the juiciness category regardless of the endpoint cook temperature. Conflicting reports regarding improvements in sensorial characteristics can be attributed to differences in variable meat source, such as amount of connective tissue found in the sample, or injection difference, such as phosphate concentration.

A pump rate and end point cook temperature interaction was found in a consumer study performed by Baublits et al., (2006a). They found that consumers rated chops pumped at 6% or 12% more acceptable in overall texture than untreated chops regardless of endpoint temperature. However, chops cooked to a low temperature (71°C) at the 12% pump rate were less acceptable for texture than chops cooked to a high temperature (82°C) at the 6% pump rate. Similarly, in regards to tenderness perception, consumers again rated the high temperature, low pump rate chop more superior. They found no difference in Warner Braztler Shear force values between the cook temperatures, but chops cooked to higher temperatures required fewer chews to swallow and had more loose particles than chops cooked at lower temperatures, therefore consumers may have perceived a more tender product from this fact.

1.4.8 Consumer Purchase Intent Towards Enhanced Meats

Small changes in consumer sensory ratings of tenderness, juiciness, and flavor have dramatic effects on the probability of acceptance of beef steaks, where acceptance decreases linearly as ratings on the above attributes decreases from 3 to 5 on a 1 to 9 hedonic scale (Platter et al., 2003). A beef quality questionnaire revealed that tenderness, flavor, and juiciness were most important to eating satisfaction, while color, price, and visible fat were the most important factors underlying actual purchase of meat (Robbins et al., 2003). Therefore, technology which can improve eating satisfaction will not assure first time purchase, but can increase the chances of repeat buying situations.

Brewer et al., (2002) found that the overall eating quality of pork was improved by needle injection of brine containing STPP and salt. Hayes et al., (2006) found that 62% of consumers preferred an enhanced pork chop over a control, and that 60% would buy the product. However, purchase intent has been observed to decrease as pump level of enhancement solution increases (Brewer et al., 2002). In pork, Brewer et al., (1999) found that a wet/dry appearance and color contributed to purchase intent, and that in general, purchase intent paralleled overall acceptability of fresh pork.

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CHAPTER 2

**IMPACT OF PH, FAT PERCENT, IODINE VALUE, AND COLOR ON FLAVOR
PROFILE OF COOKED PORK CHOPS**

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ABSTRACT

Crude fat content (IMF) have been documented as a major variable within muscle which impacts eating quality. A study was conducted on flavor attributes of boneless pork loin chops of known pH, IMF, color, and saturated fat content based on calculated iodine values (IV) to determine how a trained sensory panel would perceive flavor attributes of each treatment. Frozen 2.54 cm thick boneless pork loin chops were grouped into treatments with high and low values for each of 4 variables (n=16). Each treatment group had a pH above or below 5.6, an IMF percent above or below 4%, above or below National Pork Producers Council (NPPC) color score 2.5, and IV value above and below 65.

Sensory flavor profiles for treatments were determined using an established pork flavor lexicon, using a trained panel (n=8) having over 1000 hours of training in the Spectrum method of descriptive analysis. Principal component analysis (PCA) biplots were constructed to visualize the impact of treatments on sensory properties. Bitter, oxidized, salt, and piggy, were identified in the loins used for language generation but these descriptors were not identified in the samples used in this study.

Flavor attributes were composed of cooked pork aroma and umami (positive dimension of PC1) and sweet (negative dimension of PC1) in PC1 (38% variability). PC2 (18%) differentiated the treatments in sour, and to a lesser degree, astringent mouthfeel and cooked pork flavor. The PCA plot indicates that most all of the treatments utilized in the study were similar, except for treatments 1 and 2, which exhibited higher sweetness and lower cooked pork aroma compared to all other treatments. These two treatments had pH below 5.6, an IMF >4%, and an IV value <65.

Of the 7 flavor attributes detected, only 3 attributes – cooked pork flavor, cooked pork aroma, and sweet were significantly different ($P < 0.05$) among treatments, and were characterized by significant three way interactions between variables. None of these interactions have a magnitude difference large enough to provide practical significance to the study; therefore, the contribution of these interactions to have an effect on the flavor of pork in the commercial market would be negligible. These data suggest that crude fat and IV value play a minor role when determining the flavor attributes of cooked pork flavor and aroma, as well as sweet attributes of pork.

2.2 Introduction

Lipid content is often reported as impacting the sensory quality of pork (Flores et al., 1999, Huff-Lonergan et al., 2002, Josell et al., 2003, Fortin et al., 2005, Rincker et al., 2008). Ultimate pH is also reported to impact quality as well (Lonergan et al., 2007). It is well accepted that multiple factors also come into the palatability discussion, including breed, time of aging, rate of pH decline, and muscle type. It is often difficult, however, to understand the simple effect of these variables across studies when many of the independent variables are different from study to study. Nonetheless, many have attempted to understand the relationship that lipid content has with ultimate pH on sensory qualities, with conflicting conclusions. Most of the work done to understand the role of ultimate pH and lipid content on eating quality has been from the perspective of texture attributes.

Flores et al., (1999) reported a close relationship between IMF (intramuscular fat) content and ultimate pH, and that ultimate pH had a close relationship with juiciness. Fortin et al., (2005) and Fernandez et al., (1999), also reported similar favorable relationships between marbling and sensory characteristics. Conversely, Josell et al., (2003) reported that muscle with high IMF was not tender, and that a direct relationship did not exist between IMF and tenderness. In agreement were Rincker et al., 2008 who found that IMF had limited effect on perceived tenderness, and juiciness, with only 13% of the variability of the treatments controlled by varying pH levels explained by IMF.

Little published work draws conclusions on ultimate pH and lipid content and its impact on flavor. Rincker et al., (2008) used a trained panel to score pork flavor, which had the strongest relationship with marbling compared to attributes of tenderness and juiciness,

but still, this relationship was weak, with a R^2 value of only .13. Fernandez et al., (1999) found that pork flavor was significantly increased when IMF values increased above approximately 2.5%. Using a beef model, Yancey et al., (2005) found that beef top blade, top sirloin and tenderloin steaks from high pH (dark cutting) carcasses had less beef flavor and less brown roasted flavor. This is when compared to meat from carcasses with normal (<5.7) pH.

There is a need to gain a greater understanding about the impact pH and lipid content has on meat flavor. Therefore, the purpose of this study was to evaluate the flavor of pork loin chops of a known pH, crude fat content, IV value, and color, and determine if any of these factors, either alone, or synergistically, impact the flavor profile.

2.3 Materials and Methods

2.3.1 Selection of Pork for Study

Pigs (n=185) representative of the commercial industry (2005) were compared to pigs representative of the commercial industry in 1980 (Fix, 2007). The 1980 genetic sample was produced from dams selected to minimize genetic improvements since 1979 and frozen semen from boars available during that year. Pigs within sex, farrowing group, and genetic sample were assigned a feeding program representative of a diet formulation from 1980 with no antibiotics, synthetic amino acids, or added dietary fat; or a diet formulation with a commercial formulation from 2005. At the termination of the feeding trial, pigs were harvested at 116kg. Carcasses were then chilled for 24 h. At 24 h post mortem, pH was measured utilizing a handheld pH meter (IQ Scientific Instruments, San Diego, CA) and

given a NPPC (2000) subjective color score (1-6). Loin adipose tissue was collected from the rib end of the boneless loin, placed in air tight bag, and stored at -20°C until further analysis for calculated iodine value (IV). Boneless loins were placed on ice and returned to North Carolina State University Processed Meat Laboratory. After arrival (approximately 1.5h transport time), fresh loins were vacuum packaged and stored at 0°C for 7 days and then stored at -25°C. Within 7 months, boneless loins were cut into 2.54 cm thick chops. Chops were cut from the anterior portion of the boneless loin, and the first chop was used for intramuscular fat analysis (IMF) in the muscle. This chop was placed in air tight bags and stored at -20°C until further analysis. Chops 2-5 were used for analytical and sensorial tests, identified with pig number and chop number, wrapped in freezer paper, and stored at -20°C. All other chops were labeled with pig number and chop number, wrapped in freezer paper, and placed back into -20°C freezer. Pork chops were grouped into treatments based on a known pH, intramuscular fat % (IMF), National Pork Producers Council (NPPC) subjective color score and calculated iodine (IV) values from this study (Fix, 2007). Samples used for this study were in frozen storage for approximately 2 years.

2.3.2 Iodine Value Analysis

Duplicate adipose samples (0.35 to .50 grams) were placed in 13 x 100 cm test tubes and heated at 80°C for one hour. Fatty acid methyl esters (FAME) were portioned by the addition of two ml of saturated sodium chloride followed by two extractions with 2 ml each of hexane with the combined extracts dried over anhydrous sodium sulfate prior to drying under a nitrogen flush at 40°C. The FAME was redissolved in hexane and transferred to Gas

Chromatography (GC) vials for analysis. A Perkin Elmer Model XL Autosystem Gas Chromatograph (PerkinElmer, Inc., Waltham, MA) equipped with a flame ionized detector and BPX-70 capillary column (SGE, Inc., Austin, TX) 30 cm in length with .25 mm i.d. and .25 micrometer film thickness was used to analysis the FAME. Helium was used as the gas carrier at 20 psi. Injection volume was 1 microliter. The injection was split with a split flow rate of 1 mL/min. Initial oven temperature was 60°C and increased 10°C per minute to 180°C and then 4°C per minute to 235°C. Total run time was 27.7 minutes. Compounds were identified using fatty acid methyl ester standards from Matreya, LLC (Plesant Gap, PA). Response factors were calculated based on the standard with peak areas of sample FAME expressed as a percentage of the total peak area of the standard.

Calculated iodine values (IV) were estimated for FAME obtained from loin based upon the AOAC Standard Method Cc-1c-85 (AOAC Methods, 1990). Iodine value= (% hexadecenoic acid * 0.950) + (% octadecenoic acid * 0.860) + (% octadecadienoic acid * 1.732) + (% octadecatienoic acid * 2.616) + (% eicosenoic acid * 0.785) + (% docosenoic acid * 0.723).

2.3.3 Proximate Analysis

Chops were sent to University of Illinois (Urbana-Champaign, IL) for chemical analysis of intramuscular fat in the loin. Proximate analysis for moisture and fat were conducted on duplicate samples similar to that of Novakofski et al., (1989). Samples were weighed in duplicate and placed into a drying oven at 110°C for at least 24 h (AOAC method 24.004). After drying, the lipids were extracted in an azeotropic mixture of chloroform and

methanol (AOAC method 24.005), and the samples were returned to the drying oven.

Percentages of moisture and extractable lipids were determined by difference.

2.3.4 Preparation of chops

Frozen pork chops, 2.54 cm thick, were grouped as treatments with high and lows for each of the 4 known variables of the (n=16). Each treatment was above or below pH 5.6, above or below IMF 4%, above or below NPPC color score 2.5 and IV value above and below 65. For each of the 16 treatments, 3 different random pig identifications were used, and allotted 2 random chop numbers per pig, allowing for a total of 6 chops to be cooked per treatment. Chops were allowed to thaw for 15 h at 3°C, and then each treatment group was assigned a random three digit sensory panel code. Cooking of chops for the sensory panel followed recommendations of AMSA, (1995). Each chop was weighed before and after cooking. Each set of 6 chops were cooked on Faberware open hearth grill model 150A (Faberware Inc., Bronx, NY). Each chop was removed from grill once internal temperature had reached 70°C, measured by HH21 hand held digital thermometer (Omega Engineering Inc., Stamford, CT) equipped with a Type T thermocouple (Cu-CuNi). Each group of chops was then cut into 1.27cm X 1.27cm X 1.27cm cubes. 4 cubes were randomly placed into each coded plastic lidded cups (n=8) (Solo, Highland Park, IL). Lidded cups were then served to panelist for analysis.

2.3.5 Trained Descriptive Panel

Panelists (n=8) were trained using the Sensory Spectrum (Meilgaard et al., 2007)

method of descriptive analysis and each having over 1000 hours of experience developed a lexicon (Table 2.1) using product specific and non-product specific references. Both food and chemical references were used for training at North Carolina State University Sensory Services. A universal intensity scale was used to quantify aroma, flavor, ranged from 0 (minimum intensity) to 15 (maximum intensity). Prior to tasting treatments, panelists were given 1 training sample, and 2 blind reference samples to begin each session. Treatments (n=16) were presented to the panelists in duplicate over 3 sessions.

Table 2.1. Definitions used by trained sensory panelists to describe the aroma and flavor of pork loins

| <u>Descriptor</u> | <u>Definition</u> |
|-----------------------------|---|
| Cooked uncured pork; aroma | Meaty aromatic associated with uncured lean pork muscle |
| Cooked uncured pork; flavor | Meaty flavor associated with uncured lean pork muscle |
| Piggy/Boar Taint | Wet pig; the musk like aroma associated with boar meat |
| Metallic | Flavor resembling tin or copper penny held in mouth; also blood or serum |
| Oxidized | General term for the oxidized characteristic(s) of food such as cardboard, painty, and stale |
| Astringent | Mouth feel sensation of shrinking, drawing, puckering of skin surfaces of the oral cavity, or tooth coating |
| Sweet | Basic taste stimulated on the tongue by sugars and high potency sweeteners |
| Salt | Salt water; also basic taste on the tongue stimulated by sodium salt |
| Sour | Pungent, sharp aromatic; also the basic taste on the tongue associated with acids |
| Bitter | Taste stimulated by substances such as caffeine or quinine when solubilized |
| Umami | Taste sensation of fullness, savoriness or meatiness elicited by glutamates present in meat |

2.3.6 Analysis of Descriptive Sensory Panel Data

The data were analyzed with a data compression technique for Principal Component Analysis in SAS for Windows (version 9.1, SAS Institute, 2000, Cary, NC). Principal Component bi-plots were constructed to visualize the impact of treatments on flavor (n=12) and texture (n=12) properties. PROC CORR was performed to determine relationships between multivariate sensory descriptors. Further statistical analysis of sensory data was performed using the GLM procedure of SAS (2000). When main effects or interactions were significant ($P < 0.05$), significant differences between treatment means were calculated using the least significant difference (LSD) test. The pH, color, IMF and IV and all interactions between the 4 factors were used in the model along with replicate and panelist to evaluate the effect on flavor descriptive traits.

2.4 Results and Discussion

The sensory language identified and differentiated the pork loins (Table 2.5) in flavor. Principal components analysis (PCA) for the descriptive flavor attributes illustrated differences between treatments and relationships between the attributes in the first two principal components (PCs). PCA is a multivariate data compression technique which plots the greatest variation among the treatments into two dimensions. PC1 and PC2 accounted for 56 % of the total variation in the data. Principal components are shown in Figure 2.1. Treatments (1-16) are plotted as a linear combination of the vertical axis (PC1), and the horizontal axis (PC2). Four terms- bitter, oxidized, salt, and piggy, were identified in the loins used for language generation. However, panelists did not detect these attributes in the

samples used in this study; therefore only 7 attributes which were detected among the samples are examined and analyzed. It is interesting to note that oxidized attribute was not detected in these samples that had been frozen for 2 years.

Flavor attributes were composed of cooked pork aroma and umami (positive dimension of the vertical line PC1) and sweet (negative dimension of the vertical line PC1) in PC1 (38% variability). PC2 (18%) differentiated the treatments in sour, and to a lesser degree, astringent mouthfeel and cooked pork flavor. Treatments 1 and 2 are plotted on the negative dimension of the vertical line PC1. These two treatments could be characterized by strong sweet flavor and weak cooked pork aroma, and the treatment mean table (Table 2.2) supports this graphical result, with both lower mean score of cooked pork aroma and higher mean for sweet attribute. The PCA plot indicates that most all of the treatments utilized in the study were similar, except for treatments 1 and 2, which exhibited higher sweetness and lower cooked pork aroma compared to all other treatments.

Pearson correlation coefficients (Table 2.3) suggest significantly ($P<0.05$) positive correlations between cooked pork aroma, umami, astringent mouthfeel, and metallic flavors. There were negative correlations ($P<0.05$) between cooked pork aroma and sweet. There were negative correlations ($P<0.05$) between sweet and astringent mouthfeel. There was not a significant correlation between cooked pork aroma and cooked pork flavor. This may suggest that while pork may deliver typical cooked pork aromas, the actual flavor of the pork may not be perceived in the same way. High pork aroma scores may not equate to high pork flavor scores. There appears to be a strong negative correlation between cooked pork aroma and sweet attribute, while there appears to be a strong positive correlation with sweet and

cooked pork flavor. Pork flavor in previous literature has not been characterized by strong sweet flavor; rather attributes of sour and astringent have been associated with pork flavor (Baublits et al., 2006). These relationships between pork aroma, pork flavor, and sweet suggest that cooked pork flavor is not determined from specific flavor attributes which are typical to pork. But, cooked pork aroma is dependent on these typically associated pork flavors. Flavor compounds contribute a higher degree of aromaticity than in actual delivery of cooked pork flavor perception. Flavor perception of pork may be due to the detection of aromas rather than actual flavor attributes.

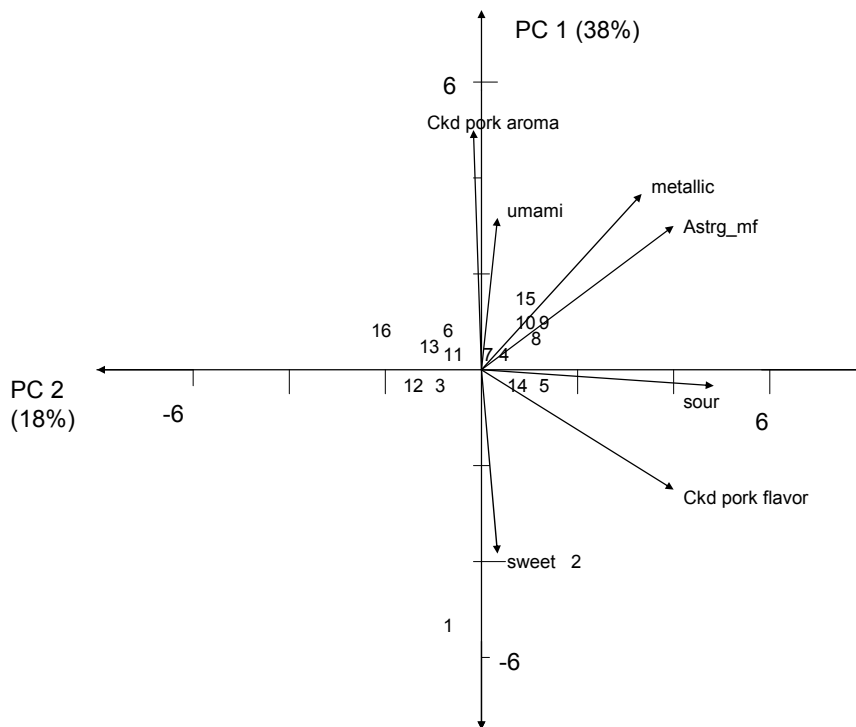


Figure 2.1. Principal component biplot of descriptive sensory flavor analysis of pork loins. Numbers represent treatments (Table 2.2) PC1= principal component 1; PC2= principal component 2.

