THE EFFECT OF HIGH PRESSURE PROCESSING ON PORK QUALITY, SHELF
LIFE, PALATABILITY, AND FURTHER PROCESSED PRODUCTS

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

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Abstract

This study evaluated the impact of High Pressure Processing (HPP) on pork

quality, shelf life, palatability, and further processed products. Pork carcasses (n=6) were

split into sides with only one side receiving HPP treatment. Data was analyzed as a

paired t test.

Carcass sides were evaluated for pH decline. Treatment caused a small, but

immediate decrease in pH. Carcass pH at 24 h was higher ($P < 0.05$) for treated sides.

Glycolytic potential analysis determined that treated *Longissimus* muscle had more

($P < 0.05$) glucose and less ($P < 0.05$) lactate suggesting that postmortem metabolism

was partially inhibited. *Longissimus, Psoas major, Triceps brachii,* and

semimembranosous muscles were evaluated for pH objective color; subjective color and

firmness; drip loss; cook loss and Warner-Bratzler shear force analysis. Ultimate pH (48

h) was higher ($P < 0.05$) for all treated muscles except the *Psoas mjor*. In general,

treatment effect was not consistent across muscles. Minolta *L*, a*,* and *b** values were

different for treated *Longissimus* chops. Drip loss % and cook loss % were improved ($P

< 0.05$) for treated *Longissimus* chops. Treatment inhibited the rate of lipid oxidation.

TBARS for ground pork samples were less ($P < 0.05$) for treated samples at storage day 7

and trending   ($P < 0.10$) at storage day 21. Warner-Bratzler shear force values were

less for treated samples at aging day 0 ($P < 0.05$). At aging day 7 and 14, treated samples

approached significance ($P < 0.07$) with chops from both days requiring less force than

controls. Western blot analysis determined protein degradation % for troponin t was less

for treated samples at aging days 7, 14, and 21. Intramuscular collagen content of

Longissimus chops was not different. Trained sensory analysis determined treated

samples were more ($P < 0.05$) tender, while juiciness and off flavor were not different ($P > 0.05$).   Salt soluble protein analysis determined that treated samples yielded less ($P < 0.05$) soluble protein. Texture profile analysis of restructured ham product indicated that treated samples received lesser ($P < 0.05$) values for hardness, fracturability, springiness, gumminess and chewiness. Additionally, treated ham samples had less ($P < 0.05$) binding strength. Muscle structure of treated samples sustained damage and disorganization to the muscle fibers indicating HPP is physically destructive.

Results indicate HPP positively impacts postmortem metabolism, lipid oxidation, and pork palatability. However, HPP did cause undesirable changes to meat color, salt soluble protein extractability, and textural properties of restructured ham product.

Acknowledgements

I am indebted to numerous people for all the assistance and guidance they have given me throughout my educational endeavors. I would like to thank my advisor Dr. John Killefer for giving me the opportunity to be a graduate student in Meat Science at the University of Illinois. Dr. Killefer your pleasant demeanor and wealth of knowledge has allowed me to enjoy my educational experience and grow intellectually. Dr. Floyd McKeith, you have been a much needed father figure who always had an encouraging word. Your sincere desire to promote the best interest of all your students will never be forgotten. Dr. Tom Carr, thank you for your participation on my defense committee. It has been a joy to observe someone who has maintained incredible passion and energy for their profession 30 years into their career. Dr. John Henson, you kindled a small interest in the meat industry into an incredible passion. Thank you for your guidance and encouragement to attend graduate school.

I would like to express my sincere gratitude to all of my colleagues: Sean Holmer, Lou Kutzler, Anna Dilger, Stacy Scramlin, Christine Leick, Demian Fernandez, Dustin Boler, Daniel Clark, Kim Varnold, Savannah Gabriel, Marcos Tavarez, Dallas Duncan, Amy Kelley, and Gail Ellis. You all have provided endless amounts of assistance and knowledge, but it is your friendship and our time together that I value most. I cannot express how thankful I am for all that you helped me accomplish.

I would like to thank my parents for all their love and support. I am infinitely proud of my roots and grateful for the "character building opportunities" provided to me as a young man. Your guidance and life lessons are reflected in every accomplishment and all of my successes. To my wife Stacie, thank you for your unconditional love and

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endless amount of patience. You have been my biggest graduate school supporter and I will never forget the sacrifices you made. I would like to thank God for all the blessings he has bestowed upon me. At times, it was he alone who guided me through the storms of this endeavor.

Finally, I would like to thank Hormel Foods particularly Dr. Jerry Cannon and Mr. John Summerfield. This project has been an incredible learning experience. It has afforded me many new skills and intellectual growth that will make me a better professional. I am sincerely appreciative for the opportunity you provided myself and the University of Illinois.

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CHAPTER 1

REVIEW OF LITERATURE

High pressure processing in the food industry

High pressure processing has become increasingly utilized in food production settings because of its ability to decrease microbial loads without the use of heat. The elimination of a thermal processing step allows for preservation of product appearance and flavor (Swientek, 1992). In 1990, a Japanese company launched a line of fruit jams that had been sterilized using high pressure only (Mozhaev et al., 1994). Additionally, several other countries such as France, Mexico, Spain, and the U.S. have instituted the use of high pressure processing in commercial settings on products ranging from fruit juices to protein-based products (de Lamballerie-Anton M. et al., 2002). It is speculated that high pressure processing will never completely replace thermal sterilization, but currently offers a feasible alternative to certain kinds of foods (Mertens, 1995).

Like other segments of the food business, the meat industry utilizes high pressure processing for its ability to reduce microbial loads (Hayman et al., 2004). A commercially available Spanish style sliced ham subjected to high pressure has extended shelf life of several weeks (de Lamballerie-Anton M. et al., 2002). Beyond extending shelf life, high pressure processing has been shown to have an effect on a host of other meat properties. The changes in functional properties of meat brought on by high pressure will be reviewed further in later sections.

High pressure in the food industry is typically used in the range of 200 to 800 MPa (de Lamballerie-Anton M. et al., 2002). Food products that are subjected to high pressure processing are generally vacuum packaged and put in a pressurized container.

Water is commonly used to fill the pressurization vessel as a pressure medium. After reaching a desired level, pressure remains constant for a set amount of time. In addition to time and temperature, other factors such as decompression time and pressurization liquid temperature vary by product. More recently, production facilities have found other uses for high pressure such as freezing and thawing of foods.

Basic chemical and thermal principles of high pressure processing

The changes in food macromolecules brought on by high pressure processing are explained by Le Chatelier's principle, which states that an increase/decrease in pressure results in a decrease/increase in volume. In the case of foods, a decrease in volume is due to changes in molecular conformation, intramolecular interactions and chemical reactions (Hoover et al., 1989; de Lamballerie-Anton M. et al., 2002). Many authors have concluded that high pressure affects the varying types of intramolecular bonds differently. It is typically regarded that weaker energy bonds such ionic and hydrophobic are broken down by high pressure. Ionic bonds are modified due to electrostriction whereas hydrophobic bonds are altered due to volume changes of water molecules near hydrophobic groups. Covalent bonds do not compress well and therefore remain unchanged when subjected to high pressure. Hydrogen bonds undergo a very small decrease in volume when subjected to pressure which results in a stabilizing affect (Van Eldik et al., 1989; Mozhaev et al., 1994; Cheftel and Culioli, 1997). The thermal properties of foods are also influenced by the use of pressure. The use of high pressure on foods is considered adiabatic compression; a process by which volume is reduced without heat flow. Despite the lack of heat energy, the temperature of foods still increases when subjected to high pressure. Compositionally, water represents the main

ingredient of most foods and therefore exhibits adiabatic temperature changes comparable to water (Ting et al., 2002). Barbosa-Cánovas and Rodríguez (2005) reported the temperature increase of water per 100 MPa pressure increase varies from 2-5º C at pressures above 200 MPa. However, it has also been shown that temperature increase of fats and oils due to pressurization is three times higher than that of water (Ting et al., 2002). Because foods are comprised of fats and water, the compression temperature of fat containing foods is higher than water, but not as high as oil (Ting et al., 2002). Understanding compression temperature increases in foods, particularly those that undergo very high levels of pressure, is very important due to the numerous effects temperature has on chemical and physical components of food.

The effect of high pressure on proteins

Proteins are integral in a food's flavor, appearance, storage and processing characteristics (Messens et al., 1997). High pressure processing alters protein by denaturation, aggregation, or gelation (Messens et al., 1997). Most proteins are denatured at pressures over 400 MPa (Tauscher, 1995). At pressurization of less than 150 MPa, studies have shown oligomeric proteins are broken into their subunits (Tauscher, 1995; Messens et al., 1997). Many authors agree that between 100-200 MPa monomeric proteins denature, unfold and aggregate (Gross and Jaenicke, 1994; Mozhaev et al., 1994; Tauscher, 1995). Proteins denatured by pressure appear to maintain a secondary-structure type due to the formation of hydrogen bonds that are promoted by high pressure (Mozhaev et al., 1996). Hydrophobic interactions are typically decreased by high pressure explaining the lack of tertiary structure (Mozhaev et al., 1996). It is also important to note that proteins subjected to high pressure do not denature in predictable

patterns varying by protein and pressure level (Cheftel and Culioli, 1997). The effect of pressure on proteins is important to understand because of the implications to the aging and further processing characteristics of meat, which will be reviewed in later sections.

The effect of high pressure on pre-rigor meat pH and postmortem metabolism

Previous studies indicate that pressurization of pre-rigor meat results in a rapid pH decline. Macfarlane (1973) determined that pH decline for pressure treated (100-150 MPa, @ 35°C, 4 min) pre-rigor ox muscles occurred faster than controls. Pressurized ovine muscle also had a large drop in pH after pressurization, but ultimate pH was higher than controls in all muscles tested except for the *Longissiumus.* Horgan (1981) found that rabbit muscle pressurized at 150 MPa at 35°C for 10 min experienced a large pH drop and a lower ultimate pH, especially in muscle comprised of predominantly white fibers.

It has also been shown that temperature plays a role in pH decline of pressurized meat. Sheep muscle that was tempered to 15° and 30°C then subjected to 150 MPa for 5 min experienced a considerable drop in pH, particularly for those samples tempered at 30°C (Macfarlane et al., 1982). The same study determined that samples tempered to 0°C and then pressurized did not undergo a large decrease in pH nor did pH differ greatly from controls during post pressurization storage (Macfarlane et al., 1982). These results suggest that glycolysis can be either accelerated or inhibited depending on temperature at the time of pressurization. These findings are of particular importance to future research. It is well documented in several documents that pork quality and some palatability issues are related to low ultimate pH. The ability to prevent large pH declines with the use of HPP would provide for pH ranges most ideal to pork quality and palatability traits.

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Macfarlane (1973) believes the large drop in pH that is caused by pressurization at 25-35°C, is a result of the completion of glycolysis. The drop in pH by pressurization is attributed to the activity of phosphorylase, phosphorylase kinase, and phosporylase phospatase; enzymes important in glycogen degradation (Horgan; Kuypers, 1983). In muscle samples tempered to 35°C and then pressurized at a 100 MPa; calcium is released and utilized by the pressure sensitive phosphorylase kinase (Horgan and Kuypers, 1983). Phoshorlyase kinase is relatively unaffected whereas phorphorylase phosphotase activity is decreased ultimately leading to greater glycogen breakdown and decrease in pH (Horgan and Kuypers, 1983). In a similar study using pre-rigor beef muscle, Elkhalifa et al. (1984), determined that differences in glycogen levels, lactic acid levels, and lactate dehydrogenase activity supported the finding of previous authors that high pressure accelerated the rate of glycolysis.

The effect of high pressure on meat color

The color of fresh meat is one the most important evaluation parameters consumers use when purchasing. Post-rigor minced beef samples were reported to develop a gray color after being pressurized at 150 MPa for 20 min with a pressurization liquid at 50°C (Carlez et al., 1993). The same study determined that pressurization up to 150 MPa for 10 min with a pressurization liquid at 20°C did not cause differences in *L*, a*,* and *b** values between treated and control samples (Carlez et al., 1993). However, when pressure was greater than 150 MPa *L** values increased (appearing lighter) and *a** decreased (less red) (Carlez et al., 1993). Carlez et al. (1995) found results similar to their earlier research, but further concluded that pressure at or above 200 MPa causes a

'whitening' effect to the meat. The same research also determined total myoglobin content was less for samples pressurized in a range of 200-300 MPa (Carlez et al., 1995).

More recently Jung et al. (2003) determined that high pressure has an effect on metmyoglobin production. At pressures up to 300 MPa, with a pressurization liquid of 10°C, the production of metmyoglobin was decreased leading to an increase in $a*$ value (Jung et al., 2003). The authors do not agree on the effect of pressure on $a*$ value, but both concur that the discoloration of meat at higher pressure >200 MPa (Carlez et al. 1995) and >325 MPa (Jung et al., 2003) are the result of denaturation to myofibrillar and sarcoplasmic proteins, particularly myoglobin.

The effect of high pressure of lipid oxidation in meat

The oxidative stability of fresh meat is important to ensure that consumers get a product of the highest sensory quality. The use of high pressure in meat has shown to accelerate lipid oxidation particularly with pressures at or above 300 MPa (Cheah and Ledward, 1996; Cheftel and Culioli, 1997; Ma et al., 2007). Beltran et al. (2003) did not concur determining that lipid oxidation in poultry was not induced at 500 MPa.

Research using pork determined that when lipids with a water activity at or above 0.55 are subjected to high pressure, there is a stabilizing effect to lipids due in part to the destruction of peroxides (Cheah and Ledward, 1995). In a subsequent study, the same authors concluded that addition of pressurized muscle to pressurized fat leads to greater lipid oxidation, suggesting that muscle tissue contains proxidants (Cheah and Ledward, 1996). Cheah and Ledward (1996) also determined that minced pork subjected to 800 MPa for 20 min at 20°C resulted in higher thiobarbituric acid reactive substance values, but minced pork samples pressurized at 300 MPa or less did not have an increased rate of

lipid oxidation. Research using beef and poultry (chicken) found that lipid oxidation

rates were five times higher at pressures above 400 MPa; samples tempered to higher

temperatures (50°, 60°, 70°C) and then pressurized, had higher TBARS in beef only and

suggested poultry was more stable at higher pressure (Ma et al., 2007).

It is suggested that high pressure causes lipid oxidation by denaturation of

myoglobin which in turn releases iron; a proxidant (Cheftel and Culioli, 1997). Cheah

and Ledward, (1996) support this claim in a previous study in which samples were

washed to remove haemoproteins before pressurization and ultimately had less lipid

oxidation than the non-washed samples. Cheftel and Culioli, (1997) refute the argument

by pointing out that although less, washed samples still had undesirable levels of lipid

oxidation, and therefore iron from haemoproteins is not likely to be the catalysis of

oxidative rancidity. Other research suggests that pressure-induced oxidation is related to

the extent of cell membrane damage; greater damage to the cell membrane results in

more rancidity (Beltran et al., 2003).

The effect of high pressure on Warner-Bratzler shear force values of pre-rigor meat

Several studies have been conducted to determine the effect of high pressure on

tenderness as it relates to Warner-Bratzler shear force values. Results from studies of

shear force evaluations in post rigor pressurized meat are conflicting as to whether or not

treatment improves tenderness (Ratcliff et al., 1977; Bouton et al., 1980; Locker and

Wild, 1984; Robertson et al., 1984; Beilken et al., 1990; Jung et al., 2000b). de

Lamballerie-Anton et al. (2002) argues that increased tenderness post-rigor meat is not

caused by of high pressure but is a result of the increased lysosomal enzyme activity

caused by high pressure. For the present research, it is most appropriate to review work in which high pressure treatment was conducted on pre-rigor meat.

Pre-rigor beef muscles subjected to 103 MPa at 30-35°C for 2-4 min had Warner-Bratzler shear force values for some muscles that were more than 50% less than post-rigor control samples (Macfarlane, 1973).   Kennick et al, (1980) subjected pre-rigor beef and lamb muscles to 103.5 MPa at 35°C for 2 min and determined that shear force values were highly significant for all muscles in both species after a week of storage time. In an experiment similar to the studies published in 1973 and 1980, Riffero and Holmes (1983) also saw an improvement in tenderness for pre-rigor beef. Because of the impracticality of hot boning in most commercial meat systems, very little to no research has been conducted on pre-rigor meat since the mid 1980s. Furthermore there is little to no information regarding the effect of pre-rigor high pressure treatment on improving pork tenderness. The potential of HPP to produce consistently tender pork without additives or mechanical alteration would be a well sought after product by today's consumers.

The effect of high pressure on enzymatic aging

Meat is believed to be tenderized by the proteolysis of myofibrillar proteins by μ-calpain (Koohmaraie, 1996). Additionally, cathespsins, particularly B and L, also play a role in postmortem aging of meat (Ouali, 1990). Homma et al. (1995) determined that calpain activity in pre-rigor rabbit meat at 100 MPa was more readily able to bind its substrate. However, as pressures reached 200 MPa, calpain activity was one-fifth of the control and at 300 MPa was almost inactivated (Homma et al., 1995). Very similar research with pre-rigor rabbit meat concluded that pressure up to 150 MPa caused hydrolyzing activity in proteasomes, which dropped off at 200 MPa (Otsuka et al., 1998).

In research using pre-rigor beef muscles, Koohmaraie et al, (1984) determined that calpain activity was decreased at pressures near 100 MPa. It was also noted by Homma et al, (1995) that calpastatin, an inhibitor of calpains, was decreased with pressure; 40% of the initial level at 100 MPa. Cheftel and Culioli (1997) argue that the effect of pressure on calpain and calpastatin is too inconclusive to determine because many of the factors that influence proteolysis also occur during pressurization i.e. release of calcium and lysosomal enzymes. Thus, it is difficult to accurately evaluate proteolytic activity post pressurization.

In regard to the effect of high pressure on cathepsin activity, Homma et al. (1994) determined that cathepsins B, D, and L were more active with increasing pressure to 400 MPa but dropped off at 500 MPa. Cathepsin H and amino peptidase B did not differ from control at 100 MPa and activity of both decreased as pressure increased (Homma et al., 1994). More recent research reached similar conclusions determining that activities of cathepsin D and acid phosphatase increased as pressure increased (Jung et al., 2000a). The increased activity of lysosomal enzymes is believed to result from the breakdown of lysosomal membranes during pressurization (Homma et al., 1994; Jung et al., 2000a). Jung et al, (2000a) confirmed this through electron microscopy that indicated the membrane integrity of lysosomes from pressurized meat is altered.

The effect of high pressure on connective tissue

The presence of connective tissue in meat contributes to what is often referred to as background toughness. Increases in tenderness from high pressure treatment are believed to be the result of changes to myofibrillar protein and research has been done to determine if there is any affect to stromal proteins. Pressurization of lamb epimysial

tissue indicated that shrinkage, isometric tension, and transition heat measurements were not different from non pressurized samples suggesting that pressure was not any more useful than heat alone in tenderizing connective tissue (Bouton et al., 1978).   Ratcliff et al. (1977) reported that connective tissue was the only contributor to overall toughness because myofibrillar toughness had been eliminated by high pressure. Beilken et al. (1990) reported that Warner-Bratzler shear force values of beef muscles pressurized at temperatures between 40-80°C indicated that treatment had no effect on background toughness other than to raise the temperature at which heat alone produced a decrease in toughness. In research involving mature dairy cows, high pressure treatment ranging from 100 to 400 MPa did not provide significant differences in eltrophoretic pattern, thermal solubility, or differential scanning calorimetry (DSC) for isolated intramuscular collagen (Suzuki et al., 1993) . Work has also been conducted examining the ultrastructure of connective tissue. Scanning electronmicrographs indicate that deformation of the honeycomb like structure of endomysium was increased at higher pressures; at 400 MPa, the mesh structure of endomysium was expanded (Ueno et al., 1999). The effect of high pressure on connective tissue has been investigated in a very small number of studies. The use of high pressure to alter intramuscular connective tissue is somewhat inconclusive and is an area in need of further research.

The effect of high pressure on meat sensory characteristics

A small number of studies have used sensory panels to evaluate the parameter of tenderness and juiciness. Macfarlane (1973) conducted a taste panel on bovine and ovine samples that were subjected to high pressure while in a pre-rigor state . For both species, panelists concluded that treatment improved tenderness and despite being less juicy,

pressurized samples were found to be more acceptable (Macfarlane, 1973). Taste panel assessment of high pressure treated beef muscle determined that tenderness scores for treated samples were significantly different while juiciness values were not (Riffero; Holmes, 1983).

In regards to specific meat flavor, Japanese researchers investigated the impact of high pressure on serum and umami (Suzuki et al., 1994). The serum flavor is attributed to reducing sugars, amino acids and peptides. As meat ages, the serum flavor increases due to the breakdown of sugars, amino acids and peptides. Suzuki et al, (1994) refers to the amount of peptides and amino acids as phenol reagent positive materials. High pressure treatment 100-400 MPa of beef muscles revealed that levels of phenol reagent positive material was only numerically higher at each level of treatment. However, the authors concluded that the breakdown of muscle protein, as estimated by phenol reagent positive material, was accelerated by pressurization (Suzuki et al., 1994). When evaluating umami, the content of inosinic acid was compared between treated and non-treated samples. No significant differences led the authors to conclude that because inosinic acid is not reduced by pressurization, treatment does not reduce the umami flavor in meat (Suzuki et al., 1994).

Sensory evaluation studies of processed meats are few, but as of the present time, it does not appear sensory characteristics are compromised by high pressure treatment. Sensory research using high pressure treated cooked sausages determined that panelists preferred treated sausages because they were better appearing, more juicy, and had better taste (Mor-Mur and Yuste, 2003). Rubio et al. (2007) concluded that sensory attributes of Spanish style dried beef were not adversely affected by high pressure treatment.

The effect of pressure on myofibrillar protein solubility

Myofibrillar proteins such as actin and myosin are important in meat processing. The effect of high pressure on the solubility of myofibrillar proteins helps to better understand many potential changes to the properties of both fresh and processed meat. Macfarlane (1974) reported that lamb muscle homogenates in saline solution subjected to 150 MPa yielded higher amounts of soluble myofibrillar protein. In a subsequent study, it was confirmed that high pressure increased protein solubility but was dependent on temperature, salt concentration and pH (Macfarlane and McKenzie, 1976). In particular, protein solubility was increased in samples pressurized at 0°C instead of 30°C (Macfarlane and McKenzie, 1976). In research using rabbit meat, it was determined that myofibrillar solubility was greater with increasing pressure reaching 2, 2.3, and 2.6 times that of the control at 150, 200, and 300MPa (Suzuki et al., 1991). SDS-PAGE from the same research indicated that thin filament protein such M-protein, actin and tropomyosin decreased as pressure increased (Suzuki et al., 1991).

The effect of high pressure on processed meats

Beef patties made with varying salt levels (0, 0-5, 1, and 3%) were subjected up to 150 MPa. Increase in binding strength was dependant on pH, pressure level, and NaCl concentration. At 150 MPa, binding strength was increased at all NaCl levels (Macfarlane et al., 1984). The raw materials for Frankfurters were subjected to 150 and 300 MPa with varying salt levels (1.5 and 2.5%) were evaluated for emulsion stability and texture profile analysis (Crehan et al., 2000). Emulsion stability was increased at lower pressure and lower salt level but a decrease in emulsion stability was seen at 300 MPa (Crehan et al., 2000).   Texture profile analysis indicated that hardness,

cohesiveness, gumminess, and chewiness were improved at 150 MPa and 1.5% NaCl, but a decrease in textural properties was seen at 300 MPa (Crehan et al., 2000). Poultry sausages subjected to 500 MPa were more cohesive and less firm than controls (Mor-Mur and Yuste, 2003). In very similar research, Yuste et al. (1999) found that pressurization of poultry sausages made them less springy and firm but more cohesive. Cheftel and Culioli (1997) hypothesize that high pressure disrupts protein functionality and impacts myosin heavy chains resulting in textural changes. In general, the affect of high pressure on processed meats varies greatly by pressure level, pressurization liquid temperature, and NaCl inclusion level.

The effect of high pressure on muscle structure

The effect of high pressure on muscle has been studied by examining changes that occur to the ultrastructure of muscle (Macfarlane and Morton, 1978; Kennick et al., 1980; Macfarlane et al., 1981; Suzuki et al., 1991; Jung et al., 2000c). It has been shown that changes to the ultrastructure of meat are dependent on postmortem condition, pressure level, pressure time, and temperature during pressurization. Macfarlane and Morton (1978) investigated the effect of 100 MPa on pre and post rigor sheep muscles concluding that electron microscopy revealed ultrastructural changes in both postmortem conditions. Pressurized post rigor muscle was removed of the M-band in the central region of the A-band as well as loss of integrity to the I-band; treated pre rigor muscle was extensively disrupted with contraction band formation, distortion of the sarcolemma, and disaggregation of compounds of thin filaments (Macfarlane and Morton, 1978). Kennick et al. (1980) found similar results concluding electron micrographs showed extensive convolution and fraying of beef *supraspinatus* fibers after muscle had been pressurized.

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In follow up research to work done in 1978, Macfarlane (1981) found similar results in pressurized beef muscle, but found that I-band disorganization was even more extensive after muscles had been cooked for 1 h at 80°C.

Research conducted on pressurized isolated myofibrils also suggested high pressure profoundly affects ultrastructure of muscle. Suzuki et al, (1991) found that as pressure increased (100, 150, 200, 300 MPa), the ultrastructure of rabbit muscle underwent greater changes. At 100 MPa, loss of M-line structure and confusion of I-filament structure was visible. At 200 MPa, micrograph images indicated structural loss of the sarcomere, disappearance of Z-lines, and I and A-filament disorganization; by 300 MPa, a mass of material was seen on each side of where the M-line had previously been (Suzuki et al., 1991).

More recent research by Jung et al. (2000c) did not completely concur with previous research; indicating that electron micrographs did not show ultrastructural differences to beef muscle at pressures of 130 MPa at 10°C. However, at 325 MPa, the authors saw modification to the ultrastructure including disorganization of filaments, loss of I-band integrity, strengthening of costameres, aggregation of filaments, and gap formation between filaments; the same effects were seen at 520 MPa. As previously noted in prior sections, the same holds true for research regarding muscle ultrastructure; very few investigations on pre-rigor meat, particularly pork, have been conducted in the last 20 years.

In summary, the use of HPP has shown its ability to alter a host of meat properties. Over the last 30 years, HPP technology has advanced to the point that it can be used in ways that have not been previously researched. Previous work has indicated

that HPP has the ability to make desirable changes to meat properties. HPP induced

stoppage of postmortem metabolism could potentially eliminate large pH declines that

cause several pork quality defects. The ability to eliminate quality issues would allow for

more consistent, consumer pleasing pork products. Additionally, it is also evident that

HPP has can tenderize meat when treatment occurs during the pre-rigor state. Therefore,

one must speculate that HPP has the potential to produce pork that is not only high in

quality but also tender. With this in mind it would be of considerable value to investigate

the potential use of HPP in the pork industry.

Objectives

With a need to understand the implications of high pressuring processing on the

pork packing and processing industry, the objectives of this study were:

1. To understand the effect of high pressure on postmortem metabolism.

2. Evaluate the effect of high pressure on pork quality and shelf life.

3. To examine the ability of high pressure to improve pork palatability.

4. To investigate the effect of high pressure on pork value added products.

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CHAPTER 2

THE EFFECTS OF HIGH PRESSURE PROCESSING ON PORK QUALITY, SHELF LIFE, PALATABILITY, AND FURTHER PROCESSED PRODUCTS

Introduction

High pressure processing (HPP) has been used on a variety of foods since the early 1990's. HPP has gained popularity because of its ability to significantly reduce foodborne illness causing pathogens (Shigehisa et al., 1991; Carlez et al., 1993; Hayman et al., 2004). Additionally, HPP is considered a non-thermal process, which allows product appearance and flavor to be preserved (Swientek, 1992). The meat industry has turned to HPP as a tool to help prevent food pathogens particularly in ready to eat deli meat type products. However, very little research particularly in the last 20 years, has investigated the effects of high pressure on other meat characteristics such as postmortem metabolism, lean color, shelf life, mechanical tenderness, sensory attributes, and integrity of value added products. Recent advancements in high pressure systems technology has created the ability to conduct experiments using conditions that have not been previously reported.

Research has been published regarding the effect of HPP on meat systems. Studies investigating pH decline has suggested that HPP causes an immediate drop in pH (Macfarlane, 1973; Horgan, 1981; Macfarlane et al., 1982). The pH drop is believed to be a result of expediting glycolysis by altering enzymes important in glycogen degradation (Horgan and Kuypers, 1983; Elkhalifa et al., 1984).

Meat color is one of the most important traits when it comes to willingness of consumers to buy. In some research, the use of HPP has decreased meat color score (Carlez et al., 1993; Jung et al., 2003). Evaluations of myoglobin content of meat concluded that pressure treated samples had significantly less than controls (Carlez et al., 1995).   It generally regarded that pressure levels greater than 200 MPa result in the denaturation of sarcoplasmic and myofibrillar proteins resulting in meat color changes (Carlez et al., 1995; Jung et al., 2003).

The oxidative stability of lipids associated with meat is also affected by HPP. Research has been somewhat inconclusive, but generally lipid oxidation rate is increased at pressures above 300 MPa (Cheah and Ledward, 1996; Cheftel and Culioli, 1997). This has serious implications to shelf life and sensory attributes of meat. It is suggested that the increase in lipid oxidation is caused by proxidants (iron) released when myoglobin is denatured or can also be attributed to extensive, pressure induced cell membrane damage (Cheftel and Culioli, 1997; Beltran et al., 2003).

In regard to tenderness, studies have been conducted to investigate the effect of HPP on Warner-Bratzler shear force values, enzymatic aging, and connective tissue. Research subjecting post-rigor meat to high pressure processing has had varied results in regards to mechanical tenderness (Ratcliff et al., 1977; Robertson et al., 1984; Beilken et al., 1990; Jung et al., 2000b). Studies conducted on pressurized pre-rigor meat have typically seen lower Warner-Bratzler shear force values for treated versus controls (Macfarlane, 1973; Kennick et al., 1980; Riffero and Holmes, 1983).   Enzymatic aging studies have found that activity of calpains, calpastatin, and cathepsins are increased, decreased, or both depending on pressure level (Koohmaraie et al., 1984; Homma et al.,

1994; Homma et al., 1995; Jung et al., 2000a). In general, HPP on enzymatic aging is not well understood and few concurring conclusions have been made. Studies investigating connective tissue have generally concluded that HPP does not decrease the amount of connective tissue level (Bouton et al., 1978; Suzuki et al., 1993). However, scanning electron micrographs have suggested that HPP alters the mesh structure of endomysium (Ueno et al., 1999).

Like many other meat parameters subjected to HPP, the effect on binding strength and textural properties of value added products are varied. Binding strength was increased in beef patties formulated with various salt concentrations (Macfarlane et al., 1984). However, the textural properties of pork and poultry sausage products were found to be positively and negatively affected by HPP (Yuste et al., 1999; Crehan et al., 2000; Mor-Mur and Yuste, 2003). The changes caused by HPP to value added products may be caused by the changes in myofibrillar proteins, which are integral to the binding of processing meats. Authors have agreed that myofibrillar protein solubility was increased when subjected to HPP (Macfarlane, 1974; Suzuki et al., 1991)

The effect of HPP on muscle structure has been shown to cause dramatic changes. Scanning electron micrographs of muscle structure consistently show disruptions of the thin filaments, loss of I-band integrity, and destruction of the sarcomere (Macfarlane and Morton, 1978; Suzuki et al., 1991; Jung et al., 2000c).

In general, research regarding HPP has been highly variable in terms of species, post mortem condition, and pressurization chamber settings (time, pressurization liquid temp, pressure level). Previous research has not investigated the use of HPP on early

post mortem (20 min) whole pork sides. The current research is to examine the effect of HPP on the pork industry from harvest to value added processing.

Materials and Methods

Animals and Treatments

Pigs (n=6) weighing approximately 95 kg were selected at random immediately after being humanely slaughtered. Pigs went through normal harvest procedures of exsanguination, dehairing, and evisceration. After carcasses were split, identification was maintained on each side in a manner so that eventual samples could be analyzed as paired data. To determine if treatment caused immediate changes in pH, a measurement was taken immediately prior to and after treatment. Pre and post treatment pH measurements were taken from the center section of the loin using a MPI pH Meter (Model C033, Meat Probes, Inc., USA).

One side from each carcass was selected randomly and subjected to High hydrostatic pressure treatment of 215 MPa for 15 seconds with a pressurization liquid (water) at approximately 33.3ºC. The non-pressure treated side served as a control. Treated and controlled sides entered the chill cooler together approximately 45 min postmortem.   At 24 h postmortem, pH was determined at the ventral side of the center section of the loin. Carcasses were then transported via refrigerated truck to the University of Illinois meat laboratory and held overnight. Carcasses were maintained at a temperature of 4ºC during transportation and holding.

Carcass Fabrication

At 48 h postmortem, sides were fabricated into bone in loins (NAMP #410); pork shoulder, picnic boneless (NAMP #405A); pork shoulder, Boston Butt, boneless (NAMP #406A); and pork leg, TBS, 3-way, Boneless (NAMP #402G). Bellies were not utilized in the study.

Bone-in loins were cut between the 10th and 11th ribs. The posterior section of the *longissimus* muscle and the entire *Psoas major* were removed for use in subjective and objective quality evaluations. The *Semimembranosous* and *Triceps brachii* were also removed from their respective primal cuts for subjective and objective quality evaluations. Remaining boneless ham sections were used for restructured ham formulation, while remaining boneless shoulder sections were used for TBARS analysis.

Quality Measurements

Subjective and objective quality evaluations for the *longissimus* and *Semimembranosous* were made on a cut surface of the anterior end; posterior end for the *Psoas major*, and the distal end for the *Triceps Brachii.* Ultimate pH was determined for each muscle using a MPI pH Meter (Model C033, Meat Probes, Inc., USA). After a bloom time of 20 min, Minolta L*, a* and b* were collected using a Minolta CR-300 utilizing a D65 light source and a 0º observer (Minolta Camera Company, Osaka, Japan). Subjective scores for color (NPPC, 1999) and firmness (NPPC, 1991) were also collected at this time.