Table 2.2. Treatments based on pH, fat %, color score, and IV of pork chops

| <u>Treatment</u> | <u>pH</u> | <u>Fat %</u> | <u>Color score</u> | <u>IV</u> |
|------------------|-----------|--------------|--------------------|-----------|
| 1 | <5.6 | >4% | >2.5 | <65 |
| 2 | <5.6 | >4% | <2.5 | <65 |
| 3 | <5.6 | >4% | >2.5 | >65 |
| 4 | <5.6 | >4% | <2.5 | >65 |
| 5 | <5.6 | <4% | <2.5 | <65 |
| 6 | <5.6 | <4% | >2.5 | <65 |
| 7 | <5.6 | <4% | <2.5 | >65 |
| 8 | <5.6 | <4% | >2.5 | >65 |
| 9 | >5.6 | >4% | <2.5 | <65 |
| 10 | >5.6 | >4% | >2.5 | <65 |
| 11 | >5.6 | >4% | <2.5 | >65 |
| 12 | >5.6 | >4% | >2.5 | >65 |
| 13 | >5.6 | <4% | <2.5 | <65 |
| 14 | >5.6 | <4% | >2.5 | <65 |
| 15 | >5.6 | <4% | <2.5 | >65 |
| 16 | >5.6 | <4% | >2.5 | >65 |

Table 2.3. Effect of pH, fat %, color, and IV on descriptive flavor attributes of pork chops

| Treatment | pH | Fat % | Color score | IV | Cooked pork aroma | Cooked pork flavor | Metallic | Astringent Mouthfeel | Sweet | Sour | Umami |
|------------------|------|-------|-------------|-----|------------------------------|-------------------------------|----------|-------------------------|--------------|-------------|--------------|
| 1 | <5.6 | >4% | >2.5 | <65 | 3.48 | 4.59 | 1.45 | 1.36 | 1.71 | 1.45 | 0.66 |
| 2 | <5.6 | >4% | <2.5 | <65 | 3.66 | 4.74 | 1.78 | 1.48 | 1.63 | 1.48 | 0.74 |
| 3 | <5.6 | >4% | >2.5 | >65 | 4.16 | 4.4 | 1.63 | 1.49 | 1.21 | 1.52 | 0.79 |
| 4 | <5.6 | >4% | <2.5 | >65 | 4.08 | 4.3 | 1.82 | 1.54 | 1.23 | 1.6 | 0.75 |
| 5 | <5.6 | <4% | <2.5 | <65 | 4.05 | 4.44 | 1.69 | 1.64 | 1.25 | 1.49 | 0.79 |
| 6 | <5.6 | <4% | >2.5 | <65 | 4.14 | 4.2 | 1.76 | 1.44 | 1.3 | 1.43 | 0.8 |
| 7 | <5.6 | <4% | <2.5 | >65 | 4.08 | 4.42 | 1.75 | 1.54 | 1.19 | 1.56 | 0.87 |
| 8 | <5.6 | <4% | >2.5 | >65 | 4.13 | 4.46 | 1.77 | 1.62 | 1.32 | 1.52 | 0.88 |
| 9 | >5.6 | >4% | <2.5 | <65 | 4.19 | 4.41 | 1.75 | 1.61 | 1.09 | 1.61 | 0.83 |
| 10 | >5.6 | >4% | >2.5 | <65 | 4.08 | 4.44 | 1.89 | 1.62 | 1.29 | 1.55 | 0.94 |
| 11 | >5.6 | >4% | <2.5 | >65 | 4.13 | 4.2 | 1.72 | 1.44 | 1.27 | 1.37 | 0.93 |
| 12 | >5.6 | >4% | >2.5 | >65 | 4.02 | 4.28 | 1.64 | 1.53 | 1.14 | 1.42 | 0.71 |
| 13 | >5.6 | <4% | <2.5 | <65 | 4.15 | 4.32 | 1.77 | 1.49 | 1.34 | 1.29 | 1.01 |
| 14 | >5.6 | <4% | >2.5 | <65 | 4.06 | 4.21 | 1.71 | 1.57 | 1.24 | 1.56 | 0.75 |
| 15 | >5.6 | <4% | <2.5 | >65 | 4.25 | 4.54 | 1.77 | 1.64 | 1.31 | 1.43 | 0.95 |
| 16 | >5.6 | <4% | >2.5 | >65 | 4.02 | 4.05 | 1.77 | 1.56 | 1.28 | 1.29 | 0.83 |
| LSD ¹ | | | | | 0.22 | 0.25 | 0.2 | 0.16 | 0.18 | 0.22 | 0.19 |

Attributes in **bold** are significant at $p < 0.05$

¹Means within a column that differ by more than the Least Significant Difference (LSD) are different ($P < 0.05$)

Table 2.4. Pearson correlation coefficient of flavor attributes¹

| <u>Attribute</u> | <u>Cooked Pork</u> <u>Aroma</u> | <u>Cooked Pork</u> <u>Flavor</u> | <u>Metallic</u> | <u>Astringent Mouthfeel</u> | <u>Sweet</u> | <u>Sour</u> | <u>Umami</u> |
|----------------------|------------------------------------|-------------------------------------|-----------------|-----------------------------|--------------|-------------|--------------|
| Cooked pork aroma | 1 | -0.48 | 0.55 | 0.57 | -0.84 | 0.06 | 0.64 |
| Cooked pork flavor | -0.48 | 1 | -0.15 | 0.01 | 0.57 | 0.34 | -0.13 |
| Metallic | 0.55 | -0.15 | 1 | 0.56 | -0.34 | 0.12 | 0.59 |
| Astringent mouthfeel | 0.57 | 0.02 | 0.56 | 1 | -0.55 | 0.35 | 0.35 |
| Sweet | -0.84 | 0.57 | -0.34 | -0.55 | 1 | -0.24 | -0.27 |
| Sour | 0.06 | 0.34 | 0.12 | 0.35 | -0.24 | 1 | -0.30 |
| Umami | 0.64 | -0.13 | 0.59 | 0.35 | -0.27 | -0.30 | 1 |

¹Values in bold represent significant correlations (P<0.05)

Of the seven flavor attributes detected, only three attributes including cooked pork flavor, cooked pork aroma, and sweet were significantly different ($P < 0.05$) among treatments (Table 2.3). Cooked pork flavor has the most published work regarding the impact of pH and lipid content, more specific attributes of pork flavor were not found in the literature. A fat*color*IV interaction ($P < 0.05$) was observed for sweet as well as a three-way interaction of pH*fat*IV ($P < 0.01$). To further understand the interaction of most interest to the objectives of this study, the pH*fat*IV interaction was plotted (Figure 2.2). The only condition where there was a significant ($P < 0.05$) pH effect on sweet was with the combination of fat $>4\%$ and IV <65 . Sweet intensity was greater with the use fat $<4\%$ and IV <65 at pH below 5.6 than with the same combination used at pH above 5.6. Cooked pork aroma also had a significant ($P > 0.05$) three-way interaction of pH*fat*IV. To understand the significant effect on cooked pork aroma, the pH*fat*IV interaction was plotted (Figure 2.3). The only condition where there was a significant ($P < 0.05$) pH effect on aroma was with the combination of fat $>4\%$ and IV <65 . Aroma intensity was less with the use of fat $>4\%$ and IV <65 at pH below 5.6 than with the same combination used at pH above 5.6. Cooked pork flavor had a significant ($P < 0.01$) interactions of fat*IV as well as a significant ($P < 0.05$) interaction of fat*color. The fat*IV interaction was of most interest to this work, and to further understand its significance, the interaction was plotted (Figure 2.4). The only condition where there was a significant ($P < 0.05$) IV effect on cooked pork flavor was with the use of fat $>4\%$. Flavor intensity was greater with the use of fat $>4\%$ at IV below 65 than with the same fat % used at IV above 65. Sheard et al., (2000) that found that polyunsaturated fats are more susceptible to oxidative breakdown, and the oxidation

products have an adverse effect on pork flavor. Oxidation products quantity would be less in a more firm fat, with higher saturation, and low IV value. Oxidation may be manifested as off odors and flavors by sensory panelists or by a red color when reacted with thiobarbituric acid (TBA value) (Sheard et al., 2000). It appears that for this study, at high IMF and high degree of saturation (low IV), cooked pork flavor is rated the highest. While this study did not study oxidation through analytical tests such as TBA, but relative to other literature, a hypothesis exists that less oxidation occurred in treatments 1 and 2 compared to other treatments and additionally, the low pH served to buffer the impact of the perception of any oxidation that occurred. Fewer oxidation products existing in this combination of high IMF and low IV may not have had the ability to highly decrease cooked pork flavor with off flavors. If oxidation was present in a higher degree in these treatments, it may have been perceived by panelists as an increase and in what may be considered off flavors or perceived as other recognized flavors within the established lexicon.

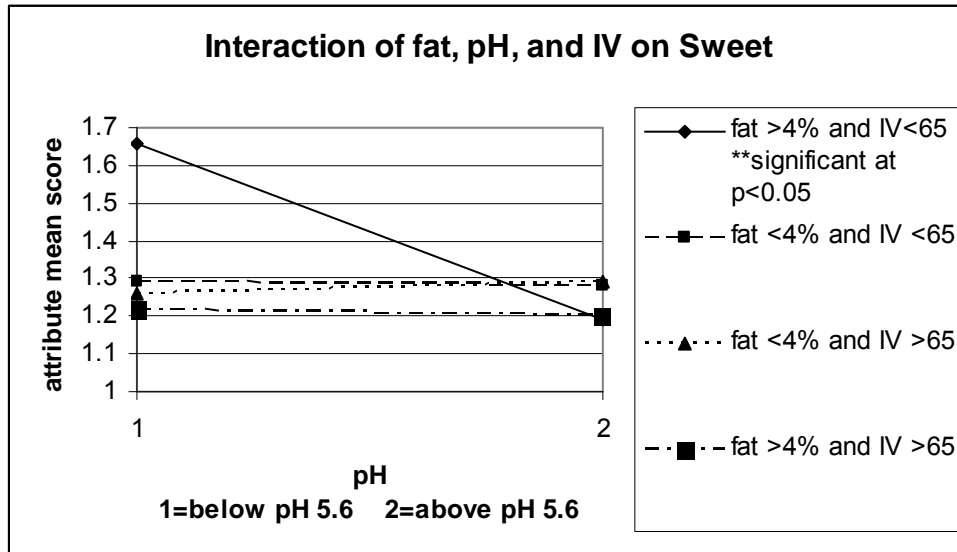


Figure 2.2. Interaction of fat, pH, and IV on sweet attribute

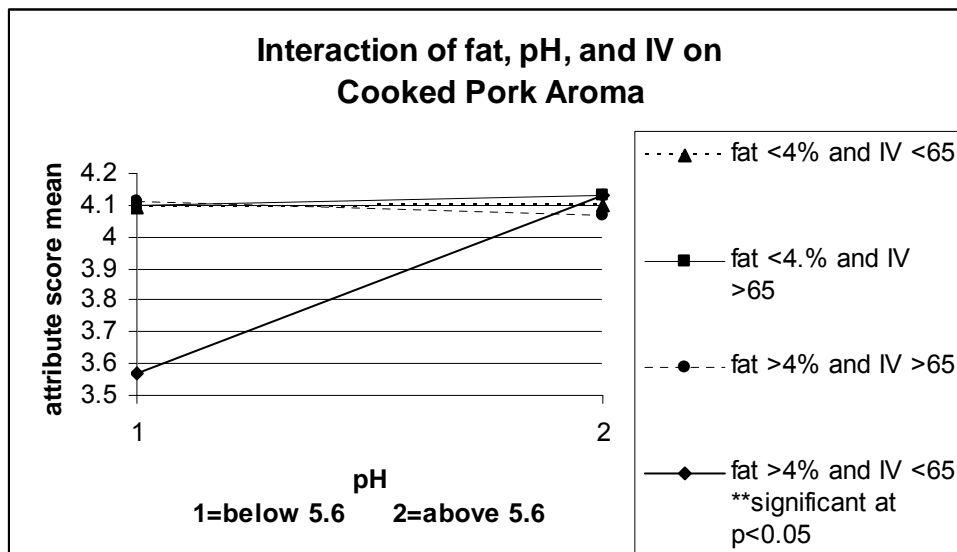


Figure 2.3. Interaction of fat, pH, and IV on cooked pork aroma attribute

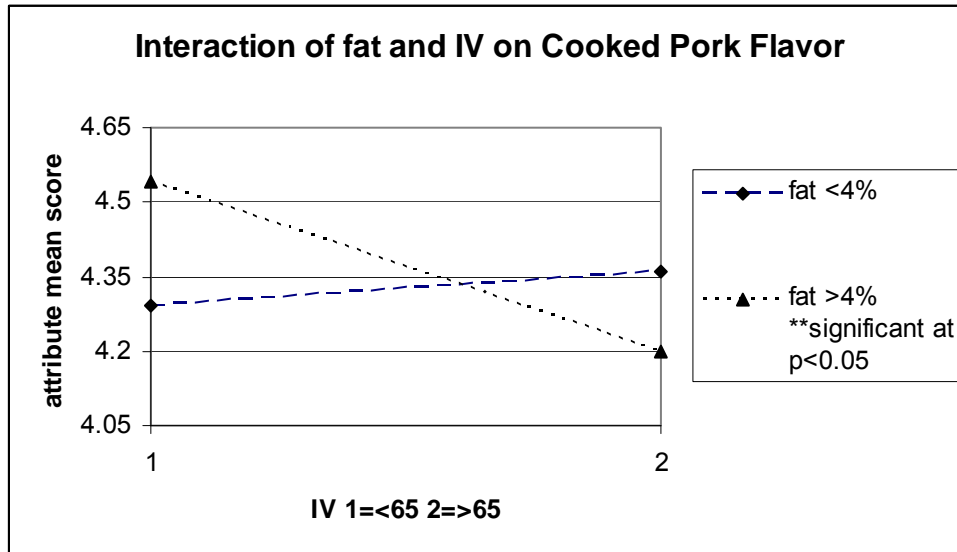


Figure 2.4. Interaction of fat and IV on cooked pork flavor attribute

Several studies have observed the effect of IMF alone on pork flavor with conflicting conclusions. Fernandez et al., (1999) grilled chops on a grill at 180C and reported that pork flavor was significantly increased when IMF values increased above approximately 2.5%. However, this work did not discuss the degree of unsaturation (IV) of lipids impacting flavor. Conversely, Rincker et al., (2008), grilled on an open hearth Faberware grill at 3 different end point cooking temperatures (62, 71, and 80°C) and found that IMF was not necessary for delivery of superior eating quality, and that there was not a threshold concentration of IMF which was needed to impact eating quality.

Fatty acid composition has been show to affect firmness of fat. Saturated fatty acids have a higher melting point than unsaturated fatty acids (Wood et al., 2008). In addition to

the melting point impacting firmness, it has been suggested that the location of fat in the perimysial connective tissue between muscle bundles is important to open the muscle structure to make chewing easier (Wood et al., 2008). Thus, the IV value may have a greater impact on pork texture to deliver a ‘soft’ or a ‘hard’ fat rather than impacting pork flavor.

These descriptive flavor results build upon the sensory texture work of pork within pH classification and lipid content by Lonergan et al., (2007). These authors reported that pH has a large role in determining the protein contribution to sensory quality, but that greater lipid content is not required for superior eating quality. Overall, they concluded that at low pH, pork is inferior texturally to high pH. Additionally, at low pH (<5.5), greater lipid content does not improve the quality. However, at intermediate pH (5.5 to 5.8) they did find that increasing lipid content enhances texture properties, but with minimal improvements. This current study did not observe the textural quality of pork as in Lonergan et al., (2007), and therefore it is difficult to make any strong comparisons to published materials.

While there are significant interactions occurring between the factors in this study, none of the interactions have a magnitude difference which could be perceived by a typical consumer, and therefore, the contribution of these interactions to have an effect on the flavor of raw pork in the commercial market is negligible. Thus, it can be concluded that neither IMF or IV value plays a significant role when determining the flavor of pork. It appears that this work will add to the sector of recent meat science work (Lonergan et al., 2007, Rincker, et al., 2008) which places low value of fat content for the eating satisfaction of pork. This work will become essential when selecting meat for future studies on pork flavor; to determine what parameters of muscle selection must be controlled in a study if they have

been previously shown to impact flavor.

The implication of this study suggests that either IMF or IV of pork does not impact pork flavor. Since IMF and IV are not impacting pork flavor, future studies with sensory analysis of pork may select raw materials for treatment assignments without concern about the content of fat in the muscle. It is well documented that pH plays a large impact on eating quality of pork. While it is difficult to single out any factor as a single impact to pork quality, it appears that pH may be used to segregate pork for sensory studies. Segregation of pork loins by pH should provide enough variability among eating quality without regards to fat content as an influence to the eating quality. These results support the notion that IMF has limited impact on eating quality of pork.

2.5 Summary

Of the 7 flavor attributes detected, only 3 attributes including cooked pork flavor, cooked pork aroma, and sweet were significantly different ($P < 0.05$) among treatments, and were characterized by significant three way interactions between variables. None of these interactions have a magnitude difference large enough to provide practical significance; therefore, the contribution of these interactions to have an effect on the flavor of pork in the commercial market would be negligible. These data suggest that crude fat and IV value play a minor role when determining the flavor attributes of cooked pork flavor and aroma, as well as sweet attributes of pork. The pH alone may be used as a factor to differentiate muscle quality when selecting pork for flavor studies.

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CHAPTER 3

EVALUATION OF SODIUM PHOSPHATE AND MEAT PROTEIN ISOLATE ENHANCEMENTS ON THE SENSORY QUALITY OF BONELESS PORK LOINS

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ABSTRACT

Pale, Soft, and Exudative (PSE) pork is documented as having a negative impact on product tenderness and juiciness. The objectives of this sensory study were to determine how a trained panel perceived both texture and flavor attributes of enhanced normal and PSE pork. Pork loins were classified as above (\geq) pH 5.5 or below (\leq) 5.5 pH. Enhancement solutions were injected at three different rates (0%, 15%, and 20%) into loins, where each loin was representative of one treatment. Sensory profiles for treatments were determined using an established pork texture and flavor lexicon, using a trained panel (n=8) having over 1000 hours of training in the Spectrum method of descriptive analysis. Principal component biplots were constructed for both studies to visualize the impact of treatments on sensory properties.

In the flavor study, attributes of enhanced pork loins (n=12), loins \geq pH 5.5 had a mean pH value of 6.23, whereas loins \leq pH 5.5 had a mean pH value of 4.95. Bitter and oxidized attributes were included in the lexicon but were not detected by panelists. Flavor attributes were composed of sour, astringent mouthfeel, and metallic flavors (positively loaded) and salty and sweet (negatively loaded) in PC1 (46% variability). PC2 (22%) differentiated the treatments in cooked pork aroma and cooked pork and piggy flavors. All four loins not receiving any enhancement solution were characterized by astringent mouthfeel and metallic flavors. All four loins with (I) treatments were characterized by sweet flavor.

In the texture study, attributes of enhanced pork loins (n=12), loins \geq pH 5.5 had a mean pH value of 6.20, whereas loins \leq pH 5.5 had a mean pH value of 5.37. The first

principal component (PC1) (68% variability) of the texture biplot was composed of moisture, juiciness, and oily texture (positively loaded) and number of chews, hardness, and fibrous texture (negatively loaded), whereas PC2 (17% variability) differentiated the treatments in cohesiveness. All four loins not receiving any enhancement solution were characterized by lower intensities of moisture and juiciness compared to P and I treatments. Loins with (P) and (I) enhancement were associated with higher intensities of moisture and juiciness. Loin with \leq pH 5.5 injected with protein isolate (I) at 15% pump rate trended with negative loading of PC1, compared to loin with \leq pH 5.5 with (I) enhanced treatments at 20% pump rates, which loaded positively towards juiciness attribute.

Sensory flavor results suggest that (I) injection may negate astringent mouthfeel and metallic flavors in pork with sweet flavor. Protein isolate (I) contributes to sweeter flavor attributes than in non-enhanced pork. Sensory texture results suggest that pork treated with either (P) or (I) injection enhance texture, moisture and juiciness. More research is needed to determine the impact of meat protein isolate enhancement on eating quality of PSE pork in order to better understand consumer acceptability.

3.2 Introduction

The use of non-meat ingredients to improve functionality of proteins is commonly used to improve overall quality of muscle. Enhancement serves to add value to fresh meats by the addition of ingredients which can impact flavor, color, and texture of the product. Salts functions in enhanced meats to develop flavor, increase moisture retention, increase ionic strength, and solubilize myofibrillar proteins, thus enhancing protein binding (Baublits et al., 2005). Sodium salt typically works synergistically with sodium phosphates in most meat enhancement systems. Sodium phosphate binding with protein results in an increase in the net negative charge of a protein, causing greater repulsion among the proteins, and therefore increasing water binding (Baublits et al., 2005). Sodium phosphates can also increase pH of a protein product to the upper limits by moving the isoelectric curve to a more alkaline environment to increase water binding (Sheard et al., 1999). Increasing the pH of meat increases its functional properties that are associated with water binding, including moisture, tenderness, and texture (Sheard et al., 1999).

These characteristics can improve cook yields and improve juiciness and tenderness characteristics (Baublits et al., 2005). In addition, salt at any concentration above a control can add flavor, while phosphates can cause off flavors at any concentration alone or with salt at levels of >5% (Sheard et al., 1999, Vote et al., 2000). While sodium phosphate delivers certain protein functionality, there are some consumers who express a concern of phosphate on the label (Brewer et al., 2002).

Pale, Soft, and Exudative (PSE) is a meat quality defect occurring in animals during harvest as the body temperature remains high but the pH drops rapidly from an abnormally

high rate of post mortem glycolysis (Josell et al., 2003). Stress and struggle increase the body temperature and speeds up the rate of glycolysis (Leheska et al., 2002) and environmental stresses such as transportation, extreme fluctuations in ambient temperature, feed withdrawal, and human handling can impact glycolysis as well (D'Souza, 1998, Leheska et al., 2002, McKee and Sams, 1998).

The physical characteristics of PSE include a pale color, a soft texture, and poor water holding abilities (Torley et al., 2000). These characteristics arise from the denaturation of myofibrillar proteins at low pH, which affects protein solubility (Torley et al., 2000). Protein solubility in turn, impacts the proteins ability for water holding capacity (WHC) (Torley et al., 2000). Water holding capacity has its lowest value at the isoelectric point of actomyosin, pH 5 (Offer, 1991). On either side of this point, the net negative charge of proteins increases, thereby increasing protein-water interaction and the WHC (Offer, 1991).

The impact of PSE on consumer's eating satisfaction includes lack of tenderness and a loss of product yield and quality upon the processors (Woelfel et al., 2002). The National Pork Quality Audit (2005) reported the incidence of pork exhibiting "classic" pale, soft, and watery characteristics was 3.34% (National Pork Board, 2006). This percentage is a decrease from past audits and could be associated with increased awareness of PSE in the industry and to the improvements that have been made over time in the area of genetics to select against lines which are more susceptible to this condition.

Processors look to new ways to improve functionality of PSE protein in fresh products. While there is an understanding that while incidence over time has been reduced, a percentage of the population continues to exhibit PSE characteristics. By improving PSE

functionality, processors provide insurance to their consumers that a quality product is being consistently delivered which ultimately provides consumer confidence and continued support of the pork industry.

There is a growing interest in the use of muscle proteins as ingredients in foods (Hultin and Kelleher, 2000) due to their wide availability and the ability to add value to waste/low value starting materials. Muscle proteins flavor can be more bland as compared to soy protein, and is superior in nutritional quality over vegetable proteins (Lanier, 1985). It has been hypothesized that the use of solubilized meat proteins could provide similar WHC functions as traditional salt sodium phosphate enhancement solutions (Kristinsson and Hultin, 2003a). Acid or alkaline extraction to create muscle protein isolate is based on the concept that muscle protein can be diluted with water and treated at either alkali (pH 11) or acidic (pH 2.5) pH to achieve a low viscosity. This low viscosity material can then be centrifuged to yield a protein free of membranes and lipids (Hutlin and Kelleher, 2000). The resulting membrane and lipid free soluble protein are then recovered by isoelectric precipitation. This is accomplished by raising the pH of the protein slurry to 5.5, resulting in a protein isolate that can be used as a functional food ingredient (Kristinsson and Hultin, 2003b). This meat protein isolate can be rehydrated into water with other ingredients such as salt and then injected into whole muscle.

The purpose of this study was to compare the use of sodium phosphate enhancement to meat protein isolate enhancement in pork and determine their effectiveness against a control. The objectives was to compare the descriptive flavor and texture attributes of pork loins above and below pH 5.5 to determine the impact of both enhancement methods on PSE

and non-PSE muscle.