Chops were taken from the *longissimus* for drip loss %, proximate composition, Warner-Bratzler shear force, protein degradation analysis, texture analysis, degree of doneness analysis, and sensory panel analysis. These chops were collected serially starting at the anterior end of the *Longissimus* muscle. Chops were collected for water

holding capacity, proximate analysis, and Warner-Bratzler shear force serially from the anterior end of the *Semimembranosous,* posterior end of the *Psoas major,* and distal end of the *Triceps Brachii,* respectively. Chops for water holding capacity and protein degradation analysis measured 1.0 cm thick, while chops cut for all other analyses measured 2.54 cm thick. Chops from the *Longissimus* for Warner-Bratzler shear force analysis and protein degradation were aged for 0, 7, 14, and 21 days at 4° C and then stored at -20° C. Aging days reflects time post fabrication, and therefore '0 d' is actually 48 h postmortem. Warner-Bratzler shear force chops taken from the *Psoas major, Semimembranosous,* and *Triceps Brachii* were aged 14 days and then stored at -20° C. *Longissimus* chops used in the degree of doneness study were aged 14 days and then stored at -20° C.

Proximate Analysis

Moisture and lipid content were determined for each muscle using the procedures described by Novakoski et al. (1989). Chops were trimmed of external fat and connective tissue and homogenized using a Cusinart Food Processor (Model DLC 5-TX, Cuisinart, Stamford, CT). Moisture content was determined by oven drying the samples at 110ºC for 48 h. Lipid content was determined by extraction using a mixture of chloroform and methanol.

Drip Loss %

Chops from each muscle were weighed, placed in a Whirl-pak bag, and suspended for 24 h at 4° C. Each chop was then reweighed and reported on a percent loss basis.

Glycolytic Potential

Glycolytic potential was conducted following a modified procedure outlined by Miller et al. (2000). Duplicate 3 g sample of ground *longissimus* tissue was homogenized in 15 ml of 0.6 N perchloric acid for 1.5 min using a homogenizer (Brinkman homogenizer, PT 10/35, Brinkman Istruments, Westbury, NY). For the amyloglucosidase digestion, 200 µL of homogenate was pipetted into 1.5 mL microcentrifuge tubes and 1 mL of amyloglucosidase solution was added along with 20 µL 5.4 N potassium hydroxide. Samples were incubated at 37° C for 3 hours inverting every 20 min to mix. After incubation, samples were cooled on ice for 10 min and then 100 µL of cold 3 N perchloric acid was added. Samples were centrifuged at 4°C at 7000 x g for 5 min. A blank solution to be used for the standard curve was made using 200 µL, 0.6 N perchloric acid, and 20 µL5.4 N potassium hydroxide.

To determine lactate level, lactate assay solution was formulated using mM glycine buffer, 2.5 mM NAD, and 16.7 units/moL lactate dehydrogenase (catalog numbers G5418, N1511, and L3916, Sigma-Aldrich, Inc., St. Louis, MO). A lactate standard curve using L-lactate ([5.0 mM/L], YSI Inc., Yellow Springs, OH) was prepared and plated in duplicate on a 96 deep well plate. Amyloglucosidase digested samples (20 µL) were plated with lactate assay solution (180 µl) bringing the total volume for each well to 200 µL. Samples were incubated at 37° C for 15 minutes before being read at 340 nm on a Synergy HT plate reader (Bio-Tek Instruments, Inc., Winooski, VT).

Glycogen levels were determined using the Glucose (HK) Assay kit (GAHK20, Sigma –Aldrich Inc., St. Louis, MO). The kit included glucose reagent and a 1 mg/mL glucose standard. A standard curve of seven concentrations was prepared using the

glucose standard and 0.6 N perchloric acid. Amyloglucosidase digested samples (20 µL) were plated in duplicate into a 96 well plate with glucose reagent (180 µL) bringing the total volume of each well to 200 µL. The glycogen standard standard curve was also plated in duplicate. Samples were incubated at 37° C for 15 minutes and then read at 340 nm using a Synergy HT plate reader.

Thiobarbituric Acid Reactive Substances (TBARS)

For each side, boneless picnic shoulders and boneless Boston Butts, excluding *Triceps Brachii,* were coarse ground together to form an approximate 70% lean to 30% fat blend. A weight for the ground material was collected and salt was added as a proxidant at 1% of the total weight. The meat and salt mixture was then finely ground through a 3/16 in plate. The grinder was rinsed thoroughly between treated and controlled samples.

Samples of finely ground meat weighing 0.5 kg were placed in a modified atmosphere package with a mixture of 80/20 of oxygen and carbon dioxide. Packages were assigned to storage durations of 0, 7, 14, or 21 d. Storage duration time reflects time post fabrication, therefore '0 d' is 48 h postmortem. The 7 d sample was placed immediately under fluorescent lighting in a retail display case and remained there for 1 week. The 14 and 21 d samples were kept in dark storage until seven days prior to their assigned storage duration time; they were then placed under fluorescent lighting in a retail display case for 1 week. At the conclusion of their display case storage times, samples were analyzed for TBARS.

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After storage time, finely ground meat samples were further homogenized using Cusinart Food Processor (Model DLC 5-TX, Cuisinart, Stamford, CT). Samples weighing 5g were prepared in duplicate and homogenized for 30 seconds in a Waring blender with 1 mL of 0.2 mg/mL BHT and 45.5 mL of trichloroacetic acid in 0.2 M phosphoric acid. The homogenate was filtered through a Whatman no. 1 filter paper and two 5 mL aliquots of filtrate were added to glass test tubes. Thiobarbituric acid was added to one tube while another tube, designated as blank, received deionized water. To determine percent recovery, two additional samples were randomly selected from both treated and control groups to serve as spiked samples. The spiked samples were homogenized with 1 mL of 0.2 mg/mL BHT, 12 mL of 10 uM 1,1,3,3-tetramethoxypropane and 32 mL of 10% trichloroacetic acid in 0.2 M phosphoric acid. Spiked samples were filtered, and aliquotted with thiobarbituric acid or water as previously described. A standard curve was prepared with varying levels of malondialdehyde (0, 1.25, 2.5, 5.0, and 7.5 mg malondialdehyde/mL), 25 uM tetramethoxypropane, 0.2 M thiobarbituric acid, and 10% tricholoroacetic acid in 0.2 M phosphoric acid. All samples were stored in dark conditions at room temperature for 18 h. Absorbance values for all samples, including standards and blanks, were measured at 530 nm using a spectrophotometer (Beckman Coulter, Inc. Fullerton, CA). Thiobarbituric acid reactive substances (TBARS) values were calculated using a standard curve and reported as mg of malondialdehyde/kg of tissue.

Cook Loss and Warner-Bratzler Shear Force

Prior to analysis chops were removed from freezer and allowed to thaw at 4° C for 24 h. Chops were then trimmed of excess fat, weighed and cooked on a Farberware Open

Hearth gill (Model 455N, Walter Kiddie, Bronx, NY). Chops were cooked on one side to an internal temperature of 35˚ C, flipped, and cooked to final internal temperature of 70˚C. Internal temperature was monitored using copper constantan thermocouples (Type T, Omega Engineering, Stanford, CT) connected to a digital scanning thermometer (Model 92000-00 Barnant Co., Barington, IL). Chops were allowed to cool to 25º C and then reweighed to determine percentage of cook loss.   Four 1.25 cm cores were removed parallel to the orientation of the muscle fibers. Cores were sheared using a Texture Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK) with a blade of 10 mm/sec and a load cell capacity of 100 kg. Shear force was determined on each core, and average of the four cores reported.

Degree of Doneness

Chops were prepped, cooked, and sheared in the same manner as the previously described shear force chop. One group of chops was cooked to an internal temperature of 63º C while another group of chops were cooked to 77º C.

Sensory Panel

A six member trained sensory panel evaluated samples for tenderness, juiciness and off flavor.   A 2.54 cm chop was prepped and cooked in the same manner as the previously described shear force chop. Two cubes of 1 cm x 1cm x 2.54 cm were served to each panelist under red lighting. Panelists rated each chop using a 15 cm unstructured line scale anchored at the center and both ends with 0 being extremely tough, extremely dry, and no off flavor, while 15 was extremely tender, extremely juicy, and intense off flavor.

Tissue Preparation for SDS PAGE and Western Blotting

Longissimus samples aged 0, 7, 14, and 21d were prepared according to a modified version of Huff-Lonergan et al. (1996). A frozen section (0.2 g) was removed from the center of *longissimus* chops and added to 1.5 mL of whole muscle protein extraction buffer (10 mM sodium phosphate, and 2% SDS, pH 7.0). Samples were extracted using a tissue lyser (Qiagen, Hilden, Germany) for 1 min. The homogenate was spun at 15,000 x g for 20 minutes at 4°C. Protein concentration of the supernatant was determined using Pierce BCA (Pierce Protein Research Products, Rockford, IL) microplate protein assay. Samples were diluted 6.25 fold with distilled water so that concentrations would fall within a readable range. Bovine serum albumin was used to generate a standard curve with points between 2mg/mL and 0.1 mg/mL. Protein concentrations from the fractions were adjusted to 2.5 mg/mL for SDS PAGE.

SDS PAGE and Western blotting

Protein electrophoresis was conducted using NuPAGE 10% (Troponin T) and NuPAGE 12% (Desmin) Bis-Tris gels with MOPS running buffer. Running buffer and reducing agent were added to samples in accordance to NuPAGE protocol (Invitrogen, USA) and 10 μg protein were loaded per lane. Magic Mark XP (Invitrogen, USA) was used as chemilluminescent molecular weight standard. Gels were run at 200 v for 70 min in water-cooled Hoefer SE260 Mighty Small II gel electrophoresis boxes (Hoefer, San Francisco, CA).

At the conclusion of protein separation by electrophoresis, gels were transferred onto nitrocellulose (0.45 μm) membranes that had previously been soaked in NuPAGE Transfer Buffer ([25 mM Bicine, 25mM Bis-Tris, 1mM EDTA, SDS, pH 7.2] Invitrogen,

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USA). Protein transfer was conducted in a water-cooled Trans-Blot Cell (Bio-Rad, USA)

for 1.5 h at a constant voltage of 100 v. After transfer, membranes were soaked in

blocking buffer ([SuperBlock (TBS) Blocking Buffer with 10% Tween-20 added]

Thermo Scientific, Rockford, IL) for 1 h. Membranes were then incubated for 1 h with

Troponin T antibody ([T6277], Sigma, USA) at 0.15 µg/mL or Desmin antibody

([D1033] Sigma, USA) at 1.5 µg/mL. Membranes were then washed 4 times for 5 min in

TBS with 10% Tween-20 added. Membranes were then incubated with goat anti-mouse

antibody as the secondary antibody at 13 ng/mL for Troponin T and 12 ng/mL for

Desmin ([Immuno Pure Antibody, 31430] Thermo Scientific, Rockford, IL). Again,

membranes were washed in TBS with 10% Tween-20 4 times for 5 min and then given a

final wash with nano pure water. Membranes were incubated 5 min in a

chemilluminescent solution and photographed using the ChemiGenius^2 Imaging System

(Syngene, UK). Protein band densities were analyzed using GeneTools (Syngene, UK).

Protein degradation percentage and intact protein were determined and compared

between treated and controls. Per gel, a standard band with a known molecular weight

was used to standardize values when determining intact protein.

Soluble and Insoluble Collagen Assay

The amount of soluble and insoluble collagen was determined in accordance to a

modified methods described by Hill (1966) and AOAC method 990.26 (AOAC

International, 1995). Homogenized *Longissimus* samples weighing 5 g were placed in 50

mL conical tubes containing 12 mL ¼ strength Ringer's solution. Tubes were incubated

in an Environmental Incubator Shaker (New Brunswick Scientific Co., Inc, Edison, N.J.)

for 2 h at a temperature of 69°C. After incubation, samples were vortexed on a low

setting and then centrifuged at max speed for 10 min at room temperature. Supernatant was decanted into a glass 70 mL test tube, which represented the first portion of soluble collagen. An additional 8 mL ¼ strength Ringer's solution (8 mL) was added to the tube containing the pellet. The pellet was broken up using a spatula, vortexed on a low setting, and centrifuged again at max speed for 15 min at room temperature. The supernatant was decanted into the glass test tube containing the first portion of soluble collagen; collectively this represented the soluble collagen fraction. The pellet was thoroughly removed from the conical tube and placed in a glass tube; this represented the insoluble collagen fraction. Both collagen fractions received 25 mL 6 N hydrochloric acid and were incubated at 110° C for 16-18 h.

The soluble hydrolysate fraction was poured into a graduated cylinder and brought to a volume of 50 mL using deionized water. The hydrolysate was filtered through Whatman #1 (12.5 cm) filter papers into 15 mL conical tubes until 8-12 mL of filtered hydrolysate had been collected. The insoluble hydrolysate fraction underwent the same procedure, except that it was brought to a volume of 100 mL before being filtered.

Soluble collagen filtrate (100 µL) and deionized water (700 µL) were combined in duplicate in a 96 deep well plate. Insoluble collagen filtrate (50 µL) and deionized water (750µL) were also combined and plated in duplicate. Oxidant solution (400 µL) was added to each well, covered with foil tape, shaken to mix and incubated at room temperature for 20 min. Color reagent (400 µL) was then added to each well, covered with foil tape, shaken to mix, and incubated in a waterbath at 60º C for 15 min. Oxidant and color reagents were mixed in accordance with AOAC methods, and samples were run against a hydroxyproline standard curve. A 200 µL volume was transferred to a 96 well

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microplate and read at 558 nm on a Synergy HT plate reader. Soluble and insoluble

collagen levels were calculated assuming 12.5% hydroxyproline content (AOAC

International, 1995).

Restructured Ham Formulation

The semitendinosous, biceps femoris, and the knuckle were removed of fat and

connective tissue, and cut into 2.54 cm chops.   Chops were macerated (Pro-9, Sir Steak

Machinery, Boxboro, MA) to increase muscle surface area and promote better protein

bind. The macerated strips were then cut into 1 in cubes and placed into the mixing bowl

of a Kitchen Aid mixer (Model# KSM150PSER, St. Joseph, MI). Curing brine using 68

kg of water, 2.05 kg of phosphate, 7.61 kg Melozyme (Griffith, Laboratories, Alsip, IL),

2.77 kg seasonings and 0.31 kg of sodium erythorbate was formulated for a pick up of

20% over green weight. The brine was added to the mixing bowl containing meat and

mixed for 5 min to simulate tumbling. Tumbled meat cubes were hand stuffed into 102

mm fibrous casings and hung on a smokehouse rack.   Ham chubs were cooked to

internal temperature of 66.6º C in an Alkar smokehouse (Lodi, Wisconsin). After

cooking, ham chubs were chilled for 12 hours in a cooler at 2º C. All equipment used in

formulation of restructured hams was rinsed thoroughly between production of control

and treated samples.

Casings were removed and 15.24 cm sections were cut from each end of the

samples leaving only the center portion of the ham chub. From the remaining center

section, four 2.54 cm slices were collected; three slices were designated for binding

strength analysis and one slice for texture profile analysis.

Ham Binding Strength

Binding strength of restructured hams was analyzed using three-point bend technique. Ham slices measuring 2.54 cm in thickness were placed on two-raised metal platforms that were the same height but had a 3.81 cm gap separating the two platforms. The ham slice was positioned on the platform so that the geometric center of the chop was situated in the gap between the two platforms. Prior to analysis, a metal cross bar was calibrated to descend between the gap in the metal platform and ascend upon fracture of the sample. The metal platforms were adjusted so that the crossbar was not closer to one platform or the other while descending and ascending. The cross bar and platform were attached to a Texture Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK) with a crossbar speed of 10 mm/sec and a load cell capacity of 100 kg. The crossbar descended and depressed sample until fracture; the peak force necessary to fracture sample was recorded. This measurement was conducted on three slices per ham with the final value being the averaged force.

Texture Profile Analysis

The Bourne analysis (Bourne, 1978) was used to evaluate the parameters of hardness, fracturability, gumminess, chewiness, springiness, cohesiveness, and resilience in both fresh chops and restructured hams. In fresh chops, three 2.54 cm cores were collected and compressed on Texture Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK). A 5.08 cm diameter plate compressed each core in two consecutive cycles of 75% strain with 2 s between cycles. The cross-head moved at a constant speed of 5 mm/s. The values for the 3 cores were

averaged to receive an overall value for each parameter. Texture profile of ham slices

was analyzed in the same manner as the previously described methods for fresh chops.

However, five 2.54 cm cores were used instead of only three.

Salt Soluble Protein Assay

Sarcoplasmic and myofibrillar proteins were extracted in accordance to a

modified method described by Lan et al. (1993).   An extraction buffer of of 0.01 M 2-

[N-Morpholino] ethanesulfonic acid (MES) was diluted in ultrapure water. Ground

Triceps brachii samples weighing 10 g was homogenized in 15 mL extraction buffer for

1 min using a homogenizer (Brinkman homogenizer, PT 10/35, Brinkman Istruments,

Westbury, NY). The homogenate was centrifuged (1500 x g) for 20 min at 4° C. The

supernatant was considered to be salt soluble proteins and was diluted 12.5 fold and

quantified using the BCA protein assay kit. Samples, along with working solution

included in the kit, were plated onto a 96 well plate. A standard curve using bovine

serum albumin was prepared and plated in duplicate. Absorbance values of the samples

were read at 550 nm using a Synergy HT plate reader. Solublized protein content was

calculated using a second order polynomial equation. Salt soluble protein quantities are

reported as percent of wet tissue weight.

Histology

Longissimus pieces were frozen in liquid nitrogen-cooled isopentane, and stored

at -80° C. Frozen muscle pieces were affixed to a cryo-cut chuck using O.C.T.

embedding compound (Tissue-Tek, Sakura Finetek, USA). Longitudinal and cross

sections measuring 10-15 μm thick were cut using a cryo-cut (American Optical Corp.)

chilled to -20° C. Cut sections were affixed to slides and stained with Toluidine blue for

3 min. Stained samples were visually examined using a microscope with 20X objective (Nikon Instruments, Inc., Melville, NY) for longitudinal sections and 10X objective for cross sections; photographs of magnified sections were taken using a Nikon D60 (Nikon Instruments Inc., Melville NY) camera.

Statistical Analysis

Statistical differences were detected using paired option of the Proc T TEST procedure of SAS (SAS Inst. Inc., Cary, N.C.). For texture profile analysis of restructured hams, only 5 pairs were analyzed. For protein degradation only five pairs were analyzed. All other variables were analyzed using 6 pairs.

Results and Discussion

pH and Water Holding Capacity

Pressurization caused an immediate and significant ($P < 0.05$) drop in pH (over 0.3 units) (Figure 2.1). The pH value at 24 h postmortem was 0.4 units less ($P < 0.05$) for control carcasses. It appears that pressurization induced contraction causing a calcium release stimulating glycolysis resulting in a small, but immediate drop in pH. However, as pressure increased glycolytic enzymes were inactivated preventing further decrease of pH.   At 24 h postmortem, treated sides experienced a small improvement (0.17 units) in pH whereas controls had declined to levels typical of fresh pork.

The pH at 48 hours postmortem was 0.48, 0.47, and 0.27 units higher ($P < 0.05$) for treated *Longissimus, Semimembranosous,* and *Triceps brachii* muscles, respectively; the *Psoas* major was not different (Table 2.1). Macfarlane (1973) reported that the ultimate pH of pressure treated pre-rigor ovine muscles were greater than controls except for the *Longissimus*. Other studies determined that ultimate pH was lower in samples

subjected to HPP (Kennick et al., 1980; Macfarlane et al., 1982; Horgan and Kuypers, 1983).

The muscles evaluated in the present research when compared to previous research were subjected to a higher pressure level (215 vs 100-150 MPa) and considerably less pressurization time (15 sec vs 4-10 min). Based on the conclusions of Horgan and Kuypers, (1983) and Elkhalifa et al. (1984), the muscles in the present research may not have completed glycolysis due to the shorter pressurization time and/or change in glycolytic enzyme functionality due to the higher pressure level. It should be noted that Macfarlane et al. (1982) determined that ultimate pH was high for muscles that were pressurized for 3-24 h with pressurization liquid at 0°C; concluding glycolysis did not occur. Although pressurization conditions for the present research were vastly different, the 1982 study suggests that glycolysis can be altered by pressurization conditions.

Drip loss percentage was 1.8% less ($P < 0.05$) for both the *Longissimus*, and *Semimembranosous*, while the *Psoas major* and *Triceps Brachii* were not different (Table 2.1). It is not surprising that no differences existed for the *Psoas major* regarding drip loss percentage given its relationship to pH, which also was not different. Fiber type differences among muscles may explain the varied results found in pH and drip loss. Horgan (1981) determined fast twitch muscles experienced a larger pH decline (0.6-0.8 units) than slow twitch muscles (0.2 units) after each were subjected to HPP. Previous work has also found that weep was significantly higher and water-holding capacity significantly lower in HPP treated pre-rigor bovine and ovine muscle (Kennick et al., 1980). Kennick et al. (1980) also reported treated muscles had an ultimate pH that was

significantly lower than non treated counter parts; thus explaining the decreased water holding capacity. Other previous studies that reported high ultimate pH due to HPP, did not investigate drip loss.

Glycolytic Potential

Glycolytic potential (Table 2.2) was not different ($P < 0.05$) between controls and treated. These results were anticipated because paired sides should have the same glycolytic potential regardless of how postmortem metabolism occurred. Treated samples had 12.88 µg/mol more ($P < 0.05$) glucose than controls. Lactate levels for treated samples were 20.3 µg/mol less ($P < 0.05$) than controls. These findings agree with previously discussed ultimate pH results. The high ultimate pH is likely a result of very little lactic acid production. This evidence suggests that HPP stops glycolysis as shown by the high level of glucose and low level of lactate. The control indicates typical postmortem metabolism in which glucose is consumed. The lactate levels for controls are higher indicating that glucose was converted to lactate. Previous research indicates that depending on pressurization chamber settings, glycolysis may be expedited or inhibited (Macfarlane, 1973; Macfarlane et al., 1982; Horgan and Kuypers, 1983; Elkhalifa et al., 1984). It appears that the pressurization conditions used in the present study did not affect phosphorylase, phosphorylase kinase, and phosphorylase phosphatase in a manner that leads to accelerated glycolysis as explained in previous research (Horgan; Kuypers, 1983). The shorter pressurization time used in the current study likely garnered less pressure induced calcium release, and inactivated glycolytic enzymes due to the higher pressure level, ultimately leading to partial inhibition of glycolysis. These results in addition to the ultimate pH results imply that the use of HPP can be useful tool

in persevering pork quality. Many meat quality attributes such as color, protein functionality and water binding properties are decreased at low pH. A technology such as HPP that prevents large pH declines and subsequent negative meat property changes would be invaluable to the meat industry.

Quality Evaluations

For subjective color score, (Table 2.1) no differences ($P > 0.05$) were found for the *Longissimus*. The *Tricpes brachii, and Semimembranosous* were trending ($P = 0.10$) with treated muscles receiving lower scores than controls. The *Psoas major* controls received higher ($P < 0.05$) color scores indicating treated *Psoas major* muscles were lighter in appearance. Subjective firmness evaluation (Table 2.1) determined the *Longissimus* and *Semimembranosous* from treated carcass sides were subjectively firmer, ($P < 0.05$) whereas firmness scores for treated *Triceps Brachii* and *Psoas major* were not different from controls. Differences that occurred between treated and controls for subjective measurements were not alarming because regardless of treatment, all muscles received acceptable color (NPPC, 1999) and firmness values (NPPC, 1991).

Minolta $L*$ color scores (Table 2.1) were statistically different ($P < 0.05$) for all four muscles. The treated *Longissimus, Tricpes brachii,* and *Psoas major* muscles received $L*$ values that were 3.87, 6.37, and 2.71units higher (lighter) ($P < 0.05$), respectively, than controls. The treated *Semimembranosous* had an $L*$ value that was 4.31 units lower (darker) ($P < 0.05$) than controls. Minolta $a*$ scores were different ($P < 0.05$) for the *Longissimus* and the *Triceps brachii,* but no differences were found for the *Psoas major* and semimembranosous. The $a*$ values for treated *Longissimus* muscles were 0.94 units lower (less red) than controls, while treated *Triceps brachii* muscles were

0.67 units higher (more green) than controls. Only the treated *Longissimus* was different

($P < 0.05$) for Minolta b^* scores, indicating its color appeared more blue.

It has been shown that higher pH is correlated with higher L^* values causing the

meat to appear darker (Brewer et al., 2001). Therefore, it was anticipated that color

results would indicate that treated samples were darker in color based on their high

ultimate pH. However, the opposite occurred for all but one muscle. Results from

previous research found similar increases in L^* values, but also saw more consistent

decreases in a^* values (Carlez et al., 1995). Carlez et al. (1995) also noted that pressures

over 200 MPa caused a 'whitening' effect to the meat. Although the color of the meat

used in the present study varied from 'typical' pork color, no whitening effect was

observed. The color differences seen in the present and past research are believed to be

related to changes in myoglobin content due to pressure induced detnaturation of

sarcoplasmic proteins (Carlez et al., 1995; Jung et al., 2003). Additionally, color

differences in the present research may be attributed to pressure induced changes to

water-protein binding properties. These changes may cause differences in the way in

which light scatters causing the appearance of lighter or darker color.

Thiobarbituric Acid Reactive Substances (TBARS)

The oxidative stability of ground pork product is depicted in Figure 2.2. At day 0,

TBARS values were not different ($P > 0.05$). Day 0 TBARS values were alarmingly

higher than expected. It is believed the addition of salt as a proxidant, as well as other

unknown factors, such as temperature abuse during transportation, led to high TBARS

values at day 0. At day 7, TBARS values for treated samples were less ($P < 0.05$) than

controls at a difference of 30%. Day 14 samples were not included in the analysis. At

day 21, TBARS values were trending ($P =0.11$) with treated values being numerically less than controls. It can be assumed that the high variability within the small sample size prevented statistical differences. Results indicate that HPP drastically inhibited the rate of lipid oxidation in ground pork samples. The findings of the present research do not completely concur with that of previous results. It is generally regarded that HPP increases the rate of lipid oxidation particularly at pressure levels above 300 MPa (Cheah and Ledward, 1996; Beltran et al., 2003). Cheah and Ledward (1996), concluded that lipid oxidation rate was unchanged at pressures less than 300 MPa which is consistent with the pressure level used in the present research. Additionally, it has been shown that TBARS values in ground pork with high ultimate pH (>6.10) are less than samples with a more typical pH (5.4-5.9) (Yasosky et al., 1984). It is theorized histidine residues in meat with low pH alter the tertiary structure reducing the proteins ability to sequester catalytic metal ions used during lipid oxidation (Yasosky et al., 1984)

Cook loss, Warner-Bratzler Shear Force and Degree of Doneness

Cook loss (Table 2.1) was different ($P < 0.05$) for *Longissimus* chops but no differences were detected for any of the other muscles. The treated *Longissimus* chops retained over 3.5% more moisture during cooking when compared to controls. The present research concurs with Macfarlane (1973) who determined cook loss was decreased in treated samples. More recently however, Jung et al. (2000b) determined that cook losses of beef *Longissimus* muscles were higher ($P < 0.05$) than controls. It should be noted that research has shown cook losses to be less for meat with high ultimate pH (>6.0) (Sayre et al., 1964; Brewer and Novakofski, 1999).

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Warner-Bratzler shear force was not different ($P > 0.05$) between treated and controls for the *Psoas major, Triceps brachii or Semimembranosous*; *Longissimus* approached significance ($P = 0.07$) with treated samples being more tender than controls (Table 2.1). The results for individual muscle Warner-Bratzler shear force values indicate HPP does not affect all muscles consistently in terms of mechanical tenderness. Macfarlane (Macfarlane, 1973) found more consistent results with individual muscles determining HPP increased mechanical tenderness in all muscles examined, including the *Longissimus* and *Semimembranous* (both aged 2 days).

Warner-Bratzler shear force values for aged *Longissimus* chops are presented in Figure 2.3. Treated 0 d chops were more ($P < 0.05$) tender than controls with values being 30% different. Treated *Longissimus* samples aged 7 and 14 days approached significance ($P = 0.07$) both days being more tender; samples aged 21 days were trending ($P = 0.12$). The similarity of mechanical tenderness values across all aging days suggests treated samples do not age or become more tender. Control samples, on the other hand, start with higher shear force values at day 0 (3.07 kg), but as aging occurs the samples become more mechanically tender (2.27 kg). These results concur with studies in which pre-rigor meat is used to evaluate Warner-Bratzler shear force values. Kennick et al. (1980) as well as Riffero and Holmes (1983) determined HPP decreased Warner-Bratzler shear force values of pre-rigor pressurized meat.

Degree of doneness (Figure 2.4) chops cooked to lower (63°C) and higher (77°C) internal endpoint temperatures were not different ($P > 0.05$) between treated and control. This suggests that HPP does alter Warner-Bratzler shear force value at lower and higher degrees of doneness.

Protein Degradation

Postmortem proteolysis of myofibrillar protein by µ-calpain has been shown to be an integral part of postmortem tenderization (Huff-Lonergan et al., 1996). It has also been shown that troponin t and desmin serve as substrates for µ-calpain during postmortem proteolysis (Lametsch et al., 2004). Therefore, it is important to understand the affect of HPP on myofibrillar protein to determine if treated samples undergo postmortem aging in a typical manner. Figure 2.5a refers to how troponin t membranes were analyzed for protein band densitometry. The % degradation of troponin t (Table 2.4) at 0 vs. 7 d, 0 vs. 14 d and 0 vs. 21 d, indicated that control samples had 30.45, 60.97, and 53.33% more ($P < 0.05$) degradation of troponin t. This indicates that over time, proteolysis of troponin t in treated samples was not nearly as extensive as control samples. These results suggest that HPP my have altered the functionality troponin t and/or µ-calpains resulting in less protein degradation. Differences of intact troponin t (Table 2.4) between controls and treated at 0 d approached significance ($P = 0.07$). Intact troponin t at 14 d was 2.5 times greater ($P < 0.05$) for treated samples. Intact troponin t was trending ($P < 0.10$) at 21 d with treated samples having nearly twice the amount of intact protein as controls. Intact troponin t at 7 d was not different ($P > 0.05$). The control troponin t values for both % degradation and intact troponin t agree with control sample Warner-Bratzler shear force values plotted on the aging curve seen in Figure 2.3. The increase in troponin t degradation and decrease in intact troponin t are reflected in the decrease of mechanical tenderness values over time.

Figure 2.5b refers to how desmin membranes were analyzed for protein band densitometry. The % degradation of desmin (Table 2.4) for 0 vs. 7 d approached

significance ($P = 0.07$) with treated samples having 50% less desmin degradation. Percent degradation of desmin at 0 vs. 14 and 0 vs. 21 were not different ($P > 0.05$) between treated and controls. Desmin % degradation was relatively unchanged over all time points for control samples. Intact desmin (Table 2.4) for 0 d was trending ($P = 0.10$) with treated samples having 4 times the amount of intact desmin. The amount of intact desmin was not different between treated and controls at 7, 14, and 21 d. Intact desmin levels for treated samples were numerically higher at all days but because of the high variability among the small sample size, statistical differences were not detected. The % degradation of desmin for control and treated samples were not consistent with Warner-Bratzler shear force values on the aging curve in Figure 2.3. The results indicate that desmin degradation for controls occurred early and did not increase greatly over time. Treated samples, however, started with very little desmin degradation but then increased over time suggesting that the mechanical tenderness values would decrease slightly over time. The aging curve, however, indicates that Warner-Bratzler values for treated samples remained relatively unchanged over time suggesting that the aging phenomenon did not occur. This suggests two possible occurrences: (1) HPP damaged myofibrillar protein so extensively that it reached maximum tenderness upon treatment; (2) changes in tenderness over time were not evident because proteolysis occurred in a such a limited capacity that they had no effect on tenderness.

Previous research investigating the effect of HPP on calpains has shown decreases in activity as pressure increased. Calpain activity has been shown to decrease at pressurization as low as 100 MPa; at 200 MPa activity was one-fifth of controls and at 300 MPA was completely inactivated (Koohmaraie et al., 1984; Homma et al., 1995). It

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is likely that calpain activity for the present study was also decreased given the lower

levels of protein degradation for both troponin t and desmin.

Soluble and Insoluble Collagen

Soluble collagen, insoluble collagen and total collagen were not different ($P >$

0.05) (Table 2.2). For this particular study, HPP did not have an impact on intramuscular

collagen. Some authors have speculated that pressurization does not affect connective

tissue or what is referred to as 'background toughness' (Ratcliff et al., 1977; Beilken et

al., 1990). Research that has been conducted on actual connective tissue has concluded

that HPP has no effect on collagen (Bouton et al., 1978; Suzuki et al., 1993)

Sensory Evaluation

Panelists used for sensory evaluation did not detect differences ($P > 0.05$)

between treated and control samples when evaluating juiciness (Figure 2.6). Tenderness

scores for treated samples were 2.1 points higher ($P < 0.05$) than control samples. Off

flavor was trending ($P < 0.10$) with treated samples receiving higher off flavor scores.

Although almost statistically different, the off flavor levels are not of concern due to their

very low level (treated=0.29 on a 1-15 scale). In terms of human perception, HPP

demonstrated the ability to increase tenderness. Researchers Macfarlane (1973) and

Riffero and Holmes (1983) reached the same conclusions regarding tenderness but

disagreed with juiciness; the 1983 study concurred with the present research that no

differences existed for juiciness. Although, no quantifiable measurement was taken,

many panelists noted a mealy texture exclusively with treated samples. It is believed this

phenomenon is a result of extensive damage caused to proteins during pressurization.

Previous work examining electron micrographs has indicated that myofibrillar protein of

HPP treated muscles becomes even more disorganized after cooking when compared to raw HPP treated samples (Macfarlane et al., 1981).

Texture Profile Analysis and Ham Binding Strength

Texture profile analysis of fresh chops (Table 2.3) determined that differences ($P < 0.05$) between controls and treated existed for cohesiveness, gumminess, and chewiness. HPP treatment did not have an effect ($P < 0.05$) on the texture properties of fresh chops for hardness, springiness, and resilience. Gumminess and chewiness were 1.30 and 0.34 kg greater for treated samples while cohesiveness was 2.5 % less for control chops. Although inconsistent across all textural parameters, it appears that HPP alters texture profile.