3.3 Materials and Methods

3.3.1 Muscle Selection

Fresh pork loins, IMPS# 413B, (1d postmortem, n=6) were obtained from a local pork processor based on pH (<5.5) utilizing a calibrated handheld ISFET probe pH meter (IQ Scientific Instruments, San Diego, CA). Fresh pork loins, IMPS# 413B, (1d postmortem, n=6) were obtained from a local processor based visually on the dark end of a Japanese export color segregation scale for pork loins. Loins from each plant were held in coolers with ice blocks and transported back to NCSU Processed Meat Laboratory. Loins were held overnight for approximately 15h at 1°C. Loins were trimmed to remove straps (latissimus dorsi and trapezius), green weights were taken (w1), and pH recorded (pH I) with handheld pH meter (IQ Scientific Instruments, San Diego, CA) at the rib end. L, a, b color values were taken at luminescence D65 using a model CR-200 Minolta colorimeter (Minolta Corp., Ramsey, NJ) at the exposed sirloin end and National Pork Producers Council (Des Moines, IA) visual color and marbling scores were recorded on the exposed end. In order to preserve the rib end of the loin for sensory analysis, all analytical work proceeded from the sirloin end. This procedure, along with following processing steps was repeated 2 weeks later for loins in a second sensory study on texture. However, due to handheld pH malfunction, processing replication 2 selected texture study loins into treatment classification by color only. A chop was cut from the rib end of loins and utilized for slurry pH 24h after enhancement to confirm visual color placement of loins for both treatment groups.

3.3.1.1 Slurry pH

A 2.54 cm thick chop was cut from the rib ends of 12 loins utilized in processing replication 2. A sample was taken from this chop to perform a 1:10 meat to water dilution. Meat was homogenized with deionized water in a model 38BL54 laboratory blender (Waring Products Division, New Hartford, CT) for 20 seconds, and pH was recorded immediately using model XL15 Fisher Scientific pH meter (Fisher Scientific, Pittsburgh, PA).

3.3.1.2 Experimental Design

A 2 X 2 X 3 complete factorial design was utilized for this study, where 2 muscle pH's below (<5.5) and above (>5.5), 2 enhancement methods (sodium phosphate or meat protein isolate), and 3 pump rates (0%, 15% and 20%) were used. Pork loins were randomly assigned 1 of 12 treatment groups within a given muscle pH from the handheld pH value in processing replication 1 or visual color in processing replication 2, where lighter color was assumed to be indicative of lower pH.

3.3.2 Preparation and Delivery of Brines

3.3.2.1 Sodium Phosphate Brine

Two 75.7 liter sodium phosphate brines were formulated for 15% (Brine 1) and 20% pickup (Brine 2), where both brines targeted for .5% sodium phosphate and .5% salt delivery in the injected loin. Sodium phosphate, Brifisol 85 Instant, (BK Giulini Corporation, Simi Valley, CA) was composed of an agglomerated blend of poly and pyrosodium phosphates containing 56% phosphorus pentoxide and a pH of 8.5 ± 0.3 in a 1% solution. Tap water was

weighed according to formulation (Table 3.1) in stainless steel bins, covered with plastic, and held overnight for 15h in 1 °C cooler. Tap water analyzed with EPA method 200.8 and contained .146 mg/L copper, .952 mg/L iron, and <.002 lead, manganese, and arsenic (Chemical and Environmental Technology Inc., RTP, NC). On the day of enhancement, bins were taken from the cooler, and sodium phosphate was slowly solubilized into water. After sodium phosphate Brifisol 85 Instant was completely solubilized; the salt, Top Flo, (Cargill, Minneapolis, MN) was added to this solution using model MP450 Turbo agitator (Robot Coupe, Jackson, MS).

Table 3.1. Formulation of sodium phosphate brines

| <u>Target pick up</u> | <u>Ingredient</u> | <u>% of brine</u> | <u>% in product</u> |
|-----------------------|--|-------------------|---------------------|
| Brine 1- 15% | Cold Water | 93.34% | 14.00% |
| | Sodium phosphate (Brifisol 85 Instant) | 3.33% | .50% |
| | Salt (Top Flo) | 3.33% | .50% |
| | Total | 100.00% | 15.00% |
| Brine 2- 20% | Cold water | 95.00% | 19.00% |
| | Sodium phosphate (Brifisol 85 Instant) | 2.50% | .50% |
| | Salt (Top Flo) | 2.50% | .50% |
| | Total | 100.00% | 20.00% |

3.3.2.2 Meat Protein Isolate Preparation

Meat protein isolate was prepared using alkali aided extraction and precipitation methodology as outlined in Kristinsson et al, 2005. Knife removal of excess fat on thawed

pork trim was preformed to ensure higher yields from having greater protein to fat ratio in the starting meat source. Pork trim (approximately 80% lean, 20% fat) was obtained as raw material to obtain meat isolate. This trim was ground in Stephan chopper model 2889 (Stephan, Columbus, OH) with cold tap water in a 1:1 ratio. To reduce viscosity, additional water was further added to initial meat slurry to achieve a 1:4 solution. To increase the meat solution pH to 11, 2N Sodium Hydroxide was added dropwise, aiding to solubilize protein. As 2N sodium hydroxide was slowly added, a plastic paddle continually mixed the slurry. The pH was measured with a calibrated handheld ISFET probe pH meter (IQ Scientific Instruments, San Diego, CA). This solution was then strained through a black plastic mesh strainer with 0.6mm hole size and the insoluble fraction was discarded. A 2 N hydrochloric acid solution was added dropwise to the resulting strained material to achieve a target pH of 5.5. At this point, flocculation is evident as protein precipitation occurs. A plastic paddle continually mixed the slurry while the pH was being reduced. This protein slurry was then dewatered through a chiffon fabric with 0.001 mm hole size. The protein slurry was placed on chiffon fabric, and remaining water was manually squeezed out of the slurry. This de-watered protein isolate was placed in resealable plastic bags, placed in 1 °C cooler for 15h until further utilized in brine preparation.

3.3.2.3 Meat Protein Isolate Brine Preparation

Preliminary work was conducted to determine the ability of a automatic brine injector, Injectamatic model PI21, (Koch Equipment, Kansas City, MO) to pull through a viscous solution. Viscosity is highly affected by the percent protein of a marinade (Shahidi

and Venugopal, 1994), such that increasing protein content increases viscosity. Preliminary work revealed that 2.1% protein solution was the required percent protein needed to maximize the efficiency of the available brine injector.

Protein isolate was estimated for moisture using a simplified method to imitate the action of a laboratory scale CEM (Matthews, NC) oven. Moisture content was determined by taking a small piece of isolate, taking its sample weight, placing on a paper towel of known weight, and placing in a General Electric turntable microwave oven serial number 3850W3W081A (Fairfield, CT) for 3 minutes on highest setting to remove water. Sample was then reweighed to calculate percent moisture content.

With known percent solids (100- percent moisture content) and a standard 2.1% protein of isolate, the grams protein per liter of isolate was determined. To match the salt percentage (2.5%) found in Brine 2 (Table 3.1), the necessary amount of salt was added to this total, and subtracted from 1 L of solution to determine the grams of water needed. By percentage, the amount of water needed for this marinade was 95.4% of the total volume. These values of isolate, salt, and water per 1 L were then proportioned to match the amount of total isolate captured from isolate preparation steps to formulate Brine 3.

Brine 3 was made by mixing salt to isolate, and then adding this mix to a preweighed amount of cold tap water, which had been stored in a stainless steel bin with plastic covering in a 1°C cooler overnight for 15h, with model MP450 Turbo agitator (Robot Coupe, Jackson, MS). Upon enhancement of treatments receiving Brine 3 the remaining Brine 3 was weighed and this weight was used to reformulate isolate enhancement solution with addition of salt to match the salt percent in Brine 1 formulation to prepare Brine 4. The percentage of brine

remaining from Brine 3 enhancement was multiplied by the percentage of salt needed to match the percentage of salt in the Brine 1 (3.33%). This value was then subtracted from the amount of salt already existing in the remaining Brine 3, calculated by multiplying the percentage of remaining solution by the original weight of salt in the total Brine 3 formula.

3.3.2.4 Enhancement With Brines for Descriptive Flavor Study

Prior to enhancement of treatment loins, loins which were not to be used as a treatment in the study were ran through an automatic brine Injectamatic model PI21 (Koch Equipment, Kansas City, MO) with sodium phosphate solution to narrow down range of .3-.6 bars of pressure on the brine injector which would achieve desired level of pick up. The pH of both sodium phosphate and isolate brines were taken (IQ Scientific Instruments, San Diego, CA), a sample for salt analysis, and a sample of isolate brine was taken for viscosity analysis utilizing model RVT Brookfield Viscometer (Brookfield Engineering Laboratories Inc., Middleboro, MA). The brine injector required approximately .3 bars of pressure to inject sodium phosphate brine and .5 bars of pressure to inject meat protein isolate brine. Viscosity of flavor loin processing protein isolate brine was 190 cps at 100 rpms.

The treatments requiring Brine 2 were run through the brine injector first, and then treatments requiring Brine 1 were injected. The brine injector was drained of sodium phosphate solution prior to enhancement of meat protein isolate enhancement Brine 3 followed by Brine 4. Loins were weighed after enhancement (w2) to determine if desired percent pickup was achieved by enhancement. After weighing, loins were placed in vacuum package bag. If percent pickup was lower than the required percent pickup needed for that

specific treatment, then the necessary percent pickup of brine which was needed to achieve required total percent pickup was added to vacuum package bag. Loins in vacuum package bags with any additional needed brine were then packaged in model MVS50 vacuum packaging machine (Minipack-Toore, Dalmine, Italy) and then placed in model ET-5 tumbler (Sipromac, Quebec, Canada) for 30 minutes at 6 revolutions per minute. Loins were then stored in 1°C cooler until 6d post enhancement for further sample processing. This time aided solution distribution, uniform aging, and to facilitate scheduling with the descriptive sensory panelists.

3.3.2.5 Enhancement With Brines for Descriptive Texture Study

Prior to enhancement of treatment loins, loins which were not to be used as a treatment in the study were ran through an automatic brine Injectamatic model PI21 (Koch Equipment, Kansas City, MO) with sodium phosphate solution to narrow down range of .3-.6 bars of pressure on the brine injector which would achieve desired level of pick up. The pH of both sodium phosphate and isolate brines were taken (IQ Scientific Instruments, San Diego, CA), a sample for salt analysis, and a sample of isolate brine was taken for viscosity analysis utilizing model RVT Brookfield Viscometer (Brookfield Engineering Laboratories Inc., Middleboro, MA). The brine injector required approximately .3 bars of pressure to inject sodium phosphate brine and .5 bars of pressure to inject meat protein isolate brine. Viscosity of texture loin processing protein isolate brine was 120 cps at 100 rpms. Both texture and flavor protein isolate brines were formulated for the same protein percent content, so the lower viscosity in the protein isolate brine used in the texture study loins could be

attributed to the time that the brine was allowed to set before the measurement was taken. The point at which a viscosity reading is taken can impact the end analysis; however, this difference was not enough to impact the brine injector's ability to inject the brine.

The treatments requiring Brine 2 were run through the brine injector first, and then treatments requiring Brine 1 were injected. The brine injector was drained of sodium phosphate solution prior to enhancement of meat protein isolate enhancement of Brine 3 followed by Brine 4. Loins were weighed after enhancement (w2) to determine if desired percent pickup was achieved by enhancement. After weighing, loins were placed in vacuum package bag. If percent pickup was lower than the required percent pickup needed for that specific treatment, then the necessary percent pickup of brine which was needed to achieve required total percent pickup was added to vacuum package bag. Loins in vacuum package bags with any additional needed brine were then packaged in model MVS50 vacuum packaging machine (Minipack-Toore, Dalmine, Italy) and then placed in model ET-5 tumbler (Sipromac, Quebec, Canada) for 30 minutes at 6 revolutions per minute. Loins were then stored in 1 °C cooler until 6d post enhancement for further sample processing. This time aided solution distribution, uniform aging, and to facilitate scheduling with the descriptive sensory panelists.

3.3.2.6 Salt Analysis

A calibrated model SAT-500 salt analyzer (DKK-TOA Corp, Tokyo, Japan) was utilized, were each of 10 different brine samples (4 brines from flavor loin processing, 4 brines from texture loin processing, and 2 brines from cook yield trial) were analyzed in 20

microliter triplicates and then averaged. The brine was placed into electrolyte solution. An electric current is passed between the silver wire and the silver electrode to form white deposits of silver chloride by electrolysis. The white deposits are formed from the dissolved silver ions and the chloride present in the sample. The chloride in the sample was determined by the instrument from the electricity required until all the chloride in the sample was depleted by electrolysis.

3.3.3 Sample Processing

At 6d post mortem, all loins (n=12) were removed from vacuum package bag and weighed (w3) to determine 6d percent pickup. Then, 15.24 cm chops were cut from the rib end of loin. This 15.24 cm piece was then further divided into six 2.54 cm chops to be assigned for instrumental analysis. The most anterior 2.54 cm chop was assigned as a back up, chop 2 was to be used for cook yield and color, chop 3 for Warner Bratzler Shear, chop 4 for water holding capacity, chop 5 for cooked proximate analysis, and chop 6 for raw proximate analysis (Appendix 6.1). The chops were individually labeled and wrapped in freezer paper, placed back into the 1°C cooler, and on 7d post mortem were placed in -15°C freezer until further analysis. The resulting loin piece was then divided transversely down through the geometric center to obtain two equal length halves for treatment allocation. These two sections were weighed and then randomly assigned an A or B along with the previously assigned enhancement treatment number (1-12 in study of flavor) and (21-32 in study of texture). These treatment numbers were then converted into a three digit random code, which would be used for identification in descriptive flavor and texture panels. Thus,

every treatment was represented in duplicate (A or B) in the descriptive panel.

3.3.3.1 Weight Changes During Processing

Weights of loins were recorded before enhancement (w1) after enhancement (w2), and 6d post enhancement (w3). Loin sections in duplicate were weighed before cooking (w4) after cooking (w5) on the day of assigned descriptive panel session. The following calculations were then made:

$$\% \text{ enhancement pickup} = [(w2-w1)/w1]*100$$

$$\% \text{ pickup after 6d equilibration} = [(w3-w1)/w1]*100$$

$$\% \text{ cook yield} = (w5/w4)*100$$

3.3.4 Descriptive Flavor and Texture Panels

3.3.4.1 Sample Preparation

Loins, assigned with a 3 digit random code, were placed in disposable aluminum baking trays and wrapped in plastic film, and held in 1 °C cooler until assigned descriptive panel session day. A post enhancement pH (pH II) was taken on the loin on the day it was to be presented to the panelists utilizing a handheld pH meter (IQ Scientific Instruments, San Diego, CA) at the rib end. Weights were taken before (w4) and after cooking (w5) to determine cook yield. Plastic film was removed from tray and 2 thermocouples Type T, thermocouple Cu-Constantan, TMQSS-040U-6 (Omega Engineering, Stamford, CT) were inserted into the meat, placing each probe 5.08 cm inside transversely at two points marked as 1/3 and 2/3 of loin total length. Thermocouples were connected to a data acquisition unit

OMB-DAQ-55 with OMB-PDQ2 Expansion Module (Omega Engineering, Stamford, CT) utilizing InstaTrend Professional Software (Dianachart Inc., Denville, NJ) on a model Armada 7790DMT laptop computer (Compaq, Houston, TX). A model DFG-100 convection oven with blower (Blodgett, Burlington, VT) was allowed to preheat for 30 minutes at 350°F. Oven was previously calibrated with thermocouples to determine if there were any uneven temperature points within the oven (Appendix 6.3) which was found to have no area within the oven that cooked at a slower rate than any other area. Also, oven had been mapped with thermocouples to determine if non-injected loins cooked at a faster rate than injected loins (Appendix 6.4). Loins with attached thermocouples were then placed into oven in time intervals to allow cooked product presentation to panelists to be staggered in at least 15 minute time periods. Loins were removed once thermocouple reading on data acquisition unit read 70°C. Loins were then cut into 1.27cm X 1.27cm X 1.27cm cubes. Cubes were intermingled and then four cubes were placed into each coded plastic lidded cups (n=8) (Solo, Highland Park, IL). Cups with sealed lids were then served to panelist for analysis.

3.3.4.2 Trained Descriptive Panel

Eight panelists were trained using the Sensory Spectrum (Meilgaard et al., 2007) method of descriptive analysis and each having over 1000h of experience developed a lexicon using product and non-product specific references. Fresh, non-enhanced pork loins were cooked to an internal temperature of 70°C and used as a reference. A universal intensity scale was used to quantify aroma, flavor, and aftertaste attributes and ranged from 0 (minimum intensity) to 15 (maximum intensity). A product specific intensity scale (also 0 to

15) was used to quantify the texture attributes. Prior to tasting treatments, panelists were given 1 training sample, and 2 blind reference samples to begin each session. Each treatment was then randomly presented to the panelists in duplicate, over 3 session days.

Table 3.2. Definitions used by trained sensory panelists to describe the aroma and flavor of cooked pork loins

| <u>Descriptor</u> | <u>Definition</u> |
|-----------------------------|---|
| Cooked uncured pork; aroma | Meaty aromatic associated with uncured lean pork muscle |
| Cooked uncured pork; flavor | Meaty flavor associated with uncured lean pork muscle |
| Piggy/Boar Taint | Wet pig; the musk like aroma associated with boar meat |
| Metallic | Flavor resembling tin or copper penny held in mouth; also blood or serum |
| Oxidized | General term for the oxidized characteristic(s) of food such as cardboard, painty, and stale |
| Astringent | Mouth feel sensation of shrinking, drawing, puckering of skin surfaces of the oral cavity, or tooth coating |
| Sweet | Basic taste stimulated on the tongue by sugars and high potency sweeteners |
| Salt | Salt water; also basic taste on the tongue stimulated by sodium salt |
| Sour | Pungent, sharp aromatic; also the basic taste on the tongue associated with acids |
| Bitter | Taste stimulated by substances such as caffeine or quinine when solubilized |
| Umami | Taste sensation of fullness, savoriness or meatiness elicited by glutamates present in meat |

Table 3.3. Definitions used by trained sensory panelists to describe the texture of cooked pork loins

| <u>Descriptor</u> | <u>Definition</u> |
|----------------------|---|
| Hardness | Force required to bring incisors together on first bite |
| Moisture Release | Degree to which juices(moisture and/or fat) exude from product |
| Cohesiveness of mass | Degree to which sample product holds together in mass |
| Juiciness | Degree to which juices(moisture and/or fat) are perceived in the product during mastication |
| Fibrous/geometrical | Degree to which sample is fibrous or stringy |
| Number of Chews | Number of chews required to prepare sample for swallowing (masticating at one chew/s) |
| Oily mouth coating | Degree to which oil is coating mouth surfaces |

3.3.4.3 Analysis of Descriptive Sensory Panel Data

The data were analyzed with a data compression technique for Principal Component Analysis in SAS for Windows version 9.1, (SAS Institute, 1999, Cary, NC). Principal Component bi-plots were constructed to visualize the impact of treatments on flavor (n=12) and texture (n=12) properties. PROC CORR was performed to determine relationships between multivariate sensory descriptors. Further statistical analysis of sensory data was performed using the GLM procedure of SAS. When main effects or interactions were significant ($P > 0.05$), significant differences between treatment means were calculated using the least significant difference (LSD) test. Muscle pH, enhancement type, pump rate, and interactions among muscle type, enhancement type, and pump rate were used in the model

along with replicate and panelist to evaluate the effect on flavor and texture descriptive traits. Due to the difficulty of analyzing multiple 3 way interactions within the full model of enhancement type, muscle pH, pump rate, enhancement*pH, enhancement*pump rate, pH*pump rate, and enhancement*pH*pump rate; the study data was divided in GLM to observe the effects of pump rate and enhancement type on muscle pH above 5.5 and also on muscle pH below 5.5.

3.3.4.4 Centrifugal Moisture Holding

On 7d post mortem, wrapped chops with assigned number 4 were analyzed for moisture holding, utilizing methods of Foegeding, (1990). A microcentrifuge collection unit (Lida Corp., Kenosha, WI) consisted of a 1.5mL plastic tube which collected the released liquid and an inner filter tube which held the meat sample. The centrifuge tube and filter tube were weighed, and then reweighed after centrifugation. Each pork chop was cut with a sharpened metal tube to obtain a cylinder sample. This sample was then placed in filter tube and weighed together. The unit with filter tube within the centrifuge tube was spun in a Beckman Microfuge 11 horizontal-rotor microcentrifuge (Beckman Instruments, Palo Alto, CA). Each treatment was centrifuged at 30,600 X g in triplicate for 15 minutes. After centrifugation was complete, the centrifuge tube and filter tube were weighed again to determine the weight of liquid released from the sample.

Held water was determined by the formula

$$HW = (\text{total g water in sample} - \text{g water released}) / \text{total g protein in sample}$$

Where water in sample and protein were determined from proximate analysis results (Tables

3.9 and 3.11).

3.3.4.5 Warner Braztler Shear Force of Descriptive Panel Chops

Warner-Braztler shear force test was conducted based on recommendations from AMSA (1995). Frozen 7d postmortem wrapped chops with assigned chop number 3, were allowed to thaw for 15h at 1°C. Chops were then cooked on an Faberware model 150A electric grill (Faberware Inc., Bronx, NY) to an internal temperature of 70°C measured by a HH21 hand held digital thermometer (Omega Engineering Inc., Stamford, CT) equipped with a Type T thermocouple (Cu-CuNi). Chop weights were collected before and after cooking. Chops were then placed in resealable bags and placed in 1°C cooler overnight. Six uniform 1.27 cm diameter cores were obtained from chilled chops removed parallel to the longitudinal orientation of the muscle fiber. Cores were kept covered until analyzed with an Instron Universal Testing Machine (model 5565, Instron Corp., Norwood, MA) with Bluehill Software and attached with Warner Braztler Shear v-notch blade (G-R Electric, Manhattan, KS) with a 225 mm/min crosshead speed. Mean shear force values for all six cores from a chop were used for analysis.

3.3.4.6 Proximate Analysis of Descriptive Panel Chops

Frozen chops (n=24), 7d postmortem age, were thawed for 15h at 1°C. Meat was homogenized in a model 38BL54 laboratory blender (Waring Products Division, New Hartford, CT) for 20 seconds. Chops were then placed in resalable bags of known weight and frozen for 15 hours. Percent moisture analysis was conducted by freeze drying samples

for 96h utilizing a minimum 5 microns vacuum pressure at -50°C on a model USM-15 freeze dryer (Virtis Company, Gardiner, New York). After drying, moisture percentage was calculated as the difference between the wet and dry sample weight divided by the wet weight. Freeze dried samples were then analyzed for crude fat (AOAC method 2003.05), protein (AOAC method 990.03) and ash (AOAC 942.05). Cooked chops (n=24) which had cores removed for Warner Braztler Shear analysis were cooled, placed in resealable bag of known weight and frozen overnight. These cooked chops were freeze dried and analyzed for crude fat, protein, and ash in similar method as raw chops.

3.4 Results and Discussion

3.4.1 Descriptive Sensory Studies

3.4.1.1 Flavor

The sensory language identified and differentiated the pork loins above (Table 3.5) and below (Table 3.6) pH 5.5 in flavor. Principal components analysis for the descriptive flavor attributes illustrated differences between treatments and relationships between the attributes in the first two principal components (PCs), which accounted for 68% of the variation in the data. Principal components of both above and below pH 5.5 loins are shown in Figure 1. Two terms, bitter and oxidized, were identified in the loins used for language generation but were not identified in the samples used in this study, therefore only 9 attributes are examined in this study.

Flavor attributes were composed of sour, astringent mouthfeel, and metallic flavors (positive dimension of PC1) and salty and sweet (negative dimension of PC1) in PC1 (46%

variability). PC2 (22%) differentiated the treatments in cooked pork aroma and cooked pork and piggy flavors. Distinct clustering of treatments can be observed. Treatments 1, 2, 7, and 8 were controls in the study, and were characterized by astringent mouthfeel and metallic flavors. Negative correlation of PC1 occurred for treatments 9, 10, 11, and 12, which had been injected with meat protein isolate. These treatments could be characterized by sweet flavor. Treatments 3, 4, 5, and 6 are sodium phosphate injected treatments. It can be observed that loins with muscle pH below 5.5 with sodium phosphate (treatment 4 and 6) are grouped near the negative dimension of PC2, characterized by lower intensities of piggy, cooked pork flavor, and cooked pork aroma. Fewer conclusions can be made regarding treatments that are above pH 5.5 muscle loins with sodium phosphate enhancement, since they are less grouped together on the PCA biplot. Sodium phosphate treatments 3, 4, 5, 6 appear to be much more affected by pH than meat protein isolate treatments which were clustered together regardless of pH. An initial hypothesis from this analysis suggests that meat protein isolates mode of action is less dependent on pH than sodium phosphate, and may work similarly on all muscle types, including a defect such as PSE.

Pearson correlation coefficients (Table 3.4) suggest significantly high ($P < 0.05$) positive correlations between sour, astringent mouthfeel, and metallic flavors. There are significantly high ($P < 0.05$) negative correlations between sour, astringent mouthfeel, and metallic flavors to sweet and salt flavor. There is a strong negative correlation between cooked pork aroma and salt attribute.

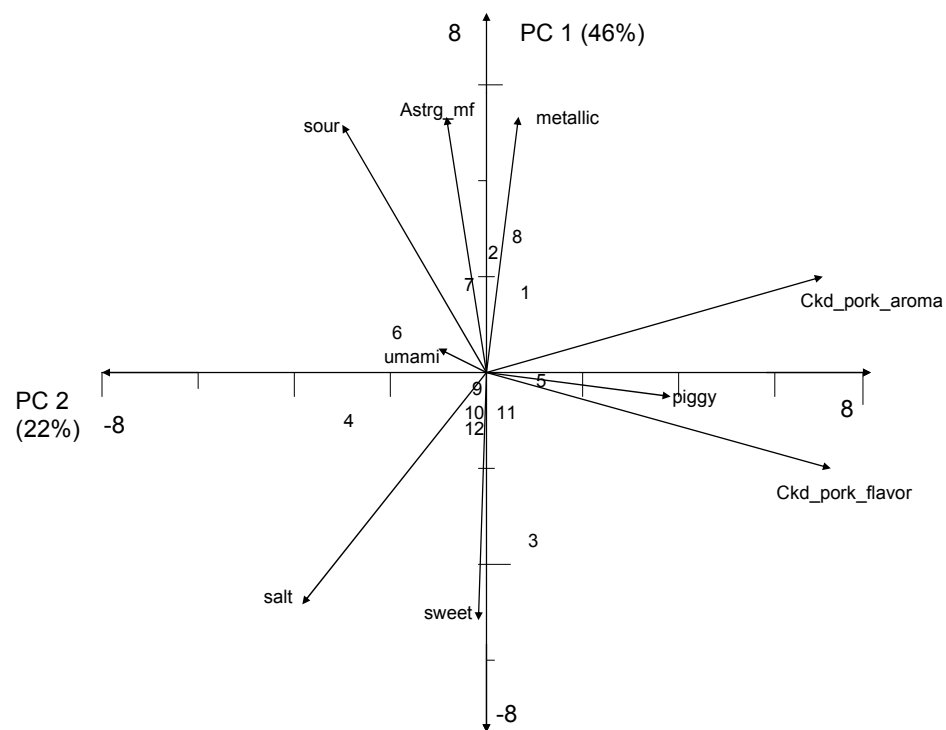


Figure 3.1. Principal component biplot of descriptive sensory flavor analysis of pork loins. Numbers represent treatments (Table 3.5 and 3.6) PC1= principal component 1; PC2= principal component 2.

Table 3.4. Pearson correlation coefficient of flavor attributes¹

| <u>Attribute</u> | <u>Cooked</u> <u>pork aroma</u> | <u>Cooked</u> <u>pork flavor</u> | <u>Piggy</u> | <u>Metallic</u> | <u>Astringent</u> <u>Mouthfeel</u> | <u>Sweet</u> | <u>Salt</u> | <u>Sour</u> | <u>Umami</u> |
|----------------------|------------------------------------|-------------------------------------|--------------|-----------------|---------------------------------------|--------------|-------------|-------------|--------------|
| Cooked pork aroma | 1 | .52 | .10 | .34 | .33 | -.37 | -.70 | .05 | .15 |
| Cooked pork flavor | .52 | 1 | .29 | -.28 | -.34 | .29 | -.22 | -.46 | -.15 |
| Piggy | .10 | .29 | 1 | .24 | -.09 | 0 | .06 | -.22 | -.39 |
| Metallic | .34 | -.28 | .24 | 1 | .82 | -.80 | -.65 | .74 | -.05 |
| Astringent Mouthfeel | .33 | -.34 | -.09 | .82 | 1 | -.91 | -.71 | .68 | .34 |
| Sweet | -.37 | .29 | 0 | -.80 | -.91 | 1 | .80 | -.73 | -.02 |
| Salt | -.70 | -.22 | .06 | -.65 | -.71 | .80 | 1 | -.48 | -.01 |
| Sour | .05 | -.46 | -.22 | .74 | .68 | -.73 | -.48 | 1 | -.15 |
| Umami | .15 | -.15 | -.39 | -.05 | .34 | -.02 | -.01 | -.15 | 1 |

¹Values in bold represent significant correlations (P<0.05)

Of the nine flavor attributes detected, only three attributes were significantly different ($P < 0.05$) among treatments with pH above 5.5 (Table 3.5). An enhancement*pump (E*P) rate interaction ($P < 0.05$) was observed for astringent mouthfeel, while salt and sweet had simple effect of pump rate ($P < 0.01$). To understand the simple effect of enhancement on pump rate, the (E*P) interaction was plotted (Figure 3.2). The only condition where there was a significant ($P < 0.05$) enhancement effect on astringent mouthfeel was with the use of a 15% pump rate. Astringent mouthfeel intensity was greater with the use of meat protein isolate pumped at 15% than with sodium phosphate pumped at the same rate. However, the significance of this interaction varied the mean score of astringent mouthfeel by only .51, therefore, this small magnitude of difference could be considered insignificant for the commercial impact of this result. While the score means may not be of practical significance, this data agrees with Baublits et al., (2006), which reported that unenhanced pork chops had higher astringent scores than chops enhanced at a sodium phosphate solution of 12%, and found no difference in metallic scores among 0%, 6% and 12% phosphate pumped pork chops.

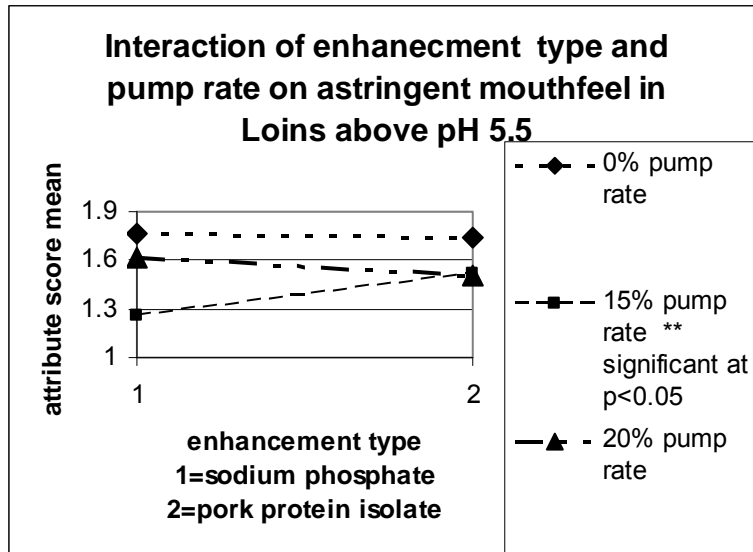


Figure 3.2. Interaction of enhancement type and pump rate on astringent mouthfeel in loins above pH 5.5

The interpretation of the simple effect of pump rate on salt flavor in loins above pH 5.5 is difficult to draw strong conclusions, since the control loins have initially significantly different ($P < 0.05$) salt means among the two enhancement types (.94 in 0% sodium phosphate vs. .51 in 0% meat protein isolate). According to the USDA National Nutrient Database (2007), raw pork loin contains .05% sodium. The ability of the trained panel to detect very small quantities of salt accurately is of concern since both of the control intensities were statistically different. There is potential that control values are different by chance from the pig to pig variation that can not be accounted. However, the variation of control salt intensities makes it difficult to assign an incremental increase in salt intensity for every

incremental increase in salt content since starting intensities of each of the controls were found to be different. While controls are different, it is interesting to note that both sodium phosphate and meat protein isolate enhancements performed statistically the same at both pump rates and that salt intensity is greatest in loins injected at 15% pump rate. All brines were formulated to deliver .5% salt in the final product. It appears that from the salt analysis of brines (Table 3.11), that salt content ranged from .53% in loins injected with 20% sodium phosphate ($2.67 \times .20$) and .6% salt in loins injected with meat protein isolate ($4.0 \times .20$) or ($2.97 \times .20$) at both pump rates. Thus, it appears that at both pump rates, delivered salt content was at most only .1% greater than the targeted salt delivery. Therefore, while it appears that salt mean score is greatest in 15% pump rate, the importance of this fact is reduced when actual salt delivery in product is calculated. This work is in agreement with Vote et al., (2000), which found that salt intensity is greater in enhanced treatments over untreated controls. Sour attribute also had a significant pump rate effect. In both enhancement types, pumping at 15% significant decreases sour intensity, but pumping at 20% begins to increase sour intensity back to intensity levels where no enhancement was used. It has been noted that sour flavor is higher at a lower end point temperature than higher end point temperature (Vote et al., 2000), but little work could be found specifically documenting the increase in sour flavor and the affect of pump rate interaction with sour flavor intensity. The significance of this pump effect on sour could be considered negligible due to small score mean difference between treatments.

Of the nine flavor attributes detected, five attributes were significantly different ($P < 0.05$) among treatments with pH below 5.5 (Table 3.6), which had characteristics of PSE

pork. Cooked pork aroma and umami had significant ($P < 0.05$) pump effects, while piggy, salt, and sour had significant ($P < 0.05$) (E*P) interactions. The use of 15% pump rate had the same effect on cooked pork aroma as the use of 20% pump rate in sodium phosphate enhancement (score of 3 at 15% vs. score 3.2 at 20%) as well as in meat protein isolate (score of 3.5 at 15% vs. score 3.4 at 20%). But, cooked pork aroma was significantly decreased from the use of sodium phosphate enhancement at any given pump rate as compared to the control (score of 3.6). Meat protein isolate enhancement did not alter the cooked pork aroma. While piggy was significantly different ($P < 0.05$) among treatments, only one loin was significant different ($P < 0.05$) than all others in the below pH 5.5 study. Loin treatment 11 does not appear to have any differences in pH, color and marbling scores, or L, a, b values than all other loins in the study (Table 3.7). Therefore, it can be concluded that this one loin, while impacting the statistical significance of the piggy attribute to the study, does not present itself to be different than the other loins, and can be considered an abnormally high value which can not be explained by the data. Piggy and acidic flavors decrease with increasing cooking temperature (Meinert et al., 2007). In this study of loins below pH 5.5, salt intensity was the same for both controls. The use of 15% pumped sodium phosphate enhancement significantly increases salt intensity, and is significantly different than all other treatment combinations. As in the study with loins above pH 5.5, it is again interesting that the perception of salt is greater in 15% (score of 3.1) than in 20% (score of 2) in sodium phosphate. The interaction plot of salt (Figure 3.3) shows that the only condition where there is a significant enhancement effect is where there is the use of 15% pump rate. One hypothesis for this result is that a dilution effect may be occurring, such that as you

inject more water, flavor compounds are dispersed in more water and thus less intensity is perceived. Salt intensity is greater with the use of sodium phosphate pumped at 15% (score of 3.1) than with the use of meat protein isolate pumped at the same rate (score of 2.4). While Baublits et al., (2006) also found that enhanced pork chop was saltier than a non-enhanced pork chop; they found that as pump rate increased, salt intensity also increased. However, this study targeted a 1% final salt delivery, while the current study targeted a .5% final salt delivery, so while it appears that this study is in disagreement with the pump rate trend found in the current study, it can not be stated that the trend of increasing salt intensity with increasing pump rate is consistent among all salt concentrations.

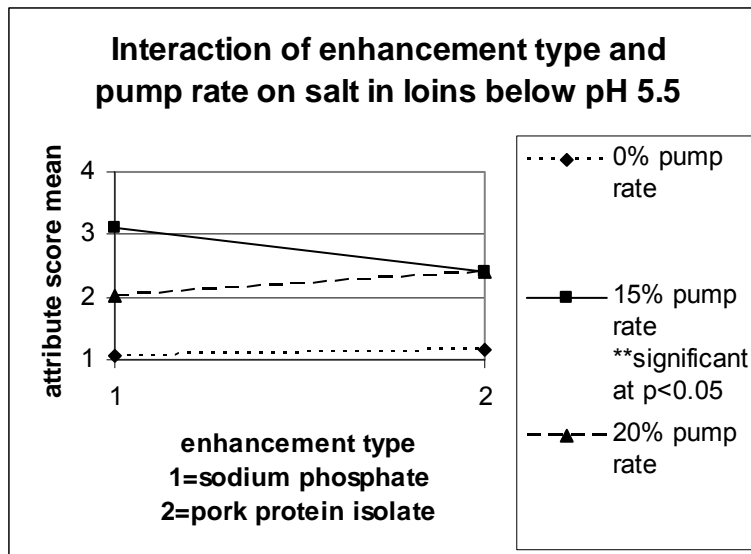


Figure 3.3. Interaction of enhancement type and pump rate on salt in loins below pH 5.5

For sour attribute, there is a trend that increasing pump in sodium phosphate enhancement increases intensity back towards control intensity, but increasing pumps in meat protein isolate decreases intensity below control intensity. In both enhancement types, the results agree with Baublits et al., (2006), that sour intensity is greatest among pork chops that were untreated. The interaction plot for sour (Figure 3.4) shows that the only condition where there is a significant enhancement effect is where there is the use of 20% pump rate. Sour intensity is greater with the use of sodium phosphate pumped at 20% (score of 2.2) than with the use of meat protein isolate pumped at the same rate (score of 1.3). While there was a simple effect of pump rate on umami, mean differences among treatments were of no strong sensorial importance due to low mean score differences within this simple effect, however, it appears that there is a trend that increasing pump rate for both enhancement types decreases umami intensity.

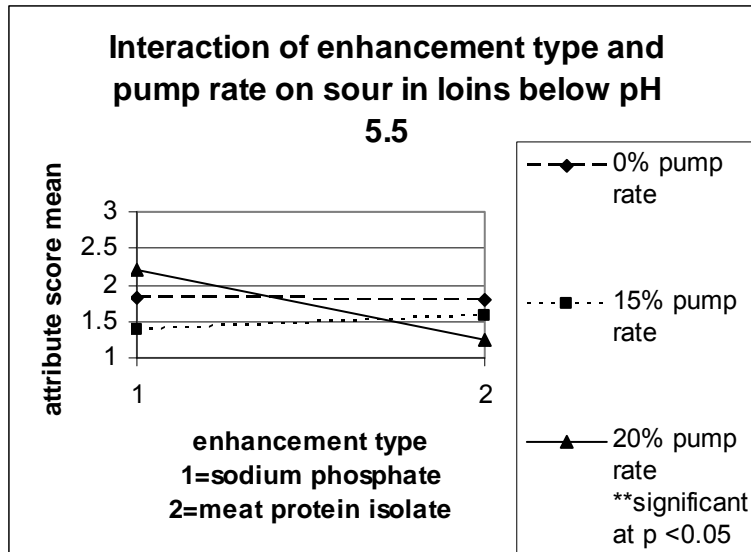


Figure 3.4. Interaction of enhancement type and pump rate on sour in loins below pH 5.5

In both studies, cooked pork flavor is not statistically different ($P < 0.05$). This disagrees with Sheard et al., (1999) who found that sodium polyphosphate injected pork loins had less intense pork flavor compared to nonenhanced pork. However, their work also disagrees with Baublits et al., (2006), who found that cooked pork flavor increased with a polyphosphate pump rate of 12%, but were not different from the control at a pump rate of 12%. There are several ideas that could impact overall cooked pork flavor in enhanced meat. First, it is thought that there a possible dilution of pork flavor as pump rate is increased from a low to higher pump rate (Sheard, et al., 1999). Secondly, there could be an impact of end point cooked temperature impacting the intensity of pork flavor (Prestat, et al., 2002a). Also, it has been suggested that soluble flavor precursors are carried away in excess marinade solution that drains from muscle immediately following pumping (Sheard et al., 1999). In

Baublits et al., (2006), there was not a reported impact of end point temperature, but Sheard et al., (1999) and Prestat et al., (2002b) found that the more intense pork flavors arise from increased internal temperature.

In both studies for above and below pH 5.5, it can be concluded that the greatest flavor differences are the perception of salt and sour. In below pH 5.5 loins, the E*P interaction describe these differences. This interaction is difficult to interpret fully due to the small difference in the magnitude of means used to generate this simple effect; however, the data supports this interaction in salt with an increase in salt for both enhancement types, particularly significant at 15% pump rate. In sour, while there is an interaction, the data helps to further explain that sodium phosphate enhancement increase sour with increasing pump, while meat protein isolate enhancement decreases sour intensity with increasing pump. In above pH 5.5, these differences in salt and sour can be explained through a simple pump rate effect. Both enhancement types reduce the sour intensity from the control, but as pump rate increases, the sour intensity increases back towards pre enhancement intensity. It appears that for salt, both enhancement types work similarly, and that salt perception is greatest at 15% pump rate of sodium phosphate enhancement.

Table 3.5. Effect of enhancement type and pump rate on descriptive flavor and texture attributes of loins above pH 5.5¹

| Treatment number | Enhancement type | | | | | | P-value ² | | |
|----------------------|------------------|------|-------|----------------------|-------|-------|----------------------|------|--------|
| | Sodium phosphate | | | Meat protein isolate | | | | | |
| | Pump rate | | | | | | | | |
| | 0% | 15% | 20% | 0% | 15% | 20% | E*P | E | P |
| 1 | 3 | 5 | 8 | 10 | 12 | | | | |
| Flavor and aroma | | | | | | | | | |
| Attribute | | | | | | | | | |
| Cooked pork aroma | 3.6 | 3.4 | 3.6 | 3.5 | 3.4 | 3.5 | .65 | .42 | .34 |
| Cooked pork flavor | 4.1 | 4.3 | 4.1 | 4.1 | 4.0 | 4.0 | .64 | .16 | .72 |
| Piggy | ND | ND | ND | ND | ND | ND | .56 | .91 | .28 |
| Metallic | 2.0 | 1.2 | 1.7 | 2.0 | 1.7 | 1.6 | .18 | .37 | .02 |
| Astringent mouthfeel | 1.8a | 1.3c | 1.6b | 1.8a | 1.5b | 1.5b | .04 | .39 | <0.01 |
| Sweet | 1.7 | 2.1 | 1.8 | 1.6 | 2.0 | 1.9 | .66 | .48 | .01 |
| Salt | .94c | 2.3a | 1.7b | .51d | 2.2a | 1.8b | .74 | .59 | <.01 |
| Sour | 1.7ab | 1.1e | 1.5bc | 1.7a | 1.2de | 1.4cd | .32 | .40 | <.01 |
| Umami | 1.7 | 1.4 | 1.6 | 1.6 | 1.7 | 1.5 | .14 | .86 | .75 |
| Texture | | | | | | | | | |
| Hardness | 7a | 4.8d | 6.3b | 6.7a | 5.6c | 6.3b | .17 | .39 | <.01 |
| Moisture | 4.6c | 7.7a | 5.1b | 4.4c | 5.4b | 5.0b | <.01 | <.01 | <.0001 |
| # of chews | 30 | 27 | 27 | 29 | 23 | 29 | .28 | .73 | .13 |
| Juiciness | 4.8c | 8.4a | 5.3b | 4.9c | 5.4b | 4.8c | <.01 | <.01 | <.0001 |
| Cohesiveness | 7.2 | 7.2 | 7.1 | 7.1 | 7.2 | 7.0 | .71 | .11 | .17 |
| Fibrous | 6.8ab | 5.5d | 6.6b | 6.9ab | 6.1c | 6.9a | .51 | .07 | <.01 |
| Oily | .65 | .76 | .74 | .63 | .64 | .70 | .54 | .18 | .28 |

^{a,b,c} Means in a row followed by different letters represent significant differences (P<0.05)

¹ Intensities scored on a 15 point Universal SpectrumTM intensity scale, where 0=none and 15=very (Meilgaard et al., 2007)

² P values from full model; full model: enhancement + pump + enhancement*pump + session + replicate + panelist
Attribute in **bold** are significantly different (P<0.05) in full model, ND= not detected