Texture profile analysis of restructured ham slices (Table 2.3) determined that significant differences ($P < 0.05$) between treated and control samples existed for the texture profile parameters of hardness, fracturability, springiness, gumminess and chewiness. The parameters of cohesiveness and resilience were not different between treated and controls. Hardness and fracturability were 2.71 and 3.00 kg less for treated samples. Control samples were 5.42% higher for springiness value. Gumminess and chewiness values were 0.72 and 0.50 kg less for treated samples.

Ham binding strength (Table 2.3) was different ($P < 0.05$) between treated and controls. The control samples required almost 2 kg of additional force to fracture the sample. This suggests that the effects of HPP resulted in a decrease in protein bind. This is further supported by the results of the texture profile analysis of restructured ham cores. In all texture profile parameters that were statistically different, the treated ham slices received lower values than its treated counterparts. It has been shown in previous

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research that correlations exist between binding strength and texture profile analysis (Herrero et al., 2007). Previous literature is conflicted regarding the results of texture analysis of value added products. Generally at lower pressure levels, (< 150 MPa) binding strength and texture parameters are improved (Macfarlane et al., 1984; Crehan et al., 2000). However, it has also been shown that as pressure level increases some textural properties are decreased (Yuste et al., 1999; Crehan et al., 2000; Mor-Mur and Yuste, 2003). Cheftel and Culioli (1997) believe that textural changes are a result of high pressure induced disruption of protein functionality, particularly myosin heavy chains, ultimately causing textural changes.

Salt Soluble Proteins

The amount of salt soluble proteins (Table 2.2) were different ($P < 0.05$) between treated and controls. Treated samples yielded a difference of 17.8% less salt soluble proteins than controls on a wet tissue basis. These results further help to explain the lower value reported for both binding strength and texture profile analysis. It appears that the treatment used in present research resulted in a decrease in myofibrillar protein functionality explaining the inability of NaCl to extract these proteins or for them to bind to each other. Previous research has indicated the opposite. Authors who have investigated myofibrillar solubility have concluded that HPP increases protein solubility even at higher pressures (Macfarlane, 1974; Macfarlane and McKenzie, 1976; Suzuki et al., 1991). However, it should be noted that all of those experiments were conducted with meat being pressurized after it had been suspended in a saline solution. This suggests that myofibrillar protein extraction was occurring prior to and was possibly improved during HPP. It is probable that functionality changes that occurred to proteins

during HPP in the current study made proteins less susceptible to extraction when introduced to saline solution during analysis.

Muscle Structure

Photographs taken of magnified longitudinal muscle tissue section of treated *longissimus* samples indicated widespread disorganization and damage to muscle tissue (Figure 2.7). Most previous research regarding muscle structure was evaluated using an electron microscope allowing for specific evaluation contractile protein structures. The muscle tissue sections for the current research were evaluated using a light microscope and only general observations could be made. Treated longitudinal sections (Figure 2.7b) had definite distortion and separation of muscle fibers. The sarcolemma of treated samples was also damaged displaying holes and areas of missing muscle structure. The z-lines of control samples (Figure 2.7a) were clearly visible, whereas z-lines for treated samples (2.7b) were only visible in areas of minimal damage suggesting. The nature of the damage seen in Figure 2.7b suggests loss of contractile protein organization as well as impaired protein functionality, for postmortem proteolysis, and myofibrillar protein solubility.

The effect of HPP on muscle structure was less conclusive when evaluating muscle tissue cross sections. Figure 2.7d indicates that HPP causes extensive damage to muscle structure. The photograph indicates widespread loss of muscle fiber integrity with some areas damaged so extensively that muscle fiber structure is indistinguishable. Unexplainably, cross sections were not as conclusive as longitudinal sections in terms of consistent damage to muscle structure in all animals. Figure 2.7f depicts a treated cross section that is very comparable to its control counterpart, Figure 2.7e. Oddly enough,

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longitudinal sections from all treated animals exhibited similar patterns of damage to muscle structure.

Previous research has consistently found that HPP, particularly at pressures > 130 MPa, cause extensive muscle structure damage (Macfarlane and Morton, 1978; Kennick et al., 1980; Macfarlane et al., 1981; Suzuki et al., 1991; Jung et al., 2000c). At pressurization levels closest to the present research, electron micrograph images indicated structural loss of the sarcomere, disappearance of Z-lines, as well as I and A-filament disorganization (Suzuki et al., 1991). The pressure-induced destruction of protein structure helps to explain the increased mechanical tenderness for treated samples. The protein degradation results are also clarified considering that a pattern of typical proteolysis could not be expected in samples where protein destruction likely occurred.

Conclusions

This study identified several HPP induced changes to meat properties. It is apparent that HPP partially inhibits postmortem metabolism. The resulting high ultimate pH suggests that HPP may be used to preserve quality in some animals that would have had abnormally high pH declines. The water holding properties associated with HPP treated meat also provide usefulness to pumped or injected products where high water retention is desired. Color results from this study indicate that HPP treatment causes meat to appear lighter. Consumers' purchasing preferences are highly based on fresh meat color. More work is needed to investigate meat color preservation when using HPP.

Lipid oxidation only increased slightly in HPP treated samples. These findings are likely attributed to the high ultimate pH. HPP also improved pork palatability

parameters. Warner-Bratlzer shear force values indicated that HPP causes increased

mechanical tenderness, which was confirmed by sensory panelists' evaluation of

tenderness. The ability of HPP to consistently produce tender products without the

addition of non-natural additives or the use of blade tenderization would be a major

breakthrough for the meat industry.   HPP did not have any effect on intramuscular

collagen indicating that the increased tenderness of HPP treated samples was likely not

due to changes in collagen.

Myofibrillar protein solubility is an area of concern regarding the use of HPP.

Results from texture profile analysis, ham binding strength, and the salt soluble protein

assay suggest that myofibrillar protein damage, or limited functionality, negatively

affects further processed pork products that rely heavily on protein-protein bind.

Photographs of magnified muscle section show that HPP treated muscle structure is

damaged and highly disorganized. This suggests that physical destruction of muscle

components is just as responsible for pressure-induced denaturation of proteins. HPP has

the possibility to be a very a useful tool in the meat industry. However, more research

must be done to maximize all the positives of HPP, while eliminating all its negatives.

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Table 2.1. Effect of HPP on Individual Muscles

	Longissimus				*Psoas major*				*Triceps brachii*				*Semimembranosous*			
	Con	**Trt**	**SEM[x]**	*P*-value	**Con**	**Trt**	**SEM**	*P*-value	**Con**	**Trt**	**SEM**	*P*-value	**Con**	**Trt**	**SEM**	*P*-value
pH	5.78[a]	6.26[b]	0.05	0.0003	6.17	6.31	0.09	0.1679	6.08[a]	6.35[b]	0.10	0.0412	6.01[a]	6.48[b]	0.07	0.0012
Drip Loss %	2.16[a]	0.30[b]	0.63	0.0328	0.54	0.60	0.12	0.6431	0.63	0.43	0.17	0.3033	2.13[a]	0.33[b]	0.62	0.0012
Color	2.67	2.50	0.31	0.6109	5.00[a]	4.00[b]	0.00	<.0001	4.17	3.50	0.33	0.1019	3.83	3.17	0.33	0.1019
Firmness	2.00[a]	3.33[b]	0.52	0.0015	2.50	2.50	0.45	1.0000	2.67	2.83	0.31	0.6109	2.50[a]	3.50[b]	0.26	0.0117
L*	46.73[a]	50.60[b]	1.48	0.0472	36.86[a]	43.23[b]	1.09	0.0020	41.49[a]	44.20[b]	0.69	0.0112	46.80[a]	42.49[b]	1.50	0.0347
a*	6.07[a]	5.13[b]	0.22	0.0085	14.80	13.76	0.55	0.1150	11.67[a]	12.34[b]	0.25	0.0423	7.26	7.01	0.73	0.7439
b*	3.06[a]	1.59[b]	0.48	0.0285	3.98	3.92	0.48	0.8993	3.95	3.82	0.18	0.4964	2.24	2.81	0.56	0.3556
Cook Loss %	20.58[a]	17.01[b]	0.98	0.0149	19.16	17.87	1.51	0.4325	18.72	20.52	2.60	0.5194	23.71	21.34	2.21	0.3331
WBSF kg[y]	2.46	1.97	0.21	0.0659	1.82	2.05	0.36	0.5449	2.35	2.35	0.08	0.9868	2.49[a]	2.11[b]	0.13	0.0292

[a,b] Means within muscle lacking a common superscript are different ($P < 0.05$)
[x] SEM is the standard error of the difference of the mean
[y] Warner-Bratzler shear force chops were aged 14 d and cooked to endpoint internal temperature of 71˚C

Table 2.2. Biochemical Analyses

	Control	Treated	SEM[y]	*P*-value
Glycolytic Potential* (μmol/g)				
Glucose	4.87[a]	17.75[b]	2.87	0.0064
Lactate	104.30[a]	84.00[b]	1.70	<0.0001
Total GP	114.08	119.48	4.80	0.3117
Collagen* (mg/g wet tissue)				
Soluble	0.99	0.73	0.14	0.1123
Insoluble	2.53	2.52	0.09	0.9259
Total Collagen	3.51	3.24	0.20	0.2330
Salt Soluble Protein[x]				
Soluble Protein	9.28	7.62	0.51	0.0223

[a,b] Means lacking a common superscript are different ($P < 0.05$)

*Glycolytic potential (GP) and Collagen analysis were conducted using *Longissimus* muscle
[x]Salt Soluble protein analysis was reported on % wet tissue basis and conducted using *Triceps brachii* muscle
[y]SEM is the standard error of the difference of the mean

Table 2.3. The effect HPP on Ham Texture Profile Analysis and Binding Strength

	Control	Treated	SEM[x]	*P*-value
Fresh Chops*				
Hardness kg	20.72	23.57	1.31	0.0821
Springiness %	25.00	25.00	1.48	1.0000
Cohesivness %	26.77[a]	29.27[b]	0.63	0.0109
Gumminess kg	5.45[a]	6.75[b]	0.40	0.0243
Chewiness kg	1.36[a]	1.70[b]	0.13	0.0509
Resilience %	17.82	17.45	0.72	0.6316
Ham Slices				
Hardness, kg	14.59[a]	11.88[b]	0.39	0.0022
Fracturability, kg	9.28[a]	6.28[b]	0.68	0.0117
Springiness, %	49.42[a]	44.00[b]	0.79	0.0024
Cohesivness, %	23.22	22.62	0.81	0.4999
Gumminess, kg	3.47[a]	2.75[b]	0.17	0.0122
Chewiness, kg	1.73[a]	1.23[b]	0.10	0.0072
Resilience, %	7.44	7.50	0.37	0.8789
Binding Strength, kg	12.93[a]	10.96[b]	0.56	0.0167

[a,b] Means within muscle lacking a common superscript are different ($P < 0.05$)

*Longissimus* chops aged 7 days were used for texture profile analysis

[x]SEM represents the standard error of the difference of the means

Table 2.4. The effect of HPP treatment on Troponin t and Desmin Degradation*

	Control	Trt	SEM[x]	*P*-value
Troponin T				
% Degradation 0 d vs 7d	48.78[a]	18.33[b]	11.67	0.0477
% Degradation 0 d vs 14d	60.97[a]	0.0[b]	14.54	0.0072
% Degradation 0 d vs 21d	61.06[a]	7.73[b]	20.76	0.0510
Intact Troponin T 0 d	5.22	4.66	0.24	0.0659
Intact Troponin T 7 d	2.67	3.74	0.64	0.1598
Intact Troponin T 14 d	2.00[a]	4.63[b]	0.78	0.0202
Intact Troponin T 21 d	2.14	4.12	0.9805	0.0995
Desmin				
% Degradation 0 d vs 7d	82.81	32.49	20.30	0.0683
% Degradation 0 d vs 14d	80.59	79.58	15.97	0.9523
% Degradation 0 d vs 21d	79.94	86.69	16.74	0.7075
Intact Desmin 0 d	1.01	4.12	1.43	0.0958
Intact Desmin 7 d	0.10	3.11	1.90	0.1871
Intact Desmin 14 d	0.07	0.86	0.44	0.1489
Intact Desmin 21 d	0.11	0.36	0.19	0.2849

[a,b] Means within muscle lacking a common superscript are different ($P < 0.05$)

[x] SEM is the standard error of the difference of the means
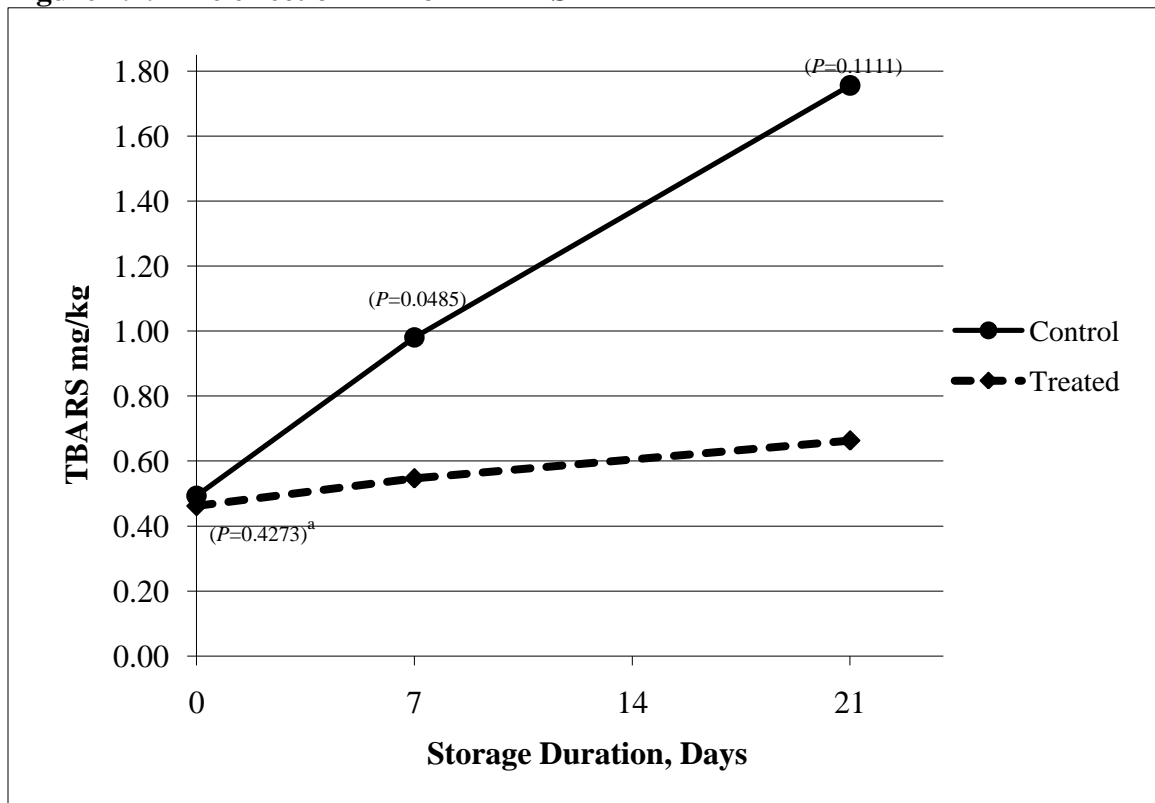
Longissimus chops were used for analysis