Table 3.6. Effect of enhancement type and pump rate on descriptive flavor and texture attributes of loins below pH 5.5¹

| Treatment number | Enhancement type | | | | | | P-value | | |
|----------------------|------------------|-------|-------|----------------------|-------|-------|---------|------|--------|
| | Sodium phosphate | | | Meat protein isolate | | | | | |
| | Pump rate | | | | | | | | |
| | 0% | 15% | 20% | 0% | 15% | 20% | E*P | E | P |
| 1 | 3 | 5 | 8 | 10 | 12 | | | | |
| Flavor and aroma | | | | | | | | | |
| attribute | | | | | | | | | |
| Cooked pork aroma | 3.6a | 3c | 3.2bc | 3.5a | 3.5a | 3.4ab | .07 | .08 | .04 |
| Cooked pork flavor | 4.0 | 3.8 | 3.9 | 3.9 | 4.0 | 4.1 | .44 | .34 | .81 |
| Piggy | ND | ND | ND | ND | ND | 1.1a | <.01 | <.01 | <.01 |
| Metallic | 2.1 | 1.5 | 2 | 1.9 | 1.7 | 1.9 | .55 | .78 | .06 |
| Astringent mouthfeel | 1.8 | 1.6 | 1.6 | 1.7 | 1.6 | 1.5 | .61 | .14 | .02 |
| Sweet | 1.7 | 1.9 | 1.8 | 1.6 | 1.9 | 1.9 | .71 | .78 | .26 |
| Salt | 1.1c | 3.1a | 2.0b | 1.2c | 2.4b | 2.4b | .04 | .64 | <.0001 |
| Sour | 1.8b | 1.4cd | 2.2a | 1.8b | 1.6bc | 1.3d | .03 | .10 | .18 |
| Umami | 1.6ab | 1.7a | 1.3c | 1.4bc | 1.8a | 1.3c | .31 | .78 | <.01 |
| Texture | | | | | | | | | |
| Hardness | 7.5b | 6.4d | 5.9e | 8.6a | 7.6b | 6.9c | .95 | <.01 | <.01 |
| Moisture | 4.0c | 4.9b | 7.2a | 2.9d | 4.7b | 6.8a | .46 | .08 | .0001 |
| # of chews | 33bc | 26e | 28de | 39a | 35b | 30cd | .25 | <.01 | <.05 |
| Juiciness | 4.2c | 4.8b | 7.9a | 3.0d | 4.6bc | 7.4a | .12 | .02 | <.0001 |
| Cohesiveness | 7.0 | 6.9 | 7.0 | 7.0 | 7.1 | 7.1 | .11 | .03 | .95 |
| Fibrous | 7.4a | 6.7b | 6c | 7.7a | 7.5a | 6.5b | .62 | .02 | <.01 |
| Oily | .57 | .79 | .83 | .58 | .71 | .84 | .81 | .77 | .03 |

^{a,b,c} Means in a row followed by different letters represent significant differences (P<0.05)

¹ Intensities scored on a 15 point Universal SpectrumTM intensity scale, where 0=none and 15=very (Meilgaard et al., 2007)

² P values from full model; full model: enhancement + pump + enhancement*pump + session + replicate + panelist
Attribute in **bold** are significantly different (P<0.05) in full model, ND=not detected

Table 3.7. Properties of pork loins for use in descriptive flavor analysis

| <u>treatment</u> | <u>L*</u> | <u>A*</u> | <u>B*</u> | <u>pH</u> | <u>pH on panel day</u> | <u>Color¹</u> | <u>Marbling²</u> |
|------------------|-----------|-----------|-----------|-----------|------------------------|--------------------------|-----------------------------|
| 1 | 52.43 | 9.48 | 5.60 | 6.16 | 5.71 | 4 | 2 |
| 2 | 70.54 | 10.7 | 11.17 | 4.95 | 5.62 | 1 | 1 |
| 3 | 53.39 | 8.8 | 5.26 | 6.09 | 6.02 | 4 | 2 |
| 4 | 65.20 | 10.13 | 8.51 | 4.86 | 5.89 | 2 | 1 |
| 5 | 55.90 | 7.05 | 6.34 | 6.23 | 6.33 | 3 | 1 |
| 6 | 68.85 | 11.56 | 10.45 | 4.93 | 5.40 | 2 | 1 |
| 7 | 63.61 | 14.9 | 12.97 | 5.00 | 5.74 | 1 | 1 |
| 8 | 55.75 | 11.46 | 7.18 | 6.12 | 5.87 | 4 | 1 |
| 9 | 66.01 | 15.15 | 11.91 | 4.97 | 5.73 | 2 | 1 |
| 10 | 49.37 | 7.81 | 4.17 | 6.42 | 6.67 | 4 | 2 |
| 11 | 52.55 | 11.86 | 8.88 | 4.97 | 6.33 | 4 | 2 |
| 12 | 52.81 | 6.13 | 4.55 | 6.33 | 6.59 | 4 | 2 |

¹Scored on the following scale (NPPC, 2000): color score (1 to 6) 1= pale pinkish gray to white and 6= darkish purple red

² Scored on the following scale (NPPC, 2000): marbling score (1 to 10) 1=1% intramuscular fat and 10=10% intramuscular fat

Table 3.8. Properties of pork loins for use in descriptive texture analysis

| <u>treatment</u> | <u>L*</u> | <u>A*</u> | <u>B*</u> | <u>pH</u> | <u>pH on panel day</u> | <u>Color¹</u> | <u>Marbling²</u> |
|------------------|-----------|-----------|-----------|-----------|------------------------|--------------------------|-----------------------------|
| 1 | 52.03 | 7.74 | 5.82 | 5.95 | 6.04 | 3.0 | 2 |
| 2 | 67.54 | 13.74 | 12.98 | 5.43 | 5.84 | 1.0 | 1 |
| 3 | 45.30 | 8.33 | 5.28 | 6.75 | 6.33 | 4.0 | 2 |
| 4 | 69.84 | 11.89 | 12.18 | 5.50 | 5.93 | 1.0 | 1 |
| 5 | 51.93 | 8.04 | 5.69 | 6.09 | 6.02 | 3.5 | 2 |
| 6 | 65.31 | 14.20 | 12.51 | 5.22 | 5.96 | 2.0 | 1 |
| 7 | 64.02 | 17.86 | 15.10 | 5.50 | 5.99 | 2.0 | 1 |
| 8 | 54.42 | 10.59 | 9.15 | 6.07 | 5.99 | 3.5 | 2 |
| 9 | 67.13 | 16.19 | 14.44 | 5.35 | 6.21 | 2.0 | 1 |
| 10 | 48.99 | 11.08 | 5.21 | 5.78 | 6.25 | 4.0 | 2 |
| 11 | 65.38 | 11.45 | 8.89 | 5.19 | 5.87 | 2.0 | 1 |
| 12 | 50.72 | 14.69 | 9.52 | 6.53 | 6.10 | 4.0 | 2 |

¹Scored on the following scale (NPPC, 2000): color score (1 to 6) 1= pale pinkish gray to white and 6= darkish purple red

² Scored on the following scale (NPPC, 2000): marbling score (1 to 10) 1=1% intramuscular fat and 10=10% intramuscular fat

Table 3.9. Warner Braztler Shear (kg) and proximate analysis of raw loins used in descriptive flavor analysis

| <u>Treatment</u> | <u>WBS (kg)</u> | <u>Held Water (g water/ g protein)¹</u> | <u>Raw Moisture (%)</u> | <u>Raw Fat (%)</u> | <u>Raw Protein (%)</u> | <u>Raw Ash (%)</u> |
|------------------|-----------------|--|-------------------------|--------------------|------------------------|--------------------|
| 1 | 2.03 | 3.3 | 75.54 | 1.0 | 22.6 | 1.0 |
| 2 | 3.27 | 2.9 | 72.59 | 1.6 | 24.6 | 1.0 |
| 3 | 1.56 | 3.9 | 76.50 | 1.9 | 19.6 | 1.7 |
| 4 | 1.76 | 4.2 | 78.26 | 0.75 | 18.5 | 1.9 |
| 5 | 1.78 | 4.1 | 78.34 | 1.1 | 19.0 | 1.5 |
| 6 | 1.81 | 3.5 | 74.11 | 2.4 | 21.3 | 1.4 |
| 7 | 2.44 | 3.1 | 73.26 | 2.0 | 23.4 | 1.8 |
| 8 | 2.34 | 3.2 | 74.39 | 2.0 | 22.8 | 1.3 |
| 9 | 2.06 | 3.2 | 74.02 | 1.5 | 22.6 | 1.5 |
| 10 | 1.00 | 3.9 | 77.38 | 1.9 | 19.8 | 1.3 |
| 11 | 1.34 | 4.0 | 78.03 | 1.2 | 19.2 | 1.4 |
| 12 | 1.39 | 3.9 | 76.97 | 2.3 | 19.4 | 1.3 |

¹Held Water= (total g water in sample- g water released)/ total g protein in sample

Table 3.10. Proximate analysis of cooked loins used in descriptive flavor analysis

| <u>Treatment</u> | <u>Cooked Moisture (%)</u> | <u>Cooked Fat %</u> | <u>Cooked Protein (%)</u> | <u>Cooked Ash (%)</u> |
|------------------|----------------------------|---------------------|---------------------------|-----------------------|
| 1 | 67.27 | 2.0 | 29.4 | 1.3 |
| 2 | 60.00 | 2.4 | 35.5 | 1.7 |
| 3 | 72.03 | 2.4 | 23.4 | 2.0 |
| 4 | 70.60 | 1.0 | 25.3 | 2.3 |
| 5 | 70.02 | 2.1 | 26.2 | 1.7 |
| 6 | 63.02 | 3.3 | 30.7 | 3.5 |
| 7 | 63.56 | 2.7 | 32.2 | 2.5 |
| 8 | 65.65 | 2.3 | 30.9 | 1.5 |
| 9 | 69.12 | 1.7 | 27.0 | 1.5 |
| 10 | 72.67 | 1.9 | 23.8 | 1.7 |
| 11 | 71.90 | 1.6 | 24.4 | 1.6 |
| 12 | 70.60 | 2.6 | 25.0 | 1.6 |

Table 3.11. Warner Braztler Shear (kg) and proximate analysis of raw loins used in descriptive texture analysis

| <u>Treatment</u> | <u>WBS (kg)</u> | <u>Held Water (g water/ g protein)¹</u> | <u>Raw Moisture (%)</u> | <u>Raw Fat (%)</u> | <u>Raw Protein (%)</u> | <u>Raw Ash (%)</u> |
|------------------|-----------------|--|-------------------------|--------------------|------------------------|--------------------|
| 1 | 2.41 | 3.3 | 74.73 | 1.6 | 22.5 | 1.2 |
| 2 | 1.80 | 3.3 | 71.68 | 4.9 | 21.3 | 1.1 |
| 3 | 1.11 | 3.8 | 76.84 | 1.3 | 20.1 | 1.6 |
| 4 | 1.45 | 4.4 | 78.37 | 1.2 | 17.6 | 1.9 |
| 5 | 1.85 | 3.9 | 77.70 | 1.1 | 20.0 | 1.6 |
| 6 | 2.77 | 4.0 | 76.51 | 1.5 | 19.1 | 1.4 |
| 7 | 2.92 | 3.3 | 74.53 | 1.2 | 22.8 | 0.94 |
| 8 | 2.02 | 3.2 | 73.97 | 2.3 | 22.9 | 1.0 |
| 9 | 2.81 | 3.5 | 75.59 | 1.1 | 21.5 | 1.1 |
| 10 | 1.05 | 3.7 | 76.99 | 1.2 | 20.6 | 1.2 |
| 11 | 2.11 | 3.8 | 76.26 | 2.0 | 20.0 | 1.4 |
| 12 | 1.03 | 3.8 | 77.28 | 1.5 | 20.0 | 1.2 |

¹Held Water= (total g water in sample- g water released)/ total g protein in sample

Table 3.12. Proximate analysis of cooked loins used in descriptive texture analysis

| <u>Treatment</u> | <u>Cooked Moisture (%)</u> | <u>Cooked Fat (%)</u> | <u>Cooked Protein (%)</u> | <u>Cooked Ash (%)</u> |
|------------------|----------------------------|-----------------------|---------------------------|-----------------------|
| 1 | 63.31 | 2.5 | 32.7 | 1.7 |
| 2 | 66.36 | 4.9 | 26.6 | 1.6 |
| 3 | 69.50 | 1.8 | 26.4 | 2.2 |
| 4 | 73.23 | 1.6 | 22.0 | 2.4 |
| 5 | 72.47 | 1.6 | 23.9 | 1.9 |
| 6 | 67.91 | 1.8 | 26.9 | 2.0 |
| 7 | 63.23 | 1.7 | 32.8 | 1.9 |
| 8 | 66.42 | 3.0 | 29.6 | 1.3 |
| 9 | 64.71 | 1.8 | 31.2 | 1.5 |
| 10 | 70.20 | 1.6 | 26.7 | 1.6 |
| 11 | 68.24 | 2.3 | 27.0 | 1.8 |
| 12 | 69.62 | 2.2 | 26.2 | 1.6 |

¹Held Water= (total g water in sample- g water released)/ total g protein in sample

Table 3.13. Properties of brines used in descriptive sensory panels and cook yield trial

| | <u>% salt of brine</u> | <u>% salt in product</u> (% salt of brine * pump rate) | <u>pH of brine</u> |
|---------------------------------|------------------------|---|--------------------|
| Flavor Panel Processing | | | |
| Sodium phosphate 15% | 3.58 | .54 | 7.43 |
| Sodium phosphate 20% | 2.67 | .53 | 7.71 |
| Meat protein isolate 15% | 4.0 | .60 | 9.91 |
| Meat protein isolate 20% | 2.97 | .59 | 9.84 |
| Texture Panel Processing | | | |
| Sodium phosphate 15% | 3.85 | .58 | 7.5 |
| Sodium phosphate 20% | 2.32 | .46 | 7.7 |
| Meat protein isolate 15% | 4.05 | .60 | 9.94 |
| Meat protein isolate 20% | 3.08 | .62 | 9.73 |
| Cook Yield Trial | | | |
| Meat protein isolate 20% | 3.43 | .69 | 10.31 |
| Sodium phosphate 20% | 3.21 | .64 | 7.75 |

3.4.1.2 Texture

The sensory language identified and differentiated the pork loins above (Table 3.5) and below (Table 3.6) pH 5.5 in texture. Principal components of both above and below pH 5.5 loins are shown in Figure 5. Principal components analysis for the descriptive texture attributes illustrated differences between treatments and relationships between the attributes in the first two principal components (PCs), which accounted for 85% of the variation in the data. Principal components of both above and below pH 5.5 loins are shown in Figure 3.1. The first principal component (PC1) (68% variability) of the texture biplot was composed of moisture, juiciness, and oily texture (positive dimension of PC1) and number of chews, hardness, and fibrous texture (negative dimension of PC1), whereas PC2 (17% variability) differentiated the treatments in cohesiveness. Treatments 1, 2, 7, and 8 were controls in the study, and were characterized by higher intensities of hardness, fibrous and number of chews

compared to sodium phosphate and meat protein isolate treatments. All loins except treatment 9 with sodium phosphate and meat protein isolate enhancement were associated with higher intensities of moisture and juiciness compared to controls. Treatment 3 has the highest degree of moisture among all treatments.

Multivariate analysis of sensory profiles can give a wide variation of results. Risvik, (1994), reported on the concern of multivariate techniques on the analysis of sensory quality of meats, and the misuse of its results to draw specific conclusions. In thier study of trained panels, they reported that multiple trained sensory studies of texture and appearance come out of a multivariate analysis along the same dimensions in PCA plots, suggesting that these two attributes confound each other in the analysis. When using descriptive data to correlate with consumer preference data, a change in meat texture could be seen as a color change to a consumer when using confounded data, so the effect of texture would be picked up by a consumer in a different way, causing disturbance in the conclusions of utilizing descriptive data to understand potential consumer preference. The author warns of indicating casual relationships among attributes in Principal Component Analysis without first having similar results in multiple similarly designed studies preformed by independent trained groups.

Pearson correlation coefficients (Table 3.12) show high ($P < 0.05$) positive correlations between hardness, number of chews, and fibrous texture. Also, Warner Braztler Shear (WBS) instrumental values have strong positive correlation to texture lexicon descriptors hardness and number of chews. Hayes et al., (2006) found strong positive correlations between WBS values and texture descriptors tenderness and juiciness. Additionally, there are high ($P < 0.05$) positive correlations between juiciness, moisture, and oily textures. As

expected, terms moisture and juiciness have strong negative correlation to hardness and fibrous.

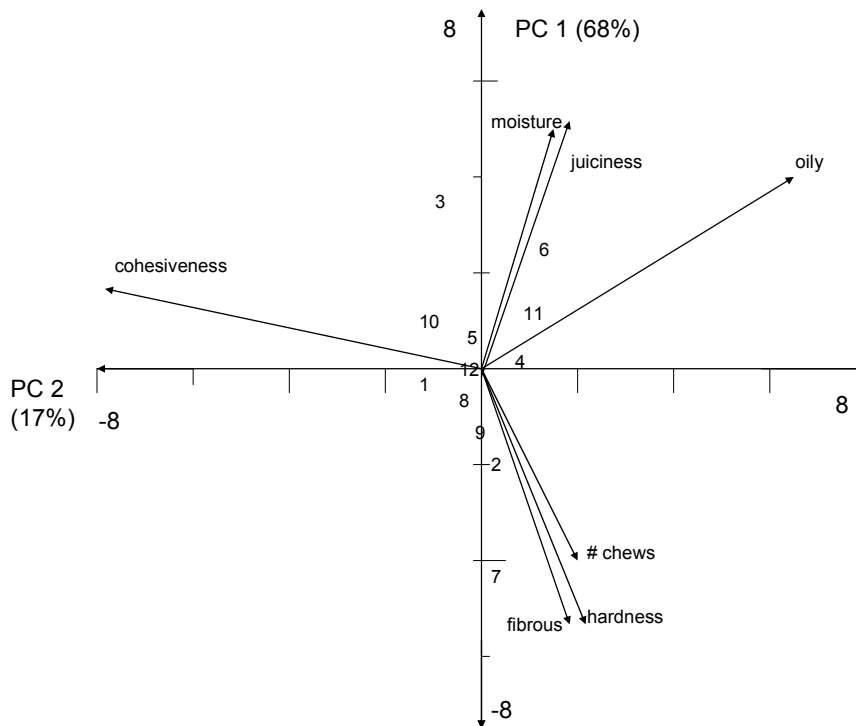


Figure 3.5. Principal component biplot of descriptive sensory texture analysis of pork loins. Numbers represent treatments (Table 3.5 and 3.6) PC1= principal component 1; PC2= principal component 2.

Table 3.14. Person correlation coefficient of texture attributes and Warner Braztler Shear (WBS) value¹

| <u>Attribute</u> | <u>Hardness</u> | <u>Moisture Release</u> | <u>Number of Chews</u> | <u>Juiciness</u> | <u>Cohesiveness</u> | <u>Fibrous</u> | <u>Oily</u> | <u>WBS</u> |
|------------------|-----------------|-------------------------|------------------------|------------------|---------------------|----------------|-------------|------------|
| Hardness | 1 | -.79 | .88 | -.75 | -.36 | .94 | -.50 | .68 |
| Moisture Release | -.79 | 1 | -.57 | .99 | .31 | -.88 | .79 | -.27 |
| Number of Chews | .88 | -.57 | 1 | -.52 | -.26 | .80 | -.42 | .70 |
| Juiciness | -.75 | .99 | -.52 | 1 | .32 | -.87 | .76 | -.20 |
| Cohesiveness | -.36 | .31 | -.26 | .32 | 1 | -.42 | -.10 | -.14 |
| Fibrous | .94 | -.88 | .80 | -.87 | -.42 | 1 | -.59 | .49 |
| Oily | -.50 | .79 | -.43 | .76 | -.10 | -.59 | 1 | -.05 |
| WBS | .68 | -.27 | .70 | -.20 | -.14 | .49 | -.05 | 1 |

¹Values in bold represent significant correlations (P<0.05)

Of the seven texture attributes detected, four attributes were significantly different ($P<0.05$) among treatments with pH above 5.5. There was a significant simple effect ($P<0.05$) of pump rate on hardness and fibrous. Among both enhancement types, there appears to be a trend that a 15% pump rate decreases intensities of these attributes, but the use of 20% pump rate begins to increase the hardness and fibrous intensities back towards control level intensities. An enhancement*pump (E*P) rate interaction ($P<0.05$) was observed for moisture and juiciness. To understand the simple effect of enhancement on pump rate on moisture, the (E*P) interaction was plotted (Figure 3.6). The only condition where there was a significant ($P<0.05$) enhancement effect on moisture was with the use of a 15% pump rate. Moisture intensity was greater (7.7) when using sodium phosphate pumped at 15% than when pumping meat protein isolate (5.4) at 15%. To understand the simple effect of enhancement on pump rate on juiciness, the (E*P) interaction was plotted (Figure 3.7). The only condition where there was a significant ($P<0.05$) enhancement effect on juiciness was also with the use of a 15% pump rate. Juiciness intensity was greater when using sodium phosphate pumped at 15% (8.4) than when pumping meat protein isolate at 15% (5.4). The contribution of this interaction to both moisture and juiciness appears to be that mean intensities are increased from the control at 15% but again follow the same pattern as in hardness and fibrous, that 20% pump rate of both enhancements begins to decrease intensities back towards the level of intensity values found in the controls. Baublits et al., (2006) found that there was no improvement in overall tenderness in beef using a pump rate of 12%, but there was an improvement at 18% pump rate. Increasing tenderness and juiciness with an increased pump rate was also found by Sheard et al., (1999) in pork loin.