Figure 2.1. The effect of HPP on pH declines of *Longissimus*

pH Declines
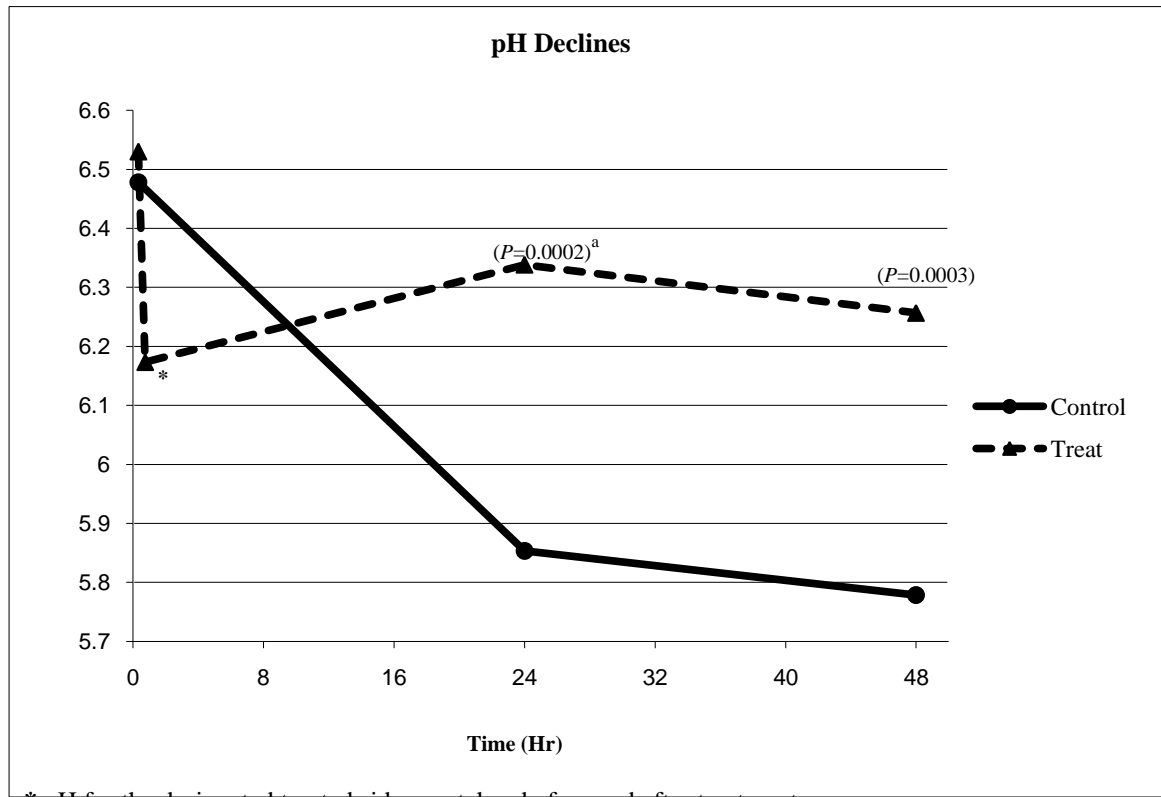
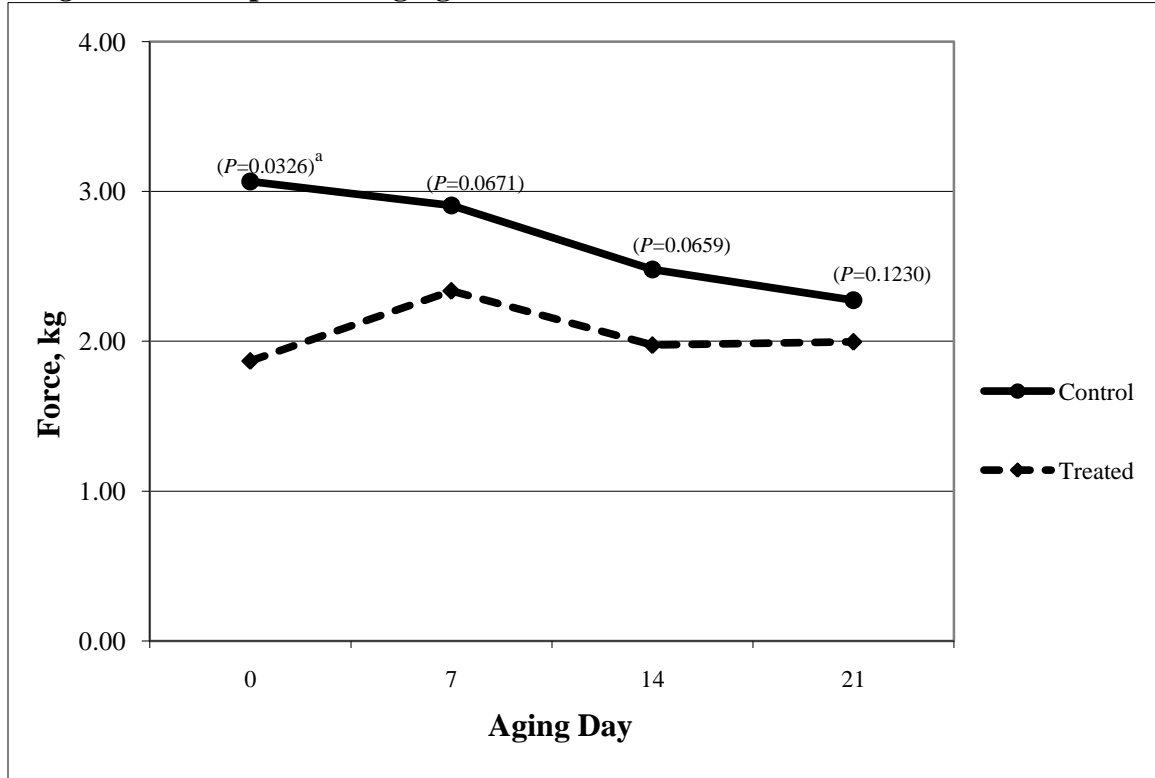* pH for the designated treated side was taken before and after treatment.
[a] P values are representative of differences between treated and controls at corresponding time point

61

Figure 2.2. The effect of HPP on TBARS

Figure 2.2. The effect of HPP on TBARS

[a]P values are representative of differences between treated and controls at corresponding time point

62

Figure 2.3. The effect of HPP on Warner-Bratzler Shear Force values of
Longissiumus **chops* after aging**

*Longissimus chops were cooked to an endpoint internal temperature of 71˚
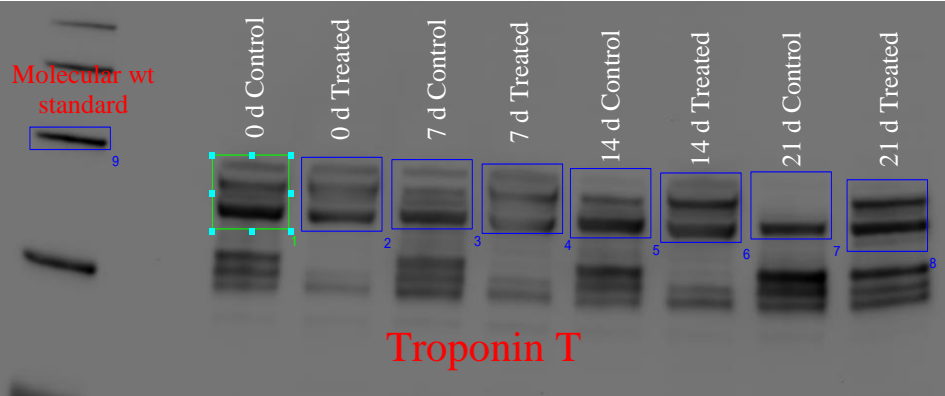
[a] P values are representative of differences between treated and controls at corresponding time point

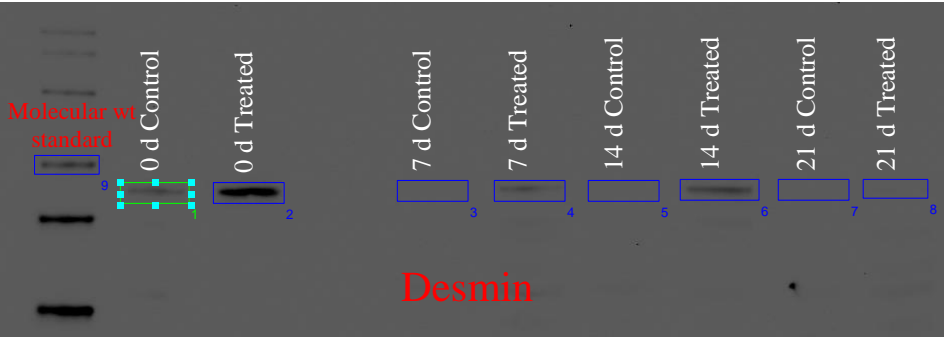**Figure 2.4. The effect of HPP on Degree of Doneness of *Longissimus** chops**
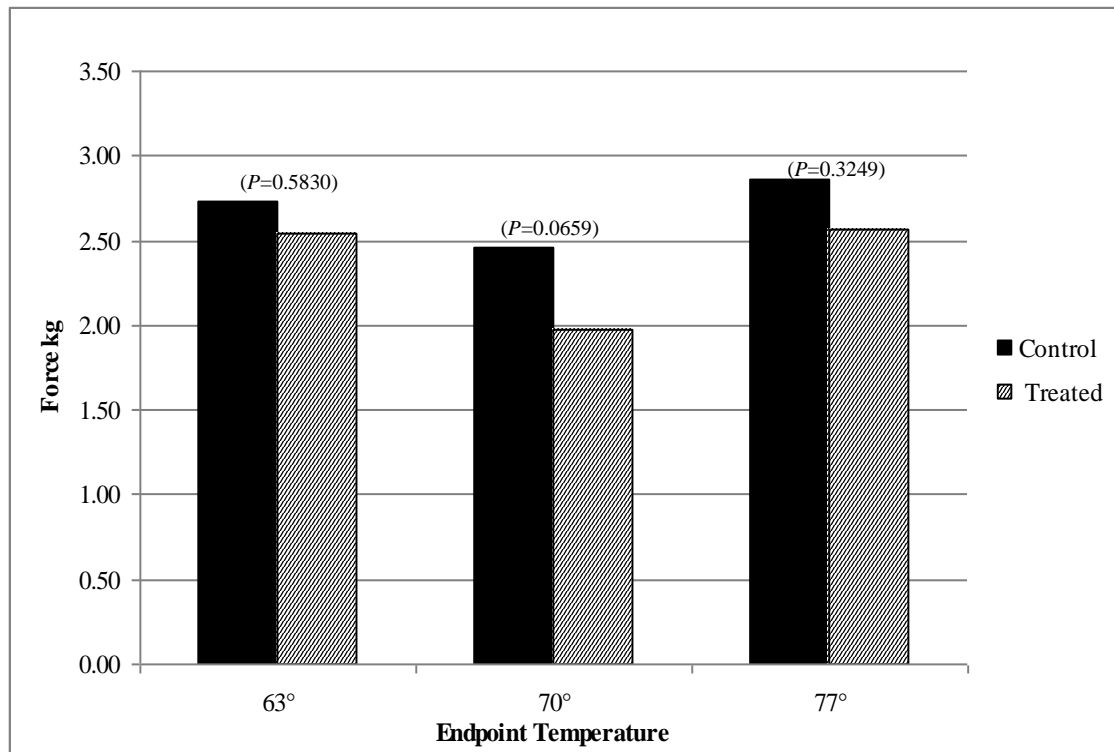
*Longissimus chops were aged 14 days.

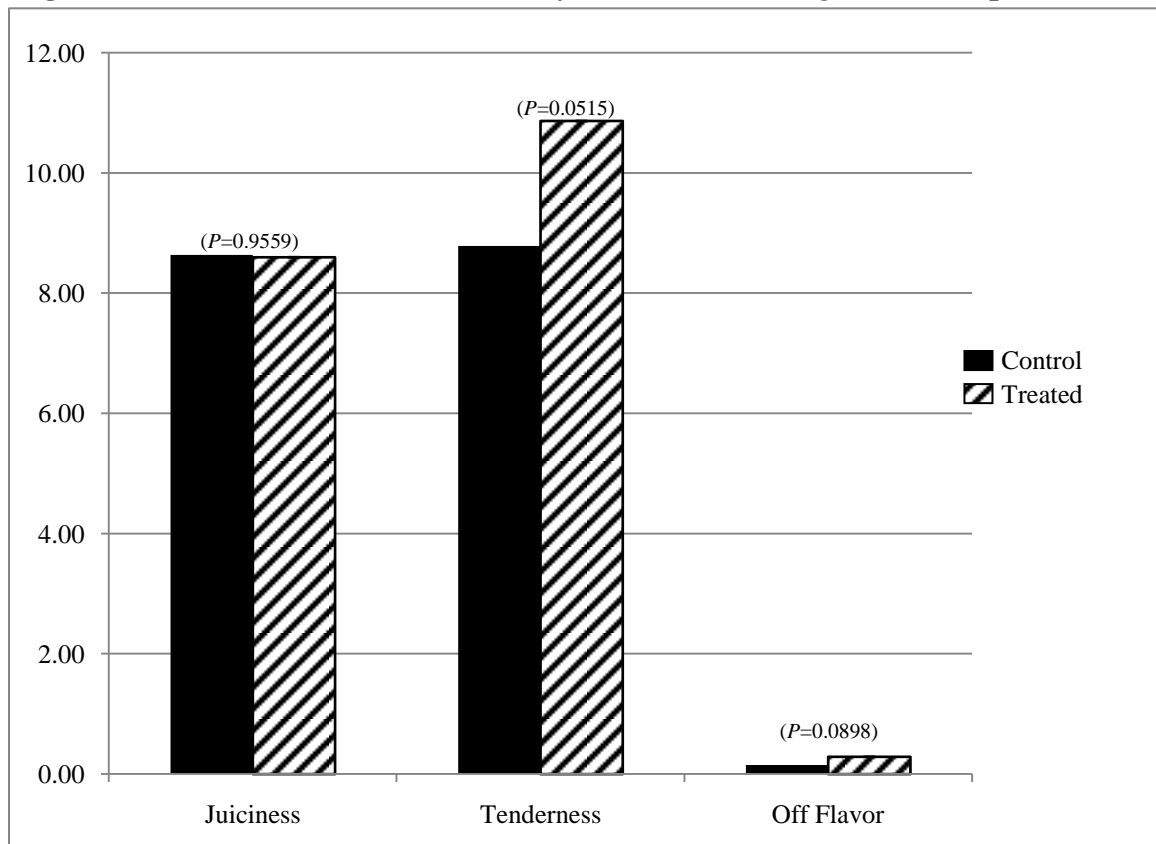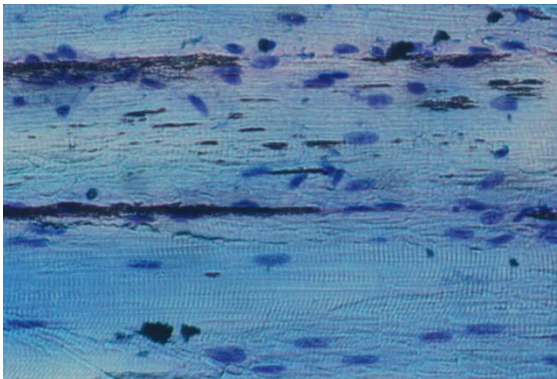**Figure 2.5. Troponin T and Desmin Western Blot Analysis**

2.5a

2.5b

Figure 2.6. The effect of HPP on sensory evaluation of *Longissimus* chops
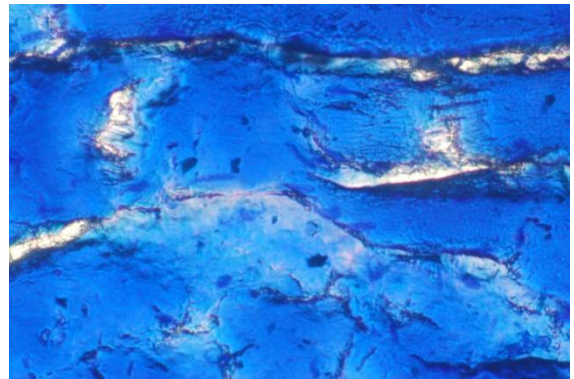
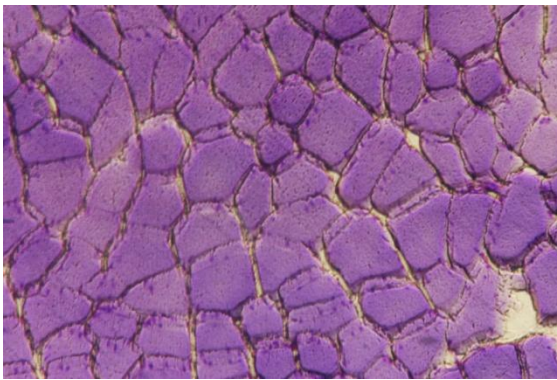

*All chops were aged 14 days

Figure 2.7. The effect HPP on muscle structure
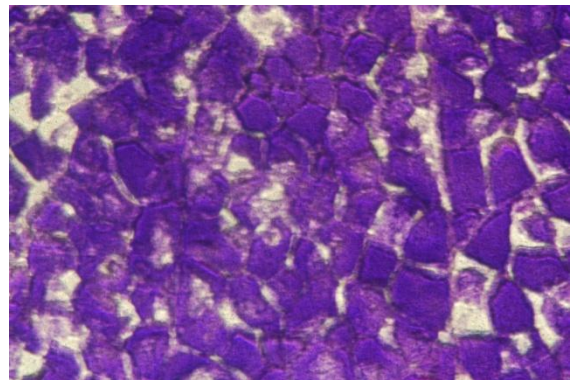

Control Longitudinal Section 40X   (7a)
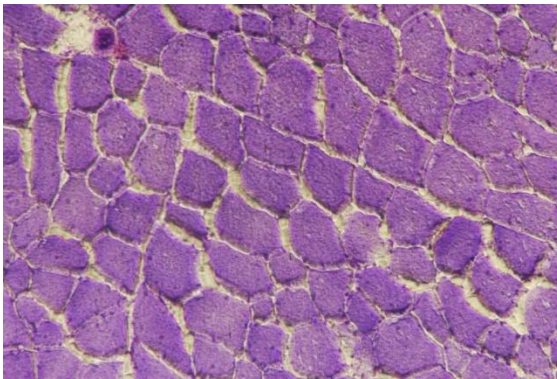

Treated Longitudinal Section 40X   (7b)

Control Cross Section 10X   (7c)

Treated Cross Section 10X   (7d)

Control Cross Section 10X   (7e)

Treated Cross Section 10X   (7f)

CHAPTER 3
THE EFFECT OF VARYING HIGH PRESSURE PROCESSING PRESSURIZATION LIQUID TEMPERATURES ON MEAT COLOR, WARNER-BRATZLER SHEAR FORCE, TEXTURAL PROFILE OF HOT DOGS AND MYOFIBRILLAR PROTEIN SOLUBILITY

Abstract

This study evaluated the impact of High Pressure Processing (HPP) treatment with varying pressurization liquid (water) temperatures on pork quality, Warner-Bratzler shear force, myofibrillar protein solubility, and textural parameters of hot dogs. HPP pressurization liquid temperatures were 15.5°C (HPP Low) and 29.4°C (HPP Med). Analyses were conducted using paired boneless loins (HPP Low 12 pairs & HPP Med 10 pairs) and paired boneless hams (HPP Low & HPP Med 6 pairs each).