While it appears that that this work with loins above pH 5.5 is in direct opposition to multiple studies, other studies did not separate muscle pHs, and had mixed pH values of both above and below pH 5.5. It is accepted that muscle pH movement away from the isoelectric point has greater water holding capacity due to increased net protein charge, and thus have a greater impact on texture of the muscle (Ouali et al., 2006). There may be a saturation point at which at a certain high muscle pH, the protein structure's ability to effectively use the ionic strength of the marinade is maximized and then plateaus. Phosphate pumping may help prevent loss of juiciness and moisture, due to both added liquid pickup and increased retention, particularly if muscle pH increases due to phosphate addition. The perceived increase in tenderness in enhanced treatments could be attributed to a weakened muscle structure from the mode of action of polyphosphate on myofibrillar protein, along with a higher water content that occurs with enhancement of brine. The perceived increase in tenderness in meat protein isolate treatment could be attributed to synergistic reactions with the protein isolate forming a gel matrix within the system, which would trap free water, which has been the proposed mechanism of hydrolyzed soy proteins functionality (Feng and Xiong, 2002). It could be concluded from these results on loins above pH 5.5 that 15% pump rate should be considered the more optimum pump rate in both enhancement types to achieve higher moisture and juiciness intensities while achieving lower hardness and fibrous intensities compared to a control. This recommendation does not follow most published work, however, since most studies support the idea that increasing pump trends towards increasing favorable texture attributes. It appears that both enhancement types are resulting in similar results with the use of lower pump rate in loins with pH above 5.5, thus, in a move

towards an “additive free” climate, meat protein isolate may be considered more favorable to consumers in delivering similar texture as sodium phosphate injected products but being more suited towards those consumers who are looking for clean ingredient statements on processed meats.

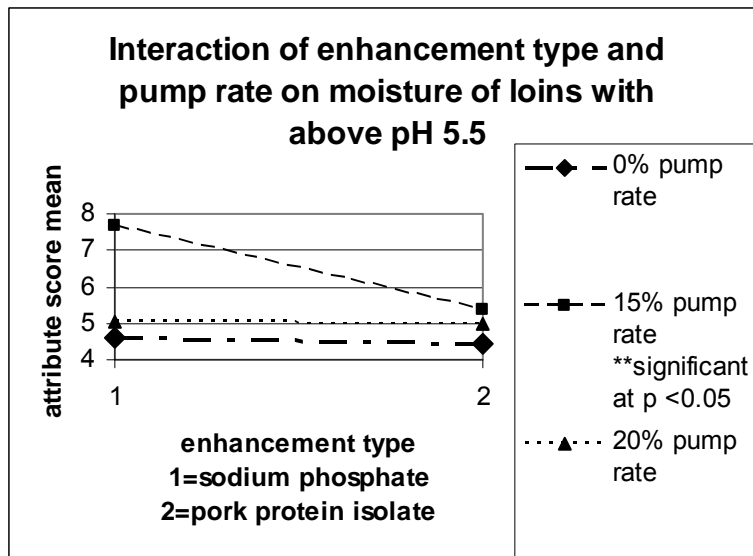


Figure 3.6. Interaction of enhancement type and pump rate on moisture in loins above pH 5.5

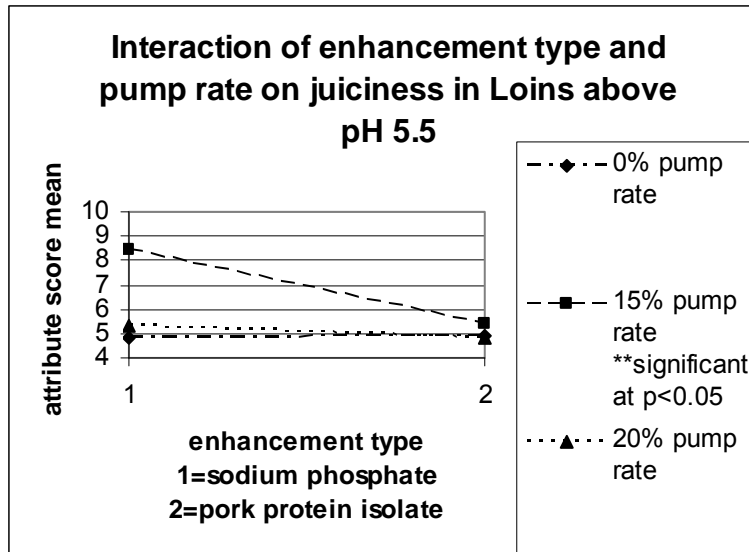


Figure 3.7. Interaction of enhancement type and pump rate on juiciness in loins above pH 5.5

Of the seven texture attributes detected, five attributes were significantly different ($P < 0.05$) among treatments with pH below 5.5 (Table 3.6). Hardness, moisture, number of chews, juiciness, and fibrous all had significant ($P < 0.05$) pump effects, and there were also significant ($P < 0.05$) enhancement effects on hardness, number of chews, and fibrous. To understand the simple effect of enhancement (Table 3.14), mean intensity scores were averaged across all pump rates. Regardless of pump rates, meat protein isolate enhancement yields higher means in hardness, number of chews, and fibrous attribute over sodium phosphate enhancement. Meat protein isolate, once injected, serves to immobilize free water and to prevent moisture loss during heating, however, this data suggests that at the protein

content and pump rates used, meat protein isolate may be binding to much water, and the impact of this over stabilization of water is a harder texture in the cooked product. Future work should investigate varying levels of protein content in the meat protein isolate brine and to see what impact protein content has on these same texture attributes within muscle that is below pH 5.5. It appears from this data that meat protein isolate is in fact effective at improving texture, but there may be a saturation point within the protein structure which does not allow for any improvement in texture, but rather a plateau and then negative impact towards texture attributes. To understand the simple effect of pump rate (Table 3.13), mean intensity scores were averaged across all enhancement types. Regardless of enhancement types, there is no difference in 15% and 20% pump rate and its effect on hardness and numbers of chews, which both perform the same in decreasing these attributes. Fibrous is also decreased compared to control, but 15% and 20% are different (7.12 vs. 6.26, respectively). As expected, moisture and juiciness intensities are increased with increasing pump rates. This work is in good agreement with Baublits et al., (2006), who found that sodium phosphate injected pork chops were less hard, less cohesive, less hard, less fibrous, and more chews to swallow than non injected pork chops. These results would suggest increased tenderness and increased juiciness with enhancement, which agrees well with multiple recent studies (Baublits et al., 2006, Prestat et al., 2002a, Sheard et al., 1999, Hayes et al., 2006). It is generally accepted that juicy and tender meats are preferred by consumers to those that are less tender and less juicy. These two attributes typically are positively correlated when determining preference as related to texture. However, the underlying mechanism behind meat texture is still open for debate among scientists.

To further complicate the discussion regarding meat texture, controversy arises in the literature in regards to the effect of ultimate pH and intramuscular fat on textural qualities such as tenderness and juiciness. Flores et al., (1999) found a close relationship between IMF (intramuscular fat) content and ultimate pH, and that ultimate pH had a close relationship with juiciness. Fortin et al., (2005) also found similar favorable relationships between marbling and sensory characteristics. Conversely, Josell et al., (2003) found that muscle with high IMF was not tender, and that a direct relationship did not exist between IMF and tenderness. In agreement were Rincker et al., (2008) who found that IMF had limited effect on perceived tenderness, juiciness, and pork flavor, with only 13% of the variability of the treatments controlled by varying pH levels explained by IMF. Unpublished work in our lab also confirms Rincker et al., (2008) and Lonergan et al., (2007), that IMF values did not have an effect on flavor of pork loins of varying pH. This result allowed for the establishment of our treatment groups in this study by above and below pH 5.5 without regard to IMF values. Additionally, there was no correlation between lipid content to juiciness in treatments controlled by pH classes (Lonergan et al., 2007), but ultimate pH classes did impact sensory quality, such that high pH (above 5.8) will have superior texture from the myofibrillar fragmentation occurring at higher pHs. Additional issues that could add to this debate include rate of pH decline, breed, and muscle type.

While this study did not address end point internal temperature and its affect on pork loin texture, it is apparent that end point temperature will have a large impact on perceived textural quality of meat. Prestat et al., (2002a), found that has endpoint temperature increased from 70°C to 80°C, that juiciness decreased in unpumped pork chops but remained

constant in pumped chops. Oppositely, Sheard et al., (1999) reported that juiciness decreased as internal temperature increased in pumped samples. Also of opposition to these selected studies is Vote et al., (2000), who showed that in beef, cooking to a higher final internal temperature increased juiciness of pumped steaks. Thus, it is apparent from these conflicting results, that study to study variability in both end point temperature, along with other factors that are controlled in experiments such as pump rate, polyphosphate type and concentration, cooking technique, as well as species type can all impact the textural quality of the study samples.

Therefore, it is difficult to make any strong conclusions regarding these descriptive texture results as they relate to previous work. Across all studies, the importance of texture to consumer satisfaction is understood. In fact, Brewer et al., (2002), found that texture most closely paralleled with purchase intent in enhanced pork. Work should be done to find optimal end point temperature, enhancement pump rate and enhancement type that will result in the greatest consumer satisfaction.

Warner Braztler Shear (WBS), water holding (WH), and proximate analysis results for flavor loin treatments are displayed in Table 3.9 and Table 3.10, while texture loin treatments are displayed in Table 3.11 and Table 3.12. Warner Braztler shear value averages for flavor loins are lowest in meat protein isolate treatments (1.4) vs. sodium phosphate (1.7) and control (2.5). In texture study loins, WBS value averages were much lower than the control (3.3), but were the same (1.8) between treatment groups. While it appears that meat protein isolate provides as good or greater tenderizing affect than sodium phosphate, WH value averages suggest that sodium phosphate treatments have the greatest degree of water

holding capacity (3.9), vs. meat protein isolate (3.8) and control (3.1) in flavor study loins.

There appears a similar trend in texture study loins, with sodium phosphate treatments having the greatest degree of water holding capacity (4.0) vs. meat protein isolate (3.7) and control (3.3). Proximate analysis results for both flavor and texture study loins show that in general, protein content is greater in meat protein isolate enhanced loins over sodium phosphate enhanced loins, but not greater than control. As expected, sodium phosphate enhanced treatments have greater ash content than in meat protein isolate and control.

Among both studies for above and below pH 5.5, it can be concluded that the use of an enhancement method can greatly impact texture attributes as compared to a control. In above pH 5.5, the use of an enhancement type at 15% pump rate is more preferred over 20% due to its ability to increase moisture and juiciness and decrease hardness and fibrous at a greater level than the use of 20% pump rate. However, in below pH 5.5, the opposite occurs, where the increasing pump rate from either enhancement type increases attributes that could be considered favorable. It can not be determined whether in above pH 5.5 if a particular enhancement type is performing more effectively, but in below pH 5.5 loins, there is a significant difference ($P < 0.05$) in the way meat protein isolate performs compared to sodium phosphate in hardness, number of chews, and fibrous.

Table 3.15. Simple effect of pump across all enhancement types on texture and flavor attributes of loins below pH 5.5

| <u>Pump rate</u> | <u>Attribute</u> | | | | | | |
|------------------|------------------|------------------------|----------------|-----------------|------------------|--------------------------|--------------|
| | <u>Hardness</u> | <u>Number of Chews</u> | <u>Fibrous</u> | <u>Moisture</u> | <u>Juiciness</u> | <u>Cooked Pork Aroma</u> | <u>Umami</u> |
| 0% | 8.01a | 35.6a | 7.52a | 3.43c | 3.58c | 3.55a | 1.51ab |
| 15% | 7.02b | 30.4b | 7.12a | 4.77b | 4.72b | 3.25b | 1.73a |
| 20% | 6.42b | 29.1b | 6.26b | 6.96a | 7.64a | 3.30ab | 1.29ab |

^{a,b,c} Means in a column followed by different letters represent significant differences (P<0.05)

Table 3.16. Simple effect of enhancement across all pump rates on texture attributes of loins below pH 5.5

| <u>Enhancement type</u> | <u>Attribute</u> | | |
|-------------------------|------------------|------------------------|----------------|
| | <u>Hardness</u> | <u>Number of Chews</u> | <u>Fibrous</u> |
| Sodium phosphate | 6.60b | 28.8b | 6.69b |
| Meat protein isolate | 7.69a | 34.7a | 7.24a |

^{a,b,c} Means in a column followed by different letters represent significant differences (P<0.05)

3.5 Summary

An E*P interaction described the flavor differences found in salt and sour attribute of below pH 5.5 loins, which were the only attributes found to be both statistically and sensorial important in flavor study. There were no differences in salt intensity of protein isolate enhancement at 15% and 20%, but salt intensity was greater at 15% than 20% in sodium phosphate. Sodium phosphate injection increases sour with increasing pump, while meat protein isolate enhancement decreases sour intensity with increasing pump. In above pH 5.5 loins, the differences in salt and sour can be explained through a simple pump rate effect. Both enhancement types reduce the sour intensity from the control, but as pump rate increases from 15% to 20%, the sour intensity increases back towards control intensity. It appears that for salt, both injection types work similarly, and that salt perception is greatest at 15% pump rate than in 20% pump rate.

In both above and below pH 5.5 loins, it can be concluded that the use of an enhancement method can greatly impact texture attributes as compared to a control. In above pH 5.5, the use of an enhancement type at 15% pump rate is more preferred over 20% due to its ability to increase moisture and juiciness and decrease hardness and fibrous at a greater

level than the use of 20% pump rate. However, in below pH 5.5, the opposite occurs, where the increasing pump rate from either enhancement type increases attributes that could be considered favorable and decreases attributes that could be considered undesirable when comparing to an initial control mean intensity. In above pH 5.5, sodium phosphate at 15% pump greatly increases moisture and juiciness attributes compared to all other pump and enhancement combinations. In below pH 5.5 loins, there is a significant difference ($P < 0.05$) in the way meat protein isolate performs compared to sodium phosphate in hardness, number of chews, and fibrous. These attributes are greater across all pump rates for meat protein isolate than in sodium phosphate.

Results of this study show that any enhancement method will improve texture and flavor attributes of boneless pork loins. While flavor does not appear to be dramatically impacted by the two methods, texture attributes which are generally accepted as positive are increased at a greater rate with the use of sodium phosphate, however, from an instrumental tenderness perspective, meat protein isolate outperforms sodium phosphate. This study lays the foundation for providing data which could aid processors in evaluation of alternatives to sodium phosphate alternatives with meat protein isolate. Much more work in the area of meat protein isolate is needed to confirm the importance of these results as they could be applied to today's meat industry.

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CHAPTER 4

EVALUATION OF SODIUM PHOSPHATE AND MEAT PROTEIN ISOLATE ENHANCEMENTS ON THE COOKING QUALITY OF BONELESS PORK LOINS

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ABSTRACT

Enhancement with sodium phosphate has become a popular practice in the meat industry. Other enhancement methods can also be effective in improving sensory characteristics as well as cooking yields and losses. Meat protein isolate prepared with alkaline solubilization and precipitation method is an emerging enhancement technology which warrants further investigation to its benefits as an enhancement method. The objective of the study was to determine the impact of both sodium phosphate (P) and meat protein isolate (I) enhancement methods on processing characteristics including drip loss, cook yields, cook losses, and cooler shrink. An additional objective was to determine the tenderizing effect of both (P) and (I) with Warner Braztler Shear (WBS).

Control (n=25), sodium phosphate (n=25) and meat protein isolate (n=25) were injected at a 20% pickup. Drip loss in (I) was less ($P<0.05$) compared to (P) (.43% vs.58%) treatments, but was not different from (C). Overall cook yields at 24h were different ($P<0.05$) among all three treatments with (P) yielding 95.93%, (I) yielding 92.65% and (C) yielding 79.61%. Cook loss was not different ($P>0.05$). Cooler shrink was different ($P<0.05$) among all three treatments, with (P) 2.27%, (I) 2.1% and (C) 1.88%.

WBS values were different ($P<0.05$) among all three treatments with (I) (1.48 kg), (P) (1.64 kg), and (C) (2.15 kg). Processing results show that yields are greatest with (P) but that (I) provide benefits in decreasing WBS. Processors can determine which value is greatest for their needs.

4.2 Introduction

The use of non-meat ingredients to improve functionality of proteins is commonly used to improve overall quality of muscle. Enhancement serves to add value to fresh meats by the addition of ingredients which can impact flavor, color, and texture of the product. Salts functions in enhanced meats to develop flavor, increase moisture retention, increase ionic strength, and solubilize myofibrillar proteins, thus enhancing protein binding (Baublits et al., 2005). Sodium salt typically works synergistically with sodium phosphates in most meat enhancement systems. Sodium phosphate binding with protein results in an increase in the net negative charge of a protein, causing greater repulsion among the proteins, and therefore increasing water binding (Baublits et al., 2005). Sodium phosphates can also increase pH of a protein product to the upper limits by moving the isoelectric curve to a more alkaline environment to increase water binding (Sheard et al., 1999). Increasing the pH of meat increases its functional properties that are associated with water binding, including moisture, tenderness, and texture (Sheard et al., 1999).

These characteristics can improve cook yields and improve juiciness and tenderness characteristics (Baublits et al., 2005). In addition, salt at any concentration above a control can add flavor, while phosphates can cause off flavors at any concentration alone or with salt at levels of >5% (Sheard et al., 1999, Vote et al., 2000). While sodium phosphate delivers certain protein functionality, there are some consumers who express a concern of phosphate on the label (Brewer et al., 2002).

There is a growing interest in the use of muscle proteins as ingredients in foods (Hultin and Kelleher, 2000) due to their wide availability and the ability to add value to

waste/low value starting materials. Muscle proteins flavor can be more bland as compared to soy protein, and is superior in nutritional quality over vegetable proteins (Lanier, 1985). It has been hypothesized that the use of solubilized meat proteins could provide similar WHC functions as traditional salt sodium phosphate enhancement solutions (Kristinsson and Hultin, 2003a). Acid or alkaline extraction to create muscle protein isolate is based on the concept that muscle protein can be diluted with water and treated at either alkali (pH 11) or acidic (pH 2.5) pH to achieve a low viscosity. This low viscosity material can then be centrifuged to yield a protein free of membranes and lipids (Hutlin and Kelleher, 2000). The resulting membrane and lipid free soluble protein are then recovered by isoelectric precipitation. This is accomplished by raising the pH of the protein slurry to 5.5, resulting in a protein isolate that can be used as a functional food ingredient (Kristinsson and Hultin, 2003b). This meat protein isolate can be rehydrated into water with other ingredients such as salt and then injected into whole muscle.

The purpose of our study was to compare the use of salt and sodium phosphate enhancement to meat protein isolate enhancement in pork and determine their effectiveness against a control. The objectives of this study was to understand the feasibility of the use of both meat protein isolate recovered using alkali precipitation and solubilization method and sodium phosphate enhancement in regards to cooking yield and losses.

4.3 Materials and Methods

4.3.1 Muscle Selection

Vacuum packed strapless pork loins, IMPS# 413B, were boxed and shipped on

refrigerated truck to NCSU Processed Meat Laboratory from local processor. Control (n=25), sodium phosphate enhanced (n=25) and meat protein isolate enhanced (n=25) were randomly assigned from this total. Loins were removed from vacuum package, and starting at the rib end, 2.54 cm chops were cut for instrumental analysis. Chop 1 was assigned for proximate and slurry pH, where one half of the chop was cut and placed in resealable bag for proximate analysis and the other half of the chop was cut and placed in resealable bag for pH analysis. Chop 1 was also utilized for L, a, b measurement using model CR-200 Minolta Colorimeter (Minolta Corp., Ramsey, NJ), and visual evaluation with National Pork Producers Council (Des Moines, IA) color and marbling score. Chop 2 was assigned for an uninjected Warner Braztler Shear (WBS) measurement and was labeled and wrapped in freezer paper (Appendix 6.2). After chops 1 and 2 were removed, a green weight was taken (w1).

4.3.2 Preparation and Delivery of Brines

4.3.2.1 Sodium Phosphate Brine Preparation

One 75.7L sodium phosphate brine was formulated for 20% pickup, where brine targeted for .5% sodium phosphate and .5% salt delivery in the injected loin (Table 4.1). Sodium phosphate, Brifisol 85 Instant, (BK Giulini Corporation, Simi Valley, CA) was composed of an agglomerated blend of poly and pyrosodium phosphate consisting of 56% phosphorus pentoxide and a pH of 8.5+/0.3 in a 1% solution. Tap water was weighed according to formulation in stainless steel bin, covered with plastic, and held for 15h in 1°C cooler. Tap water analyzed with EPA method 200.8 and contained .146 mg/L copper, .952

mg/L iron, and <.002 lead, manganese, and arsenic (Chemical and Environmental Technology Inc., RTP, NC). On the day of enhancement, bins were taken from the cooler, and sodium phosphate was slowly solubilized into water. After sodium phosphate Brifisol 85 Instant was completely solubilized; the salt, Top Flo, (Cargill, Minneapolis, MN) was added to this solution using model MP450 Turbo agitator (Robot Coupe, Jackson, MS).

4.3.2.2 Meat Protein Isolate Preparation

Meat protein isolate was prepared using alkali aided extraction and precipitation methodology as outlined in Kristinsson et al, 2005. Knife removal of excess fat on thawed pork trim was preformed to ensure higher yields from having greater protein to fat ratio in the starting meat source. Pork trim (approximately 80% lean, 20% fat) was obtained as raw material to obtain meat isolate. This trim was ground in Stephan chopper model 2889 (Stephan, Columbus, OH) with cold tap water in a 1:1 ratio. To reduce viscosity, additional water was further added to initial meat slurry to achieve a 1:4 solution. To increase the meat solution pH to 11, 2N Sodium Hydroxide was added dropwise, aiding to solubilize protein. As 2N sodium hydroxide was slowly added, a plastic paddle continually mixed the slurry. The pH was measured with a calibrated handheld ISFET probe pH meter (IQ Scientific Instruments, San Diego, CA). This solution was then strained through a black plastic mesh strainer with 0.6mm hole size and the insoluble fraction was discarded. A 2 N hydrochloric acid solution was added dropwise to the resulting strained material to achieve a target pH of 5.5. At this point, flocculation is evident as protein precipitation occurs. A plastic paddle continually mixed the slurry while the pH was being reduced. This protein slurry was then

dewatered through a chiffon fabric with 0.001 mm hole size. The protein slurry was placed on chiffon fabric, and remaining water was manually squeezed out of the slurry. This dewatered protein isolate was placed in resealable plastic bags, placed in 1 °C cooler for 15h until further utilized in brine preparation.

4.3.2.3 Meat Protein Isolate Brine Preparation

Preliminary work was conducted to determine the ability of a automatic brine injector, Injectamatic model PI21, (Koch Equipment, Kansas City, MO) to pull through a viscous solution. Viscosity is highly affected by the percent protein of a marinade (Shahidi and Venugopal, 1994), such that increasing protein content increases viscosity. Preliminary work revealed that 2.1% protein solution was the required percent protein needed to maximize the efficiency of the available brine injector.