Loins were evaluated for pH, purge loss, objective color, subjective color and firmness; and changes to Minolta $L*$, $a*$, and $b*$ during a 20 min bloom time. Both HPP treatment levels resulted in higher ($P < 0.05$) ultimate pH and less ($P < 0.05$) purge loss %. Subjective color was lighter ($P < 0.05$) for HPP Low but not different from controls for HPP Med. Samples from both treatment levels received higher ($P < 0.05$) subjective firmness scores. Pre-bloom and post-bloom $L*$ values were not different ($P > 0.05$) for either treatment level when compared to their controls. Pre-bloom and post-bloom $a*$ and $b*$ values were less ($P < 0.05$) for both treatment levels. Change in bloom analysis determined $L*$ and $a*$ blooming ability for both treatment levels was not different ($P < 0.05$) from controls. Blooming ability of $b*$ was decreased ($P > 0.05$) for both HPP treatment levels. Texture profile analysis of hot dogs determined that springiness was less ($P = 0.10$) for HPP low treated samples when compared to controls. Fracturability of HPP Med samples was also less ($P = 0.10$) than controls. Warner-Bratzler shear force

values of *Longissimus* chops taken from anterior, mid, and posterior positions were not different from controls for either HPP treatment level. HPP Med mid *Longissimus* chops were more ($P < 0.05$) mechanically tender than controls. When salt concentration levels were pooled, salt soluble proteins extractability was less ($P < 0.05$) for both HPP treatment levels. There was no HPP treatment*salt concentration interaction for salt soluble protein analysis. Protein band densitometry on SDS PAGE gels determined an HPP treatment level*salt concentration level interaction ($P < 0.05$) for protein product bands at 250 and 75 kDa. In general, salt soluble protein yields were less for both treatment levels when compared to controls at most molecular weights.

Introduction

High pressure processing (HPP) is considered a novel food technology that has gained popularity in the last two decades. Several countries such as France, Japan, Mexico, Spain, and the U.S. have instituted the use of high pressure processing in commercial settings on products ranging from fruit juices to protein-based products (de Lamballerie-Anton et al., 2002). HPP is most widely used for its ability to significantly reduce foodborne illness causing pathogens (Shigehisa et al., 1991; Carlez et al., 1993; Hayman et al., 2004). HPP is a non-thermal process because there is only a small product temperature increase that allows for preservation of product flavor and appearance (Swientek, 1992). Just like many other segments of the food business, the meat industry also uses HPP to eliminate food borne illness-causing pathogens. Besides food safety, research has indicated that HPP may offer other benefits to meat properties.

Previous investigations have indicated that pressurization level (MPa), time, and pressurization liquid temperature provide great variability in changes that meat properties

undergo. In order to determine how to obtain the ideal changes in meat properties, HPP

conditions must be further investigated. This chapter will investigate the effect of

varying pressurization liquid temperature on the parameters of pork quality, Warner-

Bratzler shear force values, textural profile of hot dogs, and the solubility of myofibrillar

proteins.

Materials and Methods

Sample Collection

Animals were humanely harvested at a commercial U.S. slaughter facility over

two days. Pigs went through normal harvest procedures. After carcasses were split into

sides, identification was maintained for each side so that paired data could be analyzed.

One side from each animal was subjected to HPP. On harvest day 1, numerous sides

were subjected to HPP using a pressurization liquid (water) with a temperature of 15.5°C

(HPP Low). On harvest day 2, several more sides were HPP treated with pressurization

liquid at 29.4° (HPP Med). Carcasses were chilled over night and fabricated the

following day into boneless loins (NAMP #413C) and bone in fresh legs (NAMP

#401A). Paired HPP Low boneless loins (12 pairs) and HPP Med boneless loins (10

pairs) were randomly selected. HPP Low and HPP Med boneless hams (6 pairs each)

were also randomly selected. Loins and fresh legs were vacuum packaged, boxed and

transported to the University of Illinois Meat Science Laboratory via a refrigerated truck.

Meat Quality

Data collection occurred 10 days postmortem for harvest day 1, and 7 days

postmortem for harvest day 2. Boneless loins were removed from packaging and cut in

70

half. In order to evaluate blooming ability, pre-bloom Minolta $L*$, $a*$, and $b*$ values were immediately collected on the freshly exposed cut lean surface using a Minolta CR-300, utilizing a D65 light source and a 0º observer (Minolta Camera Company, Osaka, Japan). After allowing loins to bloom for 20 minutes, post-bloom Minolta $L*$, $a*$, and $b*$ values were collected. The difference between pre-bloom and post-bloom values were calculated and analyzed. Subjective color (NPPC, 1999) and firmness (NPPC, 1991) as well as pH (MPI pH Meter Model C033, Meat Probes, Inc., USA) were also taken from the center section of the loin. Three Warner-Bratzler shear force chops measuring 2.54 cm were obtained from the anterior, mid, and posterior sections of the *Longissimus*. The anterior chop was obtained approximately 10.5 cm from the anterior end of the *Longissimus*; the mid chop came from the centermost portion of the *Longissimus,* while the posterior chop was obtained immediately anterior to the *Gluteus medius* muscle. Warner-Bratzler shear force chops were vacuum packaged, aged till 14 d postmortem, and then stored at -20°C.

Purge Loss

Boneless pork loins were weighed prior to (in bag) and after removal of their packaging material (out of bag). The ten bags were washed, dried and weighed to determine bag weight. The average bag weight was subtracted from the 'in bag' weights and a purge loss % was calculated.

Fresh Leg Fabrication

Fresh legs were skinned; all subcutaneous fat was kept for use in hot dog formulation. Fresh legs were fabricated into *Semimembranosous, Biceps femoris,*

Semitendinosous, and the knuckle. All muscles were removed of connective tissue; fat was also removed and kept for hot dog formulation. Meat from the shank and light butt were not used for any evaluation. A 2.54 cm chop to be used for salt soluble protein analysis was removed from the center of the *Semimembranosous.* Salt soluble protein chops were placed in whirl pack bags and stored -20°C. All other fabricated muscles were used for hot dog formulation.

Proximate Compostion

Moisture and lipid content were determined for each muscle using the procedures described by Novakoski et al. (1989). Chops were trimmed of external fat and connective tissue and homogenized using a Cusinart Food Processor (Model DLC 5-TX, Cuisinart, Stamford, CT). Moisture content was determined by oven drying the samples at 110ºC for 48 h. Lipid content was determined by extraction using a mixture of chloroform and methanol.

Hot Dog Formulation

A total of 8 hot dog batches were formulated. The muscles from the Low temp treatment group were pooled into 2 treated batches and 2 control batches. Med temp treatment group was formulated in the same manner. Identification was maintained so that parity of samples remained. The *Biceps femoris, semitendinosous,* and knuckles of each batch were cut into small pieces, mixed together, and coarsely chopped in a bowl chopper (Maschinenfabrik Meissner and Co, Biedenkopf Wallau, Germany). All fat collected from fresh legs was also coarsely chopped. Lean and fat samples were collected for proximate composition analysis. The proximate composition results were

used to determine the appropriate fractions of raw materials for a 75:25 lean to fat ratio.

Additional pork fat was needed to reach desired fat inclusion levels. None of the batches

required greater than 5% of an outside fat source. Lean and fat were placed in a bowl

chopper and chopped for 30 seconds before Leggs Bologna/Frank seasoning (Blend

#125, Calera, AL) and water were added. The ingredients were emulsified for an

additional 3 ½ min until hot dog batter reached approximately 12-14°C. Hot dog batter

was stuffed into 22 mm cellulose casings using a Handtmann stuffer ([Model# VF 80]

Biberach, Germany). Hot dogs were cooked in an Alkar smokehouse (Lodi, Wisconsin)

to an internal temperature of 67.8°C and chilled in a cooler at 2°C. After chilling, hot

dogs were peeled, vacuum packaged, and stored at 4°C. Hot dog sections (2) measuring

2.54 cm were cut from the center of the hot dogs and used for texture profile analysis.

The remaining hot dog sections were evaluated on the cut surface for Minolta L^*, a^*, and

b^* and pH values.

Hot Dog Texture Profile Analysis

The Bourne analysis (Bourne, 1978) was used to evaluate the parameters of

hardness, fracturability, chewiness, springiness, cohesiveness, and resilience in hot dogs.

Ten 2.54 cm sections per batch were compressed on Texture Analyzer TA.HD Plus

(Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK). A

5.08 cm diameter plate compressed each section in two consecutive cycles of 75% strain

with 2 s between cycles. The crosshead moved at a constant speed of 5 mm/s. The values

for the 10 sections were averaged to receive an overall value for each parameter per

batch.

Cook Loss and Warner-Bratzler Shear Force

Prior to analysis, chops were removed from freezer and allowed to thaw at 4˚C for 24 h. Chops were then trimmed of excess fat, weighed, and cooked on a Farberware Open Hearth gill (Model 455N, Walter Kiddie, Bronx, NY). Chops were cooked on one side to an internal temperature of 35˚C, flipped, and cooked to final internal temperature of 70˚C. Internal temperature was monitored using copper constantan thermocouples (Type T, Omega Engineering, Stanford, CT) connected to a digital scanning thermometer (Model 92000-00 Barnant Co., Barington, IL). Chops were allowed to cool to 25ºC and then reweighed to determine percentage of cook loss.   Four 1.25 cm cores were removed parallel to the orientation of the muscle fibers. Cores were sheared using a Texture Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK) with a blade of 10 mm/sec and a load cell capacity of 100 kg. Shear force was determined on each core, and the average of the four cores reported.

Salt Soluble Protein Assay and SDS PAGE Sample Prep

Sarcoplasmic and myofibrillar proteins were extracted in accordance to a modified method described by Lan et al. (1993). An extraction buffer of 0.01 M 2-[N-Morpholino] ethanesulfonic acid (MES) was diluted in ultrapure water. The master mix was then aliquotted into 4 beakers with increasing salt concentrations (sodium chloride). Concentrations were 0.5% (0.09M), 1.5% (0.26M), 2.5% (0.43M) and 3.5% (0.6M) salt by volume. Homogenized *Semimembranosous* samples weighing 100 mg were re-homogenized in 1.5 mL of extraction buffer for 3 min using a Tissuelyzer II (Qiagen, Hilden, Germany). The homogenate was centrifuged (1500 x g) for 20 min at 4°C. The supernatant was considered to be salt soluble proteins and was diluted 12.5 fold and

quantified using the BCA protein assay kit. Samples along with the working solution

included in the kit were plated onto a 96 well plate. A standard curve using bovine serum

albumin was prepared and plated in duplicate. Absorbance values of the samples were

read at 550 nm using a Synergy HT plate reader. Solubilized protein content was

calculated using a second order polynomial equation. Salt soluble protein quantities were

reported as percent of wet tissue weight. Protein concentrations from the fractions were

adjusted to 3.0 mg/mL for SDS PAGE.

SDS PAGE

Protein electrophoresis was conducted using NuPAGE 4-12% Bis-Tris gradient

gels with MOPS running buffer. Running buffer and reducing agent were added to

samples in accordance to NuPAGE protocol (Invitrogen, USA) and 10 μg protein were

loaded per lane. Kaleidoscope Standards (#161-0375 Bio-Rad, Hercules, CA) was used

as a molecular weight standard. Gels were run at 200 v for 70 min in water-cooled Hoefer

SE260 Mighty Small II gel electrophoresis boxes (Hoefer, San Francisco, CA). After

electrophoresis, gels were washed 3 times for 5 min in ultra pure water and then stained

for 1 h in Gelcode blue stain (#24592, Thermo-Scientific, Rockford, IL). Samples were

washed again in ultrapure water for twenty minutes and then photographed using the

ChemiGenius[2] Imaging System (Syngene, UK). Images were analyzed using GeneTools

(Syngene, UK). Protein bands corresponding with molecular weights of >250, 250, 100-

150, 100, and 75 kDa were measured for protein band density. Per gel, every band was

standardized using a standard with a known molecular weight.

Statistical Analysis

Statistical differences for all variables unless otherwise noted were detected using paired option of the Proc T TEST procedure of SAS (SAS Inst. Inc., Cary, N.C.). Paired T analysis for HPP Low vs. controls was conducted with 12 pairs of *Longissimus* samples. Paired T analysis for HPP Med vs. controls was conducted using 10 pairs of *Longissimus* samples. Paired T test for hot dog variables were conducted using 2 pairs for both HPP Low vs. controls and HPP Med vs. controls

Salt soluble protein yields were analyzed using the mixed procedure of SAS. Treatment (HPP Low, HPP Med, and controls), and salt concentration level were considered to be fixed effects. Dependent variables that were measured for several salt concentrations on the same experimental unit were analyzed using repeated measures. Covariance structures evaluated and the most appropriate was used. Residuals were examined for normality using the UNIVARIATE procedure of SAS. Any non-normal data was transformed using a natural log or square root transformation. The actual values were used to report data.

Results and Discussion

pH, Purge Loss, Color

Longissimus pH (Table 3.1) of samples subjected to HPP Low was 0.46 units higher ($P < 0.05$) than controls. HPP Med treated samples also had a higher pH being 0.57 units higher than its controls. These findings indicate that lower pressurization liquid temperature results in higher ultimate pH. Previous research does not concur by finding that pressurization liquid at 0°C for 3-24 h prevents an immediate pH drop at pressurization and causes ultimate pH to remain high several hours post postmortem (Macfarlane et al., 1982). The current study pressurized samples for only 15 seconds

suggesting length of pressurization time may be as influential as pressurization liquid temperature.

Purge loss (Table 3.1) was 1.15% less ($P < 0.05$) for HPP Low samples when compared to controls. Purge loss for HPP Med was 0.78% less for treated samples. These results are odd in that purge loss % is greater for HPP Med, which has a higher pH value than HPP Low. Generally, water-holding capacity is correlated with pH; therefore, samples bind more water at higher pH. Previous studies in which ultimate pH values were comparable to the present research did not evaluate water-binding characteristics.

Subjective firmness (Table 3.1) was nearly a full score higher ($P < 0.05$) for both levels of pressurization liquid temperature. It is believed the increased water binding ability of treated samples promotes a firmer feel when touched. Macfarlane (1973) also noted pressure treated muscles had a firmer feel when pressed by the fingers. Subjective color score was not different ($P > 0.05$) for HPP Low, but was trending ($P < 0.10$) for HPP Med. Controls and HPP Med were separated by just under a third of a color score and would be acceptable in practical applications.

Objective color results are reported in Table 3.1. Pre-bloom $L*$ values were not different ($P > 0.05$) for either treatment level. Pre-bloom $a*$ values were 0.93 and 0.74 units higher ($P < 0.05$) for control samples. These results indicate that HPP treatment causes freshly exposed lean to appear less red than controls. Pre-bloom $b*$ values were 1.13 and 1.20 units less ($P < 0.05$) for HPP Low and HPP Med, respectively. These results indicate that pre-bloom meat color is bluer in color for treated samples. Post-bloom $L*$ values were not different for either treatment level when compared to controls. These results are not consistent with the findings in Souza (Chapter 2, 2009), which

determined HPP increased (appeared lighter) *Longissimus L** value. It appears that the

cooler pressurization liquid temperatures used for the present study prevented a change in

L* value. Post-bloom *a** values were 1.28 and 0.92 units less ($P < 0.05$) for treated

samples. This suggests that after allowing samples to bloom, lean color is still

objectively less red for treated samples. Zhu and Brewer (1999) determined that

consumers could detect changes to meat redness with a* change of 0.589 under

illuminant A or 0.386 under illuminant F. The *a** results agree with research conducted

in Souza (Chapter 2, 2009), which determined that HPP decreased *a** in the *Longissimus*

muscle. Based on the current findings and the results of Souza (Chapter 2, 2009),

varying pressurization liquid temperature did not prevent a decrease in *a** value. Post-

bloom *b** values for samples were 2.41 and 2.17 units less ($P < 0.05$) than controls for

HPP Low and HPP Med samples. These results suggest that after the blooming period,

the color of the lean from treated samples is bluer in color. These results agree with the

findings in Souza (Chapter 2, 2009) regarding the effect of HPP on *b** value and suggest

that *b** value of the *Longissiums* decreases even when cooler pressurization liquid

temperatures are used. Change-in-Bloom for *L** was not different ($P > 0.05$) between

treated and controls for either HPP temperature level indicating that treated samples

behaved comparably to controls during the blooming period. Change-in-Bloom for *a**

was also not different   ($P > 0.05$) when comparing both HPP treatment levels to their

controls.   Change-in-Bloom for *b** was 1.28 and 0.97 units higher ($P < 0.05$) for

controls. These results indicate that both treatment levels decrease the *b** blooming

ability. Therefore treatment effect on *b** is twofold: 1 it decreases overall *b** values; 2 it

decreases *b** blooming ability.