Protein isolate was estimated for moisture using a simplified method to imitate the action of a laboratory scale CEM (Matthews, NC) oven. Moisture content was determined by taking a small piece of isolate, taking its sample weight, placing on a paper towel of known weight, and placing in a General Electric turntable microwave oven serial number 3850W3W081A (Fairfield, CT) for 3 minutes on highest setting to remove water. Sample was then reweighed to calculate percent moisture content.

With known percent solids (100- percent moisture content) and a standard 2.1% protein of isolate, the grams protein per liter of isolate was determined. To match the salt percentage (2.5%) found in the sodium phosphate brine, the necessary amount of salt was added to this total, and subtracted from 1 L of solution to determine the grams of water

needed. By percentage, the amount of water needed for this marinade was 95.4% of the total volume. One 75.7L meat protein isolate brine was formulated for 20% pickup, with 2.1% protein, 2.5% salt, and 95.4% water. Meat protein isolate brine was made by mixing known amount of salt to meat protein isolate. This mixture of meat protein isolate and salt was and then added to a preweighed amount of cold tap water, which had been stored in a stainless steel bin with plastic covering in a 1 °C cooler overnight for 15h, with model MP450 Turbo agitator (Robot Coupe, Jackson, MS).

Table 4.1. Formulation of sodium phosphate and meat protein isolate brines

| <u>Target pick up</u> | <u>Ingredient</u> | <u>% of brine</u> |
|-----------------------|--|-------------------|
| Brine 1- 20% | Cold Water | 93.34% |
| | Sodium phosphate (Brifisol 85 Instant) | 3.33% |
| | Salt (Top Flo) | 3.33% |
| | Total | 100.00% |
| Brine 2- 20% | Cold water | 95.40% |
| | Meat protein isolate | 2.10% |
| | Salt (Top Flo) | 2.50% |
| | Total | 100.00% |

4.3.2.4 Enhancement with Brines

Prior to enhancement of treatments, loins which were not to be used as a treatment in the study were run through an automatic brine Injectamatic model PI21 (Koch Equipment, Kansas City, MO) with salt sodium phosphate solution to narrow down range of .3-.6 bars of

pressure on brine injector which would achieve desired level of pickup. The pH of both sodium phosphate and meat protein isolate brines were taken (IQ Scientific Instruments, San Diego, CA), a sample for salt analysis, and a sample of isolate brine was taken for viscosity analysis utilizing model RVT Brookfield Viscometer (Brookfield Engineering Laboratories Inc., Middleboro, MA). Protein isolate brine was 150 cps, and needed approximately .5 bar of pressure to pull through the brine injector. Loins were passed through brine injector, targeting a 20% pickup. Meat protein isolate treatments were processed last. Loins were weighed after enhancement (w2), and a percent enhancement pickup was determined. Chop 3 was removed from the rib end and assigned for a post injected Warner Braztler Shear measurement. Warner Braztler Shear chops were labeled and wrapped, placed in 1°C carcass cooler, until 7d post mortem when WBS chops were moved to -15°C freezer. The loin was weighed after chop 3 was removed (w3). All loins were then placed in lugs with plastic wrap covering the tops, and held for 18h in 1°C cooler. An 18h post enhancement weight was determined (w4). Loins were then placed randomly on a smokehouse truck, identified with a tag and loin location on smokehouse truck mapped on paper. Ten thermocouples (Type T thermocouple Cu-Constantan, TMQSS-040U-6, Omega Engineering, Stamford, CT), attached to a data acquisition unit utilizing Personal DqView 2.0.4 software, were inserted randomly at sirloin end centers. Truck was inserted into model CVU-650 smokehouse (Enviropak, Clackamas, OR) programmed for a 3 step water and steam cook cycle (Appendix 6.5). Loins were cooked to an internal temperature of 70°C, which took approximately 4h. Loins were weighed again after 1.5h post cooking (w5), and then moved into cooked meats cooler at 1°C for 24h. A 24h post cooking weight was then taken (w6)

after this chilling time.

4.3.2.5 Weight Changes During Processing and Cooking

Weights of loins were recorded before enhancement (w1), and after enhancement (w2), after post enhanced WBS chop was removed (w3), after 18h overnight in cooler (w4), 1.5h post cooking (w5), and after 24h post cooking (w6). The following calculations were then made:

$$\% \text{ enhancement pickup (A)} = [(w2-w1)/w1]*100$$

$$\text{Post enhanced WBS chop weight (B)} = (w2-w3)$$

$$\text{Weight of purge in WBS chop (C)} = [B*(A/100)]$$

$$\text{Weight of unenhanced WBS chop (D)} = (B-C)$$

$$\text{Adjusted green weight (accounting for cut of post enhanced WBS chop) (E)} = (w1 - D)$$

$$\% \text{ pickup after 18h equilibration} = [(w4-E)/E]*100$$

$$\% \text{ drip loss} = [(w3-w4)/w3]*100$$

$$\% \text{ 1.5h cook yield} = (w5/w4)*100$$

$$\text{Overall \% 1.5h cook yield (F)} = (w5/E)*100$$

$$\% \text{ 24h cook yield} = (w6/w4)*100$$

$$\text{Overall \% 24h cook yield (G)} = (w6/E)*100$$

$$\% \text{ cook loss} = [(w4-w6/w4)]*100$$

$$\% \text{ cooler shrink} = (F-G)$$

4.3.3 Analytical Procedures

4.3.3.1 Salt Analysis

A calibrated model SAT-500 salt analyzer (DKK-TOA Corp, Tokyo, Japan) was utilized, were each of 10 different brine samples(4 brines from flavor loin processing, 4 brines from texture loin processing, and 2 brines from cook yield trial) were analyzed in 20 microliter triplicates and then averaged. The brine was placed into electrolyte solution. An electric current is passed between the silver wire and the silver electrode to form white deposits of silver chloride by electrolysis. The white deposits are formed from the dissolved silver ions and the chloride present in the sample. The chloride in the sample was determined by the instrument from the electricity required until all the chloride in the sample was depleted by electrolysis.

4.3.3.2 Warner Braztler Shear Analysis

Warner Braztler Shear Analysis followed methods of AMSA (1995). Frozen chops, 2.54cm thick, with 7d postmortem age, were thawed for 15h at 1°C and cooked to an internal temp of 70°C in a model 1132 impingement oven (Lincoln, Fort Wayne, IN) utilizing a belt speed of 14 minutes and an internal oven temperature of 400°F. Chop weights were taken before and after cooking. Chops were then placed in resalable bags and placed in cooler for 15h. Six uniform 1.27 cm diameter cores were obtained from chilled chops removed parallel to the longitudinal orientation of the muscle fiber. Cores were kept covered until analyzed with an Instron Universal Testing Machine (model 5565, Instron Corp., Norwood, MA) with Bluehill Software, attached with Warner Braztler Shear v-notch blade (G-R Electric,

Manhattan, KS) with a 225 mm/min crosshead speed. Mean shear force values for all cores from a chop were used for analysis.

4.3.3.3 Proximate Analysis

Cooked chops (n=75), which had cores removed from Warner Braztler Shear analysis chops, were cooled, and then were homogenized in a model 38BL54 laboratory blender (Waring Products Division, New Hartford, CT) for 20 seconds. Ground pork was then placed in resealable bag of known weight and frozen. Percent moisture analysis was conducted by freeze drying samples for 96h utilizing a minimum 5 microns vacuum pressure at -50°C on a model USM-15 freeze dryer (Virtis Company, Gardiner, New York). After drying, moisture percentage was calculated as the difference between the wet and dry sample weight divided by the wet weight. Freeze dried samples were then analyzed for crude fat with ether extraction (AOAC 2003.05), protein with combustion method (AOAC 990.03) and ash with oven drying method (AOAC 942.05).

4.3.4 Statistical Methods

4.3.4.1 Analysis of Cook Yield Data

The data were analyzed by analysis of variance using the generalized linear model (GLM) procedure in SAS for Windows version 9.1, (SAS Institute, 1999, Cary, NC). When main effects or interactions were significant ($P < 0.05$), significant differences between treatment means were calculated using the least significant difference (LSD) test. In an initial analysis, measured 18h post enhanced weight (w3) was included as a covariate in the

AOV of the variables. However, these analyses showed that the 18h post enhanced weight had no significant effect ($P < 0.05$) on the AOV of the variables, and thus was not included as a covariate in the AOV. Additionally, partial correlations were calculated using MANOVA statement in GLM to account for treatment effect.

4.3.4.2 Analysis of WBS Data

The data were analyzed by analysis of variance using the generalized linear model (GLM) procedure in SAS for Windows version 9.1, (SAS Institute, Cary, NC), where pre enhanced WBS values were used as covariate.

4.4 Results and Discussion

While loins were not classified as above or below pH 5.5 and separated into 2 groups as in the descriptive sensory study, it appears from the pH and color averages of treatment of control, sodium phosphate enhanced, and meat protein enhanced treatments, that all treatment groups appear to have PSE characteristics (Table 4.2). PSE classification varies among published work, but low pH and high L coordinates dictate this classification. Flores et al., (1999) defined PSE has ultimate 24 hour pH has less than 5.8 and L value greater than 50. Torley et al., (2000), defined PSE has having below pH 5.9, and Alvarado and Sams, (2003), defined PSE has having L^* value greater than 53 and pH less than 6. Pork color has been highly correlated with precipitation of sarcoplasmic proteins in PSE muscle, indicating poor protein solubility from the denaturation of proteins (Joo et al., 1999). Water holding capacity of muscle is affected by denaturation of myofibrillar proteins and low ultimate pH

(Van Laack et al., 2000). Van Laack et al., (2000) found strong correlation between pH and L* value, pH and marinade uptake, sarcoplasmic protein solubility and L* value, and sarcoplasmic protein solubility and cooking yield.

Table 4.2. Properties of pork loins prior to enhancement

| <u>Treatment</u> | <u>L</u> | <u>A</u> | <u>B</u> | <u>Slurry pH</u> | <u>Color</u> ¹ | <u>Marbling</u> ² |
|----------------------|----------|----------|----------|------------------|---------------------------|------------------------------|
| Control | 54.96a | 4.97a | 6.68a | 5.41b | 2.76a | 1.84a |
| Sodium phosphate | 52.89a | 12.18a | 3.52a | 5.46ab | 2.76a | 1.92a |
| Meat protein isolate | 53.60a | 7.47a | 6.21a | 5.51a | 2.84a | 1.88a |
| Significance level | ND | ND | ND | * | ND | ND |
| LSD | 2.78 | 9.23 | 4.91 | .095 | .286 | .296 |

^{a,b,c} Means in a column followed by different letters represent significant differences (P<0.05)

LSD Least Significant Difference

* Significance at p<0.05

ND= no difference

¹Scored on the following scale (NPPC, 2000): color score (1 to 6) 1= pale pinkish gray to white and 6= darkish purple red

² Scored on the following scale (NPPC, 2000): marbling score (1 to 10) 1=1% intramuscular fat and 10=10 % intramuscular fat

While 20% pickup was the target for both brines, it is evident that sodium phosphate injected loins achieved greater than 20% pickup (22.13 %), while meat protein isolate enhanced loins achieved less than 20% pickup (18.84 %) (Table 4.3). This initial enhancement pickup variability is due to the low precision of the brine injector used in this study. Its ability to precisely deliver target pump rates is low. The difference between equilibrated and initial pickup is greater in meat protein isolate treatments (2.06%) over

sodium phosphate treatments (1.44%). This would indicate that meat protein isolate is more effective at retaining the addition of solution to the muscle. Additionally, drip loss is less among meat protein isolate enhanced treatments (.43%) vs. sodium phosphate enhanced treatments (.58%). Sodium phosphate treatments were not different from the control in drip loss percentage. Both the lower drip loss, and higher difference between initial and equilibrated pickup percentage suggests that retention of brine solution containing meat protein isolate is greater than the sodium phosphate's effectiveness to hold brine within the muscle.

Table 4.3. Enhancement levels achieved and drip losses from loins injected with control, sodium phosphate, or meat protein isolate

| <u>Treatment</u> | <u>Initial pickup (%)¹</u> | <u>Equilibrated pickup (%) at 18h²</u> | <u>Drip Loss (%)³</u> |
|----------------------|---------------------------------------|---|----------------------------------|
| Control | .075c | .006c | .68b |
| Sodium phosphate | 22.13a | 20.69a | .58b |
| Meat protein isolate | 18.84b | 16.78b | .43a |
| Significance level | *** | *** | *** |
| LSD | 1.76 | 1.71 | .389 |

^{a,b,c} Means in a column followed by different letters represent significant differences (P<0.05)

LSD Least Significant Difference

*** Significance at p<0.0001

¹Initial enhancement % pickup = (after enhancement weight-before enhancement weight/before enhancement weight)*100

²Equilibrated enhancement pickup % 18h= (18 hr pumped weight-adjusted green weight)/adjusted green weight)*100

³Drip loss %= (weight of pumped loin after WBS chop was removed- 18hr pumped weight/weight of pumped loin after WBS chop was removed)*100

Both sodium phosphate and meat protein injected loins have greater cook yields at 1.5 and 24 h than the control. While initial brine retention immediately after pumping and after an equilibration period is superior in meat protein isolate over sodium phosphate, sodium phosphate injected treatments have cook yields which are statistically ($P<0.05$) greater than meat protein isolate injected treatments (Table 4.4). While these two treatments are significantly different from each other, it is apparent that either enhancement type outperforms the control by at least 13% yield. It has been theorized that improved yield are observed in polyphosphate/salt enhanced products due to the function of salt addition increasing the ionic strength (Torley et al., 2000, Baublits et al, 2006). Meat water binding increases with or without the inclusion of phosphates with increasing ionic strength (Torley et al., 2000). Salt inclusion can allow for chloride ion induced protein swelling and greater water retention within the protein network (Baublits et al., 2006). It is the synergism between phosphate and salt that will ultimately cause protein dissociation and solubilization (Prestat et al., 2002b).

Cook losses were not different ($P<0.05$) between the treatments (Table 4.4), which is in agreement with Hayes et al., (2006) and Sheard et al., (1999). This would indicate that all treatments equally were capable of retaining the natural juices and added solution under the given cooking conditions. In some reports, cook loss is reduced for an enhanced muscle over the control (Prestat et al., 2002a). Even a small reduction of 2-4% cook loss compared to uninjected controls is of commercial significance. Cook loss percent was much lower in this study's sodium phosphate treatments injected at 20% target pump rate (20.5%) than in sodium phosphate treatments (30.5%) injected at 12% and 18% pump rate used in Baublits et

al., 2006, and Hayes et al., (2006) which had cook loss of salt phosphate enhanced treatment at 30.7%, but closer in range to Prestat et al., (2002b), which had 23.82% cook loss in phosphate enhanced chops injected at 10%. Our lower cook losses compared to other studies could be attributed to varying polyphosphate concentration used in different studies along with differences in pump rate and cooking method.

Some attribute the differences in cook yields in varying studies mainly as an effect to the changes in ionic strength (Baublits et al., 2006, Torley et al., 2000), such that cook loss decreases as ionic strength is increased. Torley et al., (2000) studied cook loss on normal and PSE muscle. They found that there was a different effect of cook loss on normal and PSE muscle. They concluded that the action of polyphosphates occurs only on the non-denatured portion of the protein, and that there is difficulty for actomyosin to be split by polyphosphates in the denatured form, and therefore there is limited myofibrillar swelling that can occur in PSE muscle. Changing the ionic strength in PSE muscle does not have the capability to renature the denatured proteins and make them functional again, and thus, PSE pork responds less to changes in ionic strength than normal pork.

Cooler shrink, measured as the difference of cook yields at 1.5 h and 24 h followed similar means separation as the cook yield results (Table 4.4); with sodium phosphate injected loins having the greatest cooler shrink (2.27%). This result seems to be in opposition of the cook yield results. While sodium phosphate has the greatest cook yields, it does not keep the ability to retain weight once cooked. Sodium phosphate loins shrink .17% more than meat protein isolate. While sodium phosphate does have higher cooler shrink over meat protein isolate, even the addition of .17% to meat protein isolate yield would not

achieve yields close to that of sodium phosphate treatment. These conflicting results of yield and cooler shrink, do not appear to override the differences seen in cook yields between the two treatments. No published work has tracked losses back to the cooked cooler, thus it is hard to make any direct associations with other research as to why this may be occurring.

Table 4.4. Final product yield (%) at 1.5h and 24h, cook loss (%) and cooler shrink (%) for loins injected with control, sodium phosphate, or meat protein isolate

| <u>Treatment</u> | <u>Overall 1.5 hr cook yield (%)¹</u> | <u>Overall 24 hr cook yield (%)²</u> | <u>Cook loss (%)³</u> | <u>Cooler Shrink (%)⁴</u> |
|----------------------|--|---|----------------------------------|--------------------------------------|
| Control | 81.50c | 79.61c | 19.9%a | 1.88c |
| Sodium phosphate | 98.20a | 95.93a | 20.5%a | 2.27a |
| Meat protein isolate | 94.7b | 92.65b | 20.7%a | 2.1b |
| Significance level | *** | *** | ND | *** |
| LSD | 2.20 | 2.18 | 1.63 | .146 |

^{a,b,c} Means in a column followed by different letters represent significant differences (P<0.05)

LSD Least Significant Difference

*** Significance at p<0.0001

ND=no difference

¹Overall % 1.5 hr cook yield= (1.5 hr post cook weight/adjusted green weight)*100

²Overall % 24 hr cook yield= (24 hr post cook weight/adjusted green weight)*100

³% cook loss= (18 hr equilibration pumped weight-24 hr post cook weight/18 hr equilibration pumped weight)*100

⁴% cooler shrink= overall % 1.5 hr yield –overall %24 hr yield

Partial correlation coefficients (Table 4.5) show several significant and highly significant relationships between selected cook yield variables, but none of which show any practical significance to the current work. Most of the correlations discovered were expected among the variables. It is interesting to note, however, that there were no correlations found among any of the selected variables to pH. This was somewhat surprising, since several (Van Laack et al., 2000, Torley et al., 2000) showed pH highly correlated to L* as well as solution uptake, and it is generally accepted that pH, with its relationship to WHC, can impact final cook yields. Both pre enhanced WBS and post enhanced WBS values are significantly ($p < 0.05$) correlated to yields at 1.5 and 24h. This observation may suggest that moisture held within a cooked muscle is a determinant for a tenderization effect. This relationship is in agreement with the hypothesis of Sheard and Tali (2004) that believed that reductions in WBS values were a result of increased water content, which ultimately contributes to myofibrillar weakening.

Warner Braztler Shear (WBS) force values were significantly ($P < 0.05$) different among all treatments (Table 4.6). It is apparent that the use of an enhancement type greatly improves shear force values compared to the control which had the highest WBS value (2.15 kgf), but meat protein isolate enhancement was the most successful at delivering the lowest shear force value (1.48 kgf). There are conflicting studies regarding the impact of sodium phosphate on WBS values. Babulits et al., (2006) found no difference in WBS values among polyphosphate enhanced treatments pumped at 12% and 18% compared to the control. Lawrence et al., (2003) also found no effect on shear force values in phosphate enhanced beef, but Zheng et al., (2000) showed a decreased WBS value with the use of

phosphate based brines in poultry breasts. Sheard and Tali (2004) found a decrease in WBS in pork loins injected with various solutions and attributed the reduction in force due to increased water content and myofibrillar structure weakening. The conflicting results of the use of sodium phosphate and its affect on Warner Braztler shear could be contributed to the muscle type used, the concentration of phosphate, or the varying pump rates used among the studies.

Table 4.5. Partial correlation coefficient of select variables of initial meat quality and cook yield determinants¹²

| Variable | <u>L</u> | <u>A</u> | <u>B</u> | <u>pH</u> | <u>1</u> | <u>2</u> | <u>3</u> | <u>4</u> | <u>5</u> | <u>6</u> | <u>7</u> | <u>8</u> | <u>9</u> | <u>10</u> | <u>11</u> |
|--------------------|-------------|-------------|-------------|-----------|------------|------------|------------|-------------|-------------|-------------|-------------|-------------|------------|-------------|-------------|
| L | 1 | -.53 | .54 | -.10 | -.12 | -.18 | .12 | .10 | .02 | -.04 | -.04 | .14 | .04 | -.04 | 0 |
| A | -.53 | 1 | -.79 | -.15 | .03 | .07 | -.10 | -.10 | .03 | 0 | -.01 | -.06 | .12 | -.11 | -.13 |
| B | .54 | -.79 | 1 | .05 | -.07 | -.06 | -.08 | .07 | 0 | -.12 | -.1 | .06 | -.18 | .02 | .07 |
| pH | -.10 | -.15 | .06 | 1 | .21 | .05 | .04 | .03 | .05 | .10 | .11 | -.11 | -.06 | -.14 | .02 |
| Color (1) | -.12 | .03 | -.07 | .20 | 1 | .21 | -.06 | 0 | .01 | 0 | 0 | 0 | -.09 | .10 | .27 |
| Marbling (2) | -.18 | .07 | -.06 | .06 | .21 | 1 | .09 | .14 | -.17 | <u>.27</u> | <u>.27</u> | -.22 | .04 | -.14 | -.10 |
| Initial pickup (3) | .12 | -.1 | -.08 | .04 | -.01 | .09 | 1 | .96 | -.07 | .51 | .5 | .11 | <u>.26</u> | .16 | .06 |
| 18hr pickup (4) | .10 | -.11 | -.07 | .03 | -.08 | .14 | .96 | 1 | <u>-.32</u> | .6 | .59 | .03 | <u>.25</u> | .15 | .05 |
| Drip loss (5) | .03 | .04 | 0 | .06 | .01 | -.17 | -.05 | <u>-.31</u> | 1 | <u>.43</u> | <u>-.43</u> | .29 | 0 | 0 | .02 |
| Yield 1.5 hr (6) | -.05 | -.07 | -.12 | .11 | 0 | <u>.27</u> | .51 | .6 | -.43 | 1 | .99 | -.77 | .16 | <u>-.25</u> | <u>-.23</u> |
| Yield 24 hr (7) | -.05 | -.2 | -.10 | .11 | 0 | <u>.27</u> | .50 | .59 | -.43 | .99 | 1 | -.78 | .10 | <u>-.25</u> | <u>-.22</u> |
| Cook loss (8) | .14 | -.06 | .06 | -.11 | -.09 | -.22 | .11 | .03 | <u>.29</u> | -.77 | -.78 | 1 | .07 | .41 | <u>.31</u> |
| Cooler shrink (9) | .04 | .13 | -.18 | -.06 | -.09 | .05 | .26 | <u>.25</u> | .01 | .16 | .10 | .07 | 1 | -.11 | -.12 |
| Pre WBS (10) | -.03 | -.11 | .02 | -.14 | .10 | -.14 | .16 | .14 | .01 | <u>-.25</u> | <u>-.25</u> | <u>.4</u> | -.11 | 1 | .67 |
| Post WBS (11) | .01 | -.13 | .08 | .02 | <u>.27</u> | -.10 | .05 | .05 | .02 | <u>-.23</u> | <u>-.22</u> | <u>.31</u> | .3 | .67 | 1 |

¹Values in italic underline represent significant correlations ($p < 0.05$).²Values in bold represent highly significant correlations ($p < .0001$).

The moisture content of enhanced pork loins increased significantly ($p < 0.05$) in comparison to control as expected (Table 4.6). The control loin had the lowest moisture content of 63.27% while sodium phosphate and meat protein isolate loins both had a rounded value of 68% moisture content. This result also agrees with Hayes et al., 2006. The significant differences found here may be due to the presence of either the phosphate (pH 7.75) and meat protein isolate (pH 10.31) brines both having alkaline pHs, which moved the protein's water holding capacity away from the isoelectric point, and thus had greater ability to retain water. Meat isolates typically have very high water content, typically 76% moisture is found in surimi protein (Kristinsson and Hultin, 2003a). Therefore it is also plausible that the meat protein isolate treatment received its high moisture from the innate moisture properties of the pork meat protein. Protein content (Table 4.6) was lower in both enhancement treatments over the control due to the dilution effect on proteins from water increase in brine pumping.

This cook yield trial, along with descriptive sensory studies on flavor and texture, utilized alkali aided precipitation and recovery of meat proteins. Alkali aided protein recovery has been shown to be more effective than acid aided recovery in multiple regards (Kristinsson et al., 2005). First, the alkali process delivers more soluble proteins at isoelectric precipitation. Secondly, alkali processing has less protein denaturation than the acid process. Additionally, when exposed to moderately high pH has exposed to lower pH, there is a greater activation of sulfhydryl groups, which are considered to be the most reactive functional group on a protein, thus, disulfide bonds become more flexible, allowing for superior functionality from a gelling perspective (Kim et al., 2003). The decision to use

alkali processing of isolate for these studies was concluded from these studies. Results may have been different if acid aided processing was utilized as the method for isolate preparation instead of the use of alkali aided processing.

Little work has been reported regarding the use of protein isolate application for enhancement of meats. Thus, it is difficult to make any comparisons to the effectiveness of meat protein isolate treatment results and to assess the usefulness of this study relative to other work. However, one recent published study (Vann and Mireles DeWitt, 2007), compared the use of sodium phosphate enhancement to solubilized protein enhancement prepared with acid aided processing in beef strips. While this study used acid aided processing of isolate as compared to our alkali aided (pH 11) processing of isolate, this work does begin to lay some framework as to the effectiveness of protein isolates as an enhancement method. This study utilized a pump rate of 10% with 3.6% sodium chloride for both brines. This salt content is considerably higher than our brines which even at 15% only had 3.33% salt in the brine. It is unknown what percent protein content was in the isolate brine used in this study, but it does report a 1:9 dilution, whereas our study utilized a 1:4 dilution. They reported that purge loss was significantly ($p < 0.05$) different in isolate brine compared to sodium phosphate brine. They attribute this to the need to increase the protein ratio for improved protein-water binding in the matrix, but also a need to increase salt content to improve interaction of solubilized proteins. These attempts to explain poor purge loss values in their study do hold some merit when applying these concepts to the current study, seeing as our salt levels were much greater, as well as our protein ratio used, and the drip loss percent in protein isolate treatment in this study had better performance than our sodium

phosphate treatment drip loss percent. Cook losses performance was lower in sodium phosphate vs. protein isolate as in agreement with our current study. Sodium phosphate performed better in regards to protein isolate, which is in disagreement with our work. A group of experienced consumers concluded that sodium phosphate samples were significantly more juicer, tender, and overall more acceptable than protein isolate beef steaks (Vann and Mireeles DeWiit, 2007). While it appears that the results of this study could be comparative to the trends seen in drip loss and cook loss in our study, there is disagreement about the tenderizing effect that protein isolate delivers in WBS. Also, it can not be determined how our descriptive sensory results could compare to the consumer data used in this study. Much more work will need to be done with protein isolate enhancement to validate its success in water holding abilities, tenderness impact, and overall sensory quality, as they compare to what is currently used in the industry today.

Table 4.6. Warner Braztler Shear (kg) and proximate analysis of loins injected with control, sodium phosphate, and meat protein isolate

| <u>Treatment</u> | <u>WBS (kg)</u> | <u>Moisture (%)</u> | <u>Fat (%)</u> | <u>Protein (%)</u> | <u>Ash (%)</u> |
|----------------------|-----------------|---------------------|----------------|--------------------|----------------|
| Control | 2.15a | 63.27b | 3.09a | 32.40a | 1.60b |
| Sodium phosphate | 1.64b | 67.6a | 2.59b | 27.59b | 2.09a |
| Meat protein isolate | 1.48c | 67.74a | 2.83ab | 27.54b | 1.71b |
| Significance level | *** | *** | * | *** | *** |
| LSD | .146 | 1.27 | .494 | 1.24 | .120 |

^{a,b,c} Means in a column followed by different letters represent significant differences (P<0.05)

LSD Least Significant Difference

*significance at p>.05

***significance p<0.0001

4.5 Summary

While initial brine retention immediately after pumping and after an equilibration period is superior in meat protein isolate over sodium phosphate, sodium phosphate enhanced treatments have 24 hour cook yields which are 3.28% greater than meat protein isolate injected treatments and 16.32% greater than the control. While it is apparent that cook yield performance is greater in sodium phosphate treatments, WBS were .16 kg lower in meat protein isolate treatment over sodium phosphate treatment, which is a significant contribution to the body of knowledge of the impact of meat protein isolate's effect on tenderness. Thus, both enhancement methods could contribute positive impact on pork loins, but in different aspects of their contribution.

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CHAPTER 5

FINAL SUMMARY

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5.1 Conclusions

Enhancement is commonly used in today's pork industry in order to provide consumers a higher degree of eating satisfaction. Enhancement serves to add value to fresh meats by the addition of ingredients which can impact flavor, color, and juiciness of the product. A sodium phosphate and salt combination is traditionally what is being used as an enhancement method. Sodium phosphate binding results in an increase in the net negative charge of a protein and salt serves to synergistically work with phosphate through promotion of greater ionic strength in the system. While phosphate and salt provide benefits, some consumers view products with these ingredients negatively. It has been hypothesized that the use of a meat protein isolate prepared through the use of alkali aided precipitation and solubilization could contribute similar benefits without adding negative elements to the product label. This study was an attempt to understand the differences between enhancement with sodium phosphate and meat protein isolate, from both a sensory quality and cooking yield perspective.

An eight member trained descriptive panel quantified the flavor and texture attributes of 12 pork loins. The muscle pH factor was utilized to observe how each enhancement method could impact the characteristics of pale, soft, and exudative (PSE) pork, which is characterized by low pH and poor water holding capacity. The classification of muscle into above and below pH 5.5 for this study without regarding other meat quality parameters including lipid content was determined from the results of a descriptive flavor study observing the differences in pork chops with high and low values of NPPC color score, IV value, crude fat %, and pH. It was found that few flavor differences existed between chops,

and that high lipid content did not influence flavor of chops at any given pH group. Thus, muscle selection was determined by pH only in the study of enhancement methods.

An E*P interaction described the flavor differences found in salt and sour attribute of below pH 5.5 loins, which were the only attributes found to be both statistically and sensorial important in flavor study. There were no differences in salt intensity of protein isolate enhancement at 15% and 20%, but salt intensity was greater at 15% than 20% in sodium phosphate. Sodium phosphate injection increases sour with increasing pump, while meat protein isolate enhancement decreases sour intensity with increasing pump. In above pH 5.5 loins, the differences in salt and sour can be explained through a simple pump rate effect. Both enhancement types reduce the sour intensity from the control, but as pump rate increases from 15% to 20%, the sour intensity increases back towards control intensity. It appears that for salt, both injection types work similarly, and that salt perception is greatest at 15% pump rate than in 20% pump rate.

In both above and below pH 5.5 loins, it can be concluded that the use of an enhancement method can greatly impact texture attributes as compared to a control. In above pH 5.5, the use of an enhancement type at 15% pump rate is more preferred over 20% due to its ability to increase moisture and juiciness and decrease hardness and fibrous at a greater level than the use of 20% pump rate. However, in below pH 5.5, the opposite occurs, where the increasing pump rate from either enhancement type increases attributes that could be considered favorable and decreases attributes that could be considered undesirable when comparing to an initial control mean intensity. In above pH 5.5, sodium phosphate at 15% pump greatly increases moisture and juiciness attributes compared to all other pump and

enhancement combinations. In below pH 5.5 loins, there is a significant difference ($P < 0.05$) in the way meat protein isolate performs compared to sodium phosphate in hardness, number of chews, and fibrous. These attributes are greater across all pump rates for meat protein isolate than in sodium phosphate.

A cook yield study on 75 pork loins was performed to see the influence of sodium phosphate and meat protein isolate compared to a control on factors including drip loss, cook yield, cook loss, cooler shrink, and also Warner Bratzler Shear (WBS). While initial brine retention immediately after pumping and after an equilibration period is superior in meat protein isolate over sodium phosphate, sodium phosphate enhanced treatments have 24 hour cook yields which are 3.28% greater than meat protein isolate injected treatments and 16.32% greater than the control. While it is apparent that cook yield performance is greater in sodium phosphate treatments, WBS were .16 kg lower in meat protein isolate treatment over sodium phosphate treatment, which is a significant contribution to the body of knowledge of the impact of meat protein isolate's effect on tenderness. Thus, both enhancement methods could contribute positive differences in pork loins, but in different aspects of their contribution.

5.2 Implications

Results of this study show that any enhancement method will improve texture and flavor attributes, WBS instrumental values, and cook yield parameters. While flavor does not appear to be dramatically impacted by the two methods, texture attributes which are generally accepted as positive are increased at a greater rate with the use of sodium

phosphate, however, from an instrumental tenderness perspective; meat protein isolate outperforms sodium phosphate. Cook yield parameters are greatly improved with any enhancement method compared to the control, with meat protein isolate initially retaining marinade at a greater level than sodium phosphate at 18h, and sodium phosphate having greater cook yields than meat protein isolate. This study lays the foundation for providing data which could aid processors in evaluation of sodium phosphate alternatives with meat protein isolates. Much more work in the area of meat protein isolate enhancement is needed to confirm the importance of these results as they could be applied to the meat industry today.

Of concern for processors when implementing any new processing method is the costs associated with the technology. While it appears that meat protein isolate offers benefits in tenderness and other sensory qualities, these benefits will have to be weighed against the lower processing performance of the meat protein isolate compared to sodium phosphate. Additionally, the initial costs of both brines should be considered when choosing which enhancement is best suited for the processor. Table 5.1 highlights approximation of enhancement per 1 kg of cooked pork. Sodium phosphate enhancement is \$4.06/kg, while meat protein isolate brine enhancement estimated is \$4.18/kg. The calculation of meat protein isolate of brine cost is dependent on the efficiency of isolate production recovery, and for this calculation a yield of 60% was used to determine the cost of isolate by weight. This yield was achieved through the laboratory recovery of isolate, but this yield may increase with improved methodology over time in a commercial setting. Therefore, while meat protein isolate does appear to provide enhancement benefits, it may not be economically

feasible for commercial implementation of traditional products. However, if a processor chooses to enter into a market where the demand for none phosphate added product has great value, than this cost would need to be compared to the gain associated with entering this specialty market.

Table 5.1. Comparison of approximation of enhancement costs

| <u>Brine</u> | <u>lb. in 20 gallons</u> | <u>Cost/lb.</u> | <u>Total cost</u> | <u>Total cost/gal³</u> | <u>Total cost/kg⁴</u> | <u>Cost per green wt.⁵</u> | <u>Cost per pumped wt.⁶</u> | <u>Cost per cooked wt.⁷</u> |
|-----------------------------|------------------------------|-------------------|-----------------------|---------------------------------------|--------------------------------------|---|--|--|
| Sodium Phosphate | | | | | | | | |
| Water | 158.46 | .001 | .158 | | | | | |
| Sodium Phosphate | 4.17 | .12 ¹ | .500 | | | | | |
| Salt | 4.17 | 1.15 ² | 4.79 | | | | | |
| Total | | | 5.45 | .273 | .072 | .014 | 3.38 | 4.06 |
| | <u>lb. 25 gallons</u> | | | | | | | |
| Meat Protein Isolate | | | | | | | | |
| Water | 204 | .001 | .204 | | | | | |
| Isolate (from pork) | 24 | .63* | 15.12 | | | | | |
| Salt | 5.98 | .12 | .718 | | | | | |
| Total | | | 16.04 | .612 | .169 | .034 | 3.48 | 4.18 |
| | <u>lb.</u> | <u>Cost/lb.</u> | | | | | | |
| *Isolate production yield | | | | | | | | |
| Starting pork trim | 75 | .45 | | | | | | |
| Isolate recovered | 45 | .63* | | | | | | |
| % yield | 60% | | | | | | | |

¹ Personal Communication, Cargill Salts, July 2008

² Personal Communications, BK Giulini, February 2008

³ Total cost/ 20 gallons

⁴ Total cost per gallon/3.78. (3.78 L/gallon)

⁵ Cost per kg *.20 (average 20% pickup)

⁶ Cost per green weight + Cost of meat at \$.31/kg raw meat

⁷ Cost per pumped weight of meat *1.20 (accounting for 20% average cook loss)

5.3 Future Work/Recommendations

The use of descriptive sensory analysis develops the sensory profile of a product. Large differences could be indicative of a need to probe consumers about the liking or preference of these differences. PCA plots help to visualize these differences from a “big picture” perspective and determine the need for consumer testing. From these plots, it is my recommendation that consumer testing not be done on these treatments.

One source of variation in marinade preparation is protein content, due to gain or loss of water during alkali aided processing of meat proteins. Foegeding, (1990), found that cooking yields are very sensitive to protein concentration. Limits to our study included the precision and power of our injection to deliver viscous fluids, which reduced the protein content of the marinade to 2.1%. If an injector could be utilized to deliver highly viscous marinades, it would be interesting to see the stepwise increase in protein content and its affect on descriptive texture attributes as well as cook yields. I would suspect that attributes that are typically associated with negative perception of meat, such as hardness, number of chews, and fibrous would decrease along with increasing protein content, which may alter the conclusions when comparing to sodium phosphate. Further, the need to optimize this protein content along with pump rate would be needed to maximize “positive attributes” while minimizing “negative attributes.”

It is unknown about the microbial quality of pork loins injected with meat protein isolate. While this technology would probably be best in cooked meats applications, aerobic plate counts and shelf life of raw product could be determined first as a baseline on raw pork to discover what changes in the system with isolate injection. Additional microbiological

work could be done to examine the growth of pathogenic organisms, such as specific salmonella strains. It is also unknown about the stability over time of cooked pork loins injected with meat protein isolate. Other parameters which could be investigated include lipid oxidation using TBARS assessment and cooked color performance using L, a, b colorimetry. Much remains to be learned regarding the application of meat protein isolates and to build the body of knowledge surrounding the use of meat isolates in food design.

5.4 References

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APPENDICES

Appendix 6.1. Map of Longissimus dorsi used for pork quality and descriptive sensory analysis

Anterior end of loin

| | | | | | | | |
|---|---|---|---|---|---|--------|--------|
| 1 | 2 | 3 | 4 | 5 | 6 | A or B | A or B |
|---|---|---|---|---|---|--------|--------|

- 1- Backup
- 2- Cooked color
- 3- Warner Braztler Shear
- 4- Water holding capacity
- 5-Cooked Proximate analysis
- 6- Raw Proximate analysis

A or B quarter loins cooked for descriptive sensory analysis. A and B are randomized. With A and B quarters, treatment was presented twice to panelist.

Appendix 6.2. Map of Longissimus dorsi used for cook yield analysis

Anterior end of loin

| | | | |
|----|---|---|------|
| 1a | 2 | 3 | Cook |
| 1b | | | |

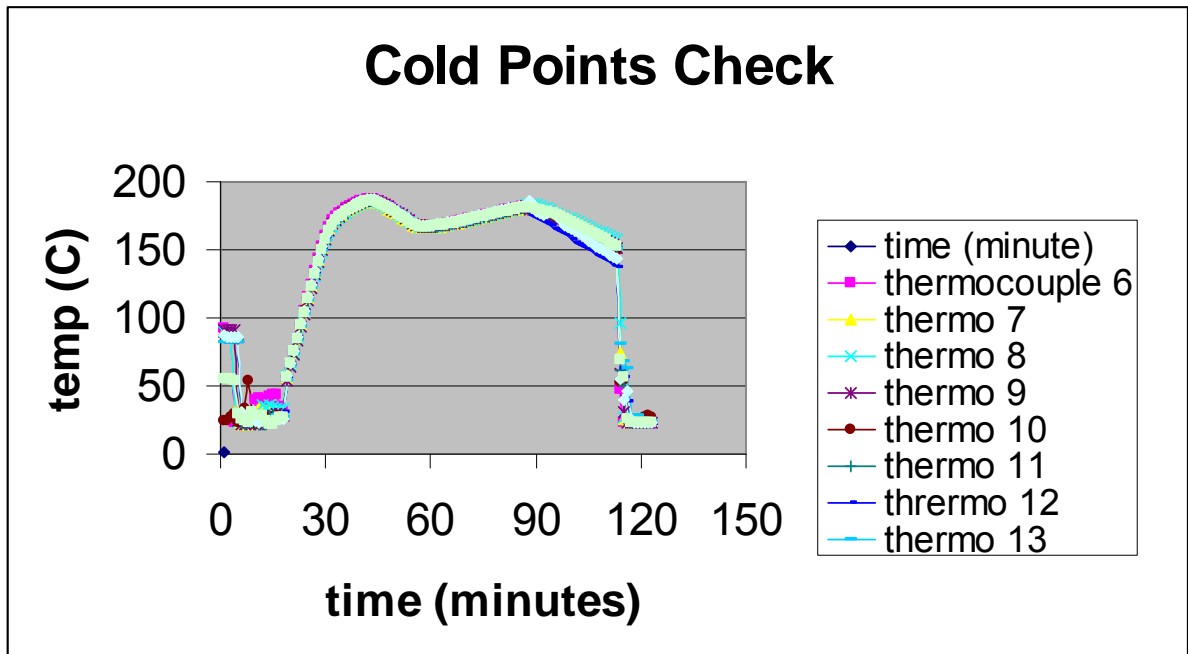
1a- proximate

1b- pH and l,a,b color

2- unenhanced Warner Braztler Shear

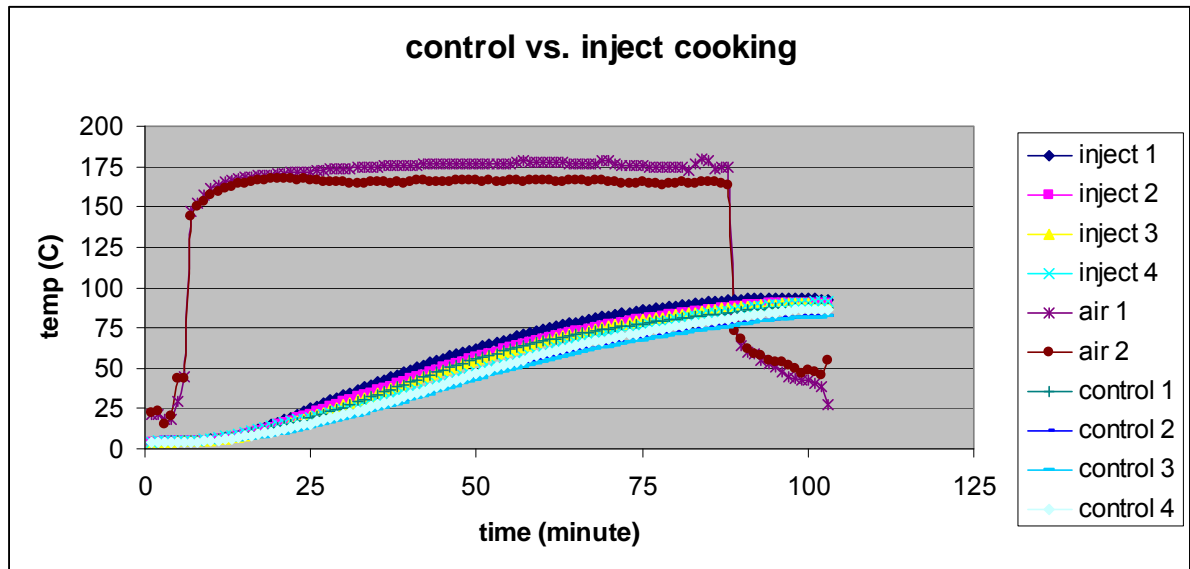
3- enhanced Warner Braztler Shear

Appendix 6.3. Temperature of oven at varying locations over time



Thermocouples (n=8) were placed randomly throughout Blodgett oven in corners at top, middle, and bottom.

Appendix 6.4. Comparison of cook rates for injected vs. noninjected loins



Thermocouples (n=8) were placed in the diametric center across the loin of both enhanced and non-enhanced loins. Thermocouples (n=2) were placed in both the top and bottom of the oven to monitor air temperature. Loins were then placed to cook in center of Blodgett oven.

Appendix 6.5. 3 Step cook cycle on Enviro-Pak smokehouse

| <u>Step In Cycle</u> | <u>Time</u> | <u>Temperature</u> | <u>Relative Humidity</u> |
|----------------------|--|--------------------|--------------------------|
| Cooking water | 1 hour | 130°F | 45% |
| Cooking water | 1 hour | 150°F | 45% |
| Cooking steam | Until internal temperature of product reaches 160°F | 175°F | 75% |