Previous literature concurs that HPP treatment causes an increase in L^* value, a decrease in a^* and no changes occurring to b^* values. (Carlez et al., 1993; Carlez et al., 1995). Other research has determined that decreases in meat redness are correlated to increases in metmyoglobin content (Jung et al., 2003). It is speculated that HPP alters the color of fresh meat by damaging the sarcoplasimic protein myoglobin (Carlez et al., 1995; Jung et al., 2003).

Hot Dog pH, Color, and Texture Profile

The results for hot dog color, pH and texture profile are shown in Table 3.2. Hot dog pH for HPP Low was trending ($P < 0.10$) being 0.22 pH units higher than controls. Hot dog pH was 0.17 units higher ($P < 0.05$) for HPP Med than controls. These results were expected given the high ultimate pH of the protein source. Minolta L^*, a^*, and b^* values were not different between HPP treatment levels and their respective controls. Other research using hot dogs formulated with HPP treated meat found no differences in $L^* a^* b^*$ values (Crehan et al., 2000). It appears that pressure induced denaturation of myoglobin that potentially occurred in treated samples does not prevent the formation of nitrosylhemochrome, which results in the distinctive cured meat color.

The textural parameter hardness was not different ($P > 0.05$) between HPP low and controls or HPP Med and controls. Fracturability between HPP Low and controls was not different ($P > 0.05$). Fracturability between HPP Med and controls was trending ($P = 0.12$) with treated samples requiring 3.67 kg less force to fracture the sample. Springiness was trending ($P = 0.11$) for HPP Low samples and controls. HPP Low samples were 6.9 percentage units less springy than controls. Springiness values for HPP Med and controls were not different ($P > 0.05$). Cohesiveness, chewiness and resilience

were not different ($P > 0.05$) between treated and controls for either pressurization liquid temperature level.

Previous studies investigating the effect of HPP on processed meats have found varied results. Crehan et al. (2000) found that hardness, springiness, cohesiveness, and chewiness were not different from non-pressurized hot dog samples at 150 MPa. However, when pressure increased to 300 MPa or if NaCl content was 2.5%, the values for the aforementioned texture variables decreased (Crehan et al., 2000). It should be noted that the same authors used a pressurization liquid temperature of 15-20°C, which is similar to HPP Low at 15.5°C. Research that used varying pressurization liquid temperature to cook poultry sausages during pressurization found varied results among texture parameters at different temperatures (Yuste et al., 1999). The research by Souza (Chapter 2, 2009) indicated that HPP treatment with a pressurization liquid of 33.3°C decreased the texture parameters of hardness, fracturability, springiness, chewiness, and cohesiveness of restructured hams.

Cook Loss % and Warner-Bratzler Shear Force

Cook loss % (Table 3.3) for anterior *Longissimus* chops was not different ($P > 0.05$) between HPP Low and controls or HPP Med and controls. HPP Med mid *Longissimus* chops retained 2.5% ($P < 0.05$) moisture during cooking than controls. HPP Low mid *Longissimus* chops were not different from controls ($P > 0.05$). Cook loss % for posterior *Longissimus* chops was not different for HPP Low and controls or HPP Med and controls. These results suggest that varying HPP temperature level does not alter cook loss % at different anatomical points in the *Longissimus* muscle. Differences were found for HPP Med mid *Longissimus,* but this is considered an anomaly due to the fact

that all other *Longissimus* positions at both treatment levels do not approach significance. These results vary from those regarding cook loss % in Souza (Chapter 2, 2009), which reported HPP to decrease cook loss %. Previous research is conflicted with some authors reporting HPP decreases cook loss % (Macfarlane, 1973; Kennick et al., 1980) while others have found the opposite (Jung et al., 2000). It is important to note that experiments reporting decreases in cook loss % used pressurization liquid temps greater than 30°C whereas the study reporting increased cook loss % had pressurization liquid temperature of 10°C.

Shear force values (Table 3.3) for anterior *Longissimus* chops were not different ($P > 0.05$) between HPP Low and controls or HPP Med and controls. HPP Low mid *Longissimus* chops were not different ($P > 0.05$) from controls. HPP Med mid *Longissimus* chops were different ($P < 0.05$) with treated chops requiring 0.25 kg less force than controls. Posterior *Longissimus* chops were not different ($P > 0.05$) between HPP Low and controls. Posterior *Longissimus* chops were trending ($P = 0.11$) with controls being 0.44 kg more tender than HPP Med treated chops.

The Warner-Bratzler shear force results from the present study do not reflect the HPP induced decreases in mechanical tenderness reported in Souza (Chapter 2, 2009). The results of the current study suggest decreased pressurization liquid temperature does not alter Warner-Bratzler shear force values. Most previous work has found that pre-rigor meat subjected to HPP results in decreased shear force values (Macfarlane, 1973; Kennick et al., 1980; Riffero and Holmes, 1983). More research needs to be done to investigate varying pressurization liquid temperature on shear force value as currently most work with pre-rigor meat was conducted at temperatures > 30°C. The differences in

shear force seen in Souza (Chapter 2, 2009) were attributed to pressure induced physical damage to muscle structure. This theory does not appear to be plausible for the current study. It should be noted that shear force values for all samples were well within the range of what is considered acceptable shear force values. There is also a need for research that investigates the ability of various HPP conditions to tenderize predetermined unacceptably tough muscles.

Salt Soluble Proteins

There was no HPP treatment*salt concentration interaction ($P > 0.05$). When salt concentrations were pooled, control samples yielded more ($P < 0.05$) salt soluble protein than either level of HPP treatment. HPP treatments did not differ ($P > 0.05$) from each other for pooled salt concentration level. Across all treatments, salt soluble protein yield increased ($P < 0.06$) with increasing salt concentration. When comparing 0.5 and 1.5% salt levels, salt soluble protein yield increased by 6.47%; between 1.5 and 2.5% there was a percent increase of 7.08%; and between 2.5 and 3.5% yields increased by 17%. These results suggest that HPP treatment, regardless of pressurization liquid temperature, decreases salt soluble protein yields. It was also shown by Souza (Chapter 2, 2009) that HPP treatment decreased salt soluble protein yields. It has been reported that protein solubility was increased at 0°C when compared to yields at 30°C (Macfarlane and McKenzie, 1976). More research using non-saline suspended muscle tissue at lower pressurization liquid temperatures is needed to determine if myofibrillar protein solubility can be improved by HPP treatment.

SDS PAGE

<u>*>250 kDa*</u>

Figure 3.1 refers to how SDS PAGE was analyzed for protein band densitometry. There was no interaction ($P > 0.05$) between HPP treatment and salt concentration for soluble proteins with a molecular weight greater than 250 kDa (Table 3.4). When averaged across all salt concentrations, control protein band densities were more ($P < 0.05$) dense than HPP Low.   HPP Med vs. controls were trending ($P < 0.10$) with control band being more dense. Protein band densities were not different ($P > 0.05$) between HPP low and HPP Med. Protein band density increased ($P < 0.05$) as salt concentration increased when treatments were pooled (Table 3.5). These results indicate HPP treatment regardless of pressurization temperature produced samples that had less salt soluble protein.

250 kDa

The results for protein band density of protein products with a molecular weight of 250 kDa are shown in Figure 3.2. Across pooled salt concentrations, control protein bands were denser ($P < 0.05$) than either HPP treatment level; HPP Low and HPP Med did not differ ($P > 0.05$) from each other. When treatments were pooled, band densities were different ($P < 0.05$) at all salt concentration levels. Unexplainably, protein band density decreased between 0.5 and 1.5% salt level. There was a significant ($P < 0.05$) interaction between HPP treatment and concentration level. At a salt concentration of 0.5% HPP Low band densities were not different ($P > 0.05$) from controls, while HPP Med was trending ($P < 0.10$) with control bands being denser. Control bands were denser ($P < 0.05$) than bands from either HPP treatment at a salt concentration level of 1.5%. At a salt concentration of 2.5% protein bands were not different ($P > 0.05$) between control and either HPP treatment level. Control protein bands at 3.5% salt were

denser ($P < 0.05$) than both HPP treatment levels. HPP treatment levels did not differ ($P > 0.05$) from each other at any salt concentration level.   These results indicate that HPP treatment regardless of pressurization temperature level decreases the yield of salt soluble protein products at salt concentrations of 1.5 and 3.5%.

100-150 kDa

When salt concentrations were pooled, protein product bands were less dense ($P < 0.05$) for both levels of HPP treatment when compared to controls; HPP Low and HPP Med did not differ from each other ($P > 0.05$) (Table 3.4). With treatments pooled, protein band density increased ($P < 0.05$) at salt concentrations of 0.5, 1.5, and 2.5%, but band density did not increase between 2.5 and 3.5% ($P > 0.05$) (Table 3.5). There was no HPP treatment *salt concentration interaction ($P > 0.05$). Salt soluble protein products weighing 100-150 kDa were not as soluble after being subjected to either level of HPP treatment.

100 kDa

Control protein product bands averaged across all salt concentrations were denser ($P < 0.05$) than both HPP treatment levels; HPP Low and HPP Med did not differ ($P > 0.05$) (Table 3.4).   Protein band density increased ($P < 0.05$) at salt levels 0.5 to 1.5% for all treatments, but from 1.5 to 2.5% and 2.5 to 3.5% protein band density did not change ($P > 0.05$) (Table 3.5). This indicates that protein products at a molecular weight of 100 kda were only extracted at an increasing rate until a salt inclusion level of 1.5%. There was no HPP treatment*salt concentration interaction ($P > 0.05$).

75 kDa

Band densities for control samples were denser ($P < 0.05$) across the pooled average of all salt concentration levels. The main effects of HPP Low and HPP Medium did not differ ($P > 0.05$) across pooled salt concentration levels. Between the 0.5, 1.5 and 2.5% salt level, protein band density averaged over all treatments increased ($P < 0.05$). Protein product band density decreased ($P < 0.07$) between 2.5 and 3.5% salt. There was an interaction ($P < 0.05$) between HPP treatment and salt concentration (Figure 3.3). At a salt level 0.5%, protein band density was not different ($P > 0.05$) between controls and either HPP treatment level. At 1.5, 2.5, and 3.5% salt levels, band density was higher ($P < 0.05$) for controls than it was for either HPP treatment levels. At 0.5% salt level, protein band density was not different ($P > 0.05$) between HPP Low and HPP Med. At 1.5, 2.5 and 3.5% salt level, HPP Low had denser ($P < 0.06$) protein product bands than HPP Med. These results suggest that HPP treatment caused decreased protein solubility when compared to controls.   However, HPP Low treatment resulted in more soluble protein than HPP Med suggesting that pressurization liquid temperature has an effect on protein products weighing 75 kDa.

Conclusions

HPP treatment with varying pressurization liquid temperature had positive and negative impacts on pork parameters evaluated in this study. The high pH and water holding capabilities of treated samples has attractive implications for pumping and further processing applications. Objective color suggests that treated samples will not appear as typical fresh meat. This may affect the willingness of consumers to purchase treated product. Hot dog texture profile results suggest that emulsified products can be made with pressurized pork without sacrifice to the textural profile. However, it is

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apparent that HPP treatment decreases salt soluble protein yields. The decreased level of myofibrillar protein solubility causes concern for the use of treated pork in further processing settings. The lower protein extracting ability of treated pork poses a risk when using it in applications where consistent protein bind is necessary. Warner-Bratzler shear force indicated that lower HPP pressurization liquid temperatures did not improve pork tenderness. In general, results were not consistent enough to indicate that one pressurization temperature level was more desirable than the other. This research has shown the continued ability of HPP treatment to change pork properties. More research is needed to better understand these changes and to identify which pressurization settings will promote the most ideal modifications to meat properties.

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Table 3.1 The effect of HPP Treatments on *Longissimus* pH, Purge loss %, and Color

	HPP Low				HPP Med			
	Control	Treated	SEM[a]	*P*-value	Control	Treated	SEM[a]	*P*-value
pH	5.59[a]	6.05[b]	0.03	< 0.01	5.67[a]	6.24[b]	0.03	< 0.01
Purge Loss %	1.55[a]	0.40[b]	0.10	< 0.01	1.31[a]	0.53[b]	0.12	< 0.01
Subjective Color	2.92[a]	2.75[b]	0.18	0.34	2.9	2.6	0.16	0.08
Subjective Firmness	2.33[a]	3.33[b]	0.14	< 0.01	2.30[a]	3.20[b]	0.13	< 0.01
Pre-bloom L*	49.71	50.19	1.25	0.60	47.31	47.98	0.69	0.17
Pre-bloom a*	6.20[a]	5.27[b]	0.31	< 0.01	5.61[a]	4.87[b]	0.3	0.02
Pre-bloom b*	1.52[a]	0.39[b]	0.30	< 0.01	1.07[a]	-0.13[b]	0.19	< 0.01
Post Bloom L*	49.49	49.31	1.19	0.88	47.36	47.86	0.62	0.46
Post Bloom a*	7.58[a]	6.30[b]	0.36	< 0.01	6.96[a]	5.77[b]	0.36	< 0.01
Post Bloom b*	4.39[a]	1.98[b]	0.38	< 0.01	3.36[a]	1.19[b]	0.22	< 0.01
Change in Bloom L*	-0.22	-0.88	0.57	0.39	0.05	-0.12	0.31	0.70
Change in Bloom a*	1.38	1.03	0.20	0.16	1.34	0.89	0.13	0.13
Change in Bloom b*	2.87[a]	1.59[b]	0.20	< 0.01	2.29[a]	1.32[b]	0.14	< 0.01

Means within a temperature group lacking a common superscript are different (*P* < 0.05)

[a]SEM is the standard error of the differences of the mean

Table 3.2 The effect of HPP Treatments on Hot Dog pH, Color and Texture Profile

Parameter	HPP Low				HPP Med			
	Control	**Treated**	**SEM^x**	**P value**	**Control**	**Treated**	**SEM^x**	**P value**
pH	6.23	6.45	0.03	0.0941	6.44^a	6.61^b	0.01	0.0436
L*	72.87	73.00	0.15	0.5454	74.30	74.43	0.05	0.2303
a*	11.01	10.74	0.12	0.2745	10.85	10.66	0.11	0.3323
b*	8.18	8.18	0.20	0.9551	7.93	7.75	0.06	0.1918
Hardness Kg	12.26	12.68	0.35	0.4419	16.45	14.80	3.04	0.6844
Fracturability kg	9.82	9.70	1.02	0.9256	14.85	11.18	0.67	0.1159
Springiness %	81.50	74.60	1.15	0.1059	78.90	75.20	4.45	0.5627
Cohesiveness %	22.30	22.90	0.20	0.2048	22.60	22.50	4.20	0.9848
Chewiness kg	2.24	2.18	0.01	0.1346	3.01	2.53	1.20	0.7584
Resilience %	6.70	6.70	0.00	1.0000	6.80	6.70	0.95	0.9665

[a,b]Means within treatment lacking a common superscript are different (P < 0.05)

[x]SEM is the standard error of the differences of the mean

Table 3.3 The effect of HPP on cook loss % and Warner-Bratzler shear force values at varying *Longissimus** positions

	HPP Low				HPP Med			
	Control	Trt	SEM[x]	*P*-value	Control	Trt	SEM	*P*-value
Cook Loss %								
Anterior	21.47	20.22	1.98	0.5372	21.14	20.56	1.46	0.6827
Mid	21.49	21.06	0.86	0.6272	23.21[a]	20.71[b]	0.86	0.0171
Posterior	21.44	21.32	0.80	0.1462	19.97	20.75	1.24	0.5443
WBSF kg								
Anterior	2.27	2.21	0.29	0.8311	2.27	2.55	0.20	0.1933
Mid	2.45	2.28	0.13	0.1939	2.50[a]	2.25[b]	0.09	0.0181
Posterior	2.46	2.80	0.28	0.2424	2.34	2.78	0.25	0.1089

[a,b] Means within treatment lacking a common superscript are different (*P*<0.05)

*Longissimus chops aged 14 days and cooked to an internal temperature of 71°C

[x] SEM represents the standard error of the difference of the means

Table 3.4 The main effects of treatment for salt soluble proteins & protein band density

	Control	HPP Low	HPP Med	SEM
SSP Yield (% wet tissue)	5.45^a	4.91^b	4.77^b	0.15
Protein Band Density				
>250 kDa*	0.75^a	0.3175^{ab}	0.4841^b	0.12
100-150 kDa	0.22^a	0.11^b	0.12^b	0.19
100 kDa	0.97^a	0.13^b	0.13^b	0.48

^{a,b} Means lacking a common superscript are different ($P<0.05$)
* Molecular weight standards were used as point of reference point when analyzing bands

Table 3.5 The main effects of salt concentration for salt soluble proteins and protein band density

	% Salt Concentration				
	0.5	**1.5**	**2.5**	**3.5**	**SEM**
SSP Yield	4.45^c	4.73^c	5.07^b	5.93^a	0.12
Protein Band Density					
>250 kDa[*]	0.18^d	0.40^c	0.62^b	0.87^a	0.07
100-150 kDa	0.05^c	0.10^b	0.21^a	0.24^a	0.10
100 kDa	0.36^b	0.42^a	0.45^a	0.42^a	0.03

[a,b,c,d] Means lacking a common superscript are different ($P<0.05$)
[*] Molecular weight standards were used as point of reference point when analyzing bands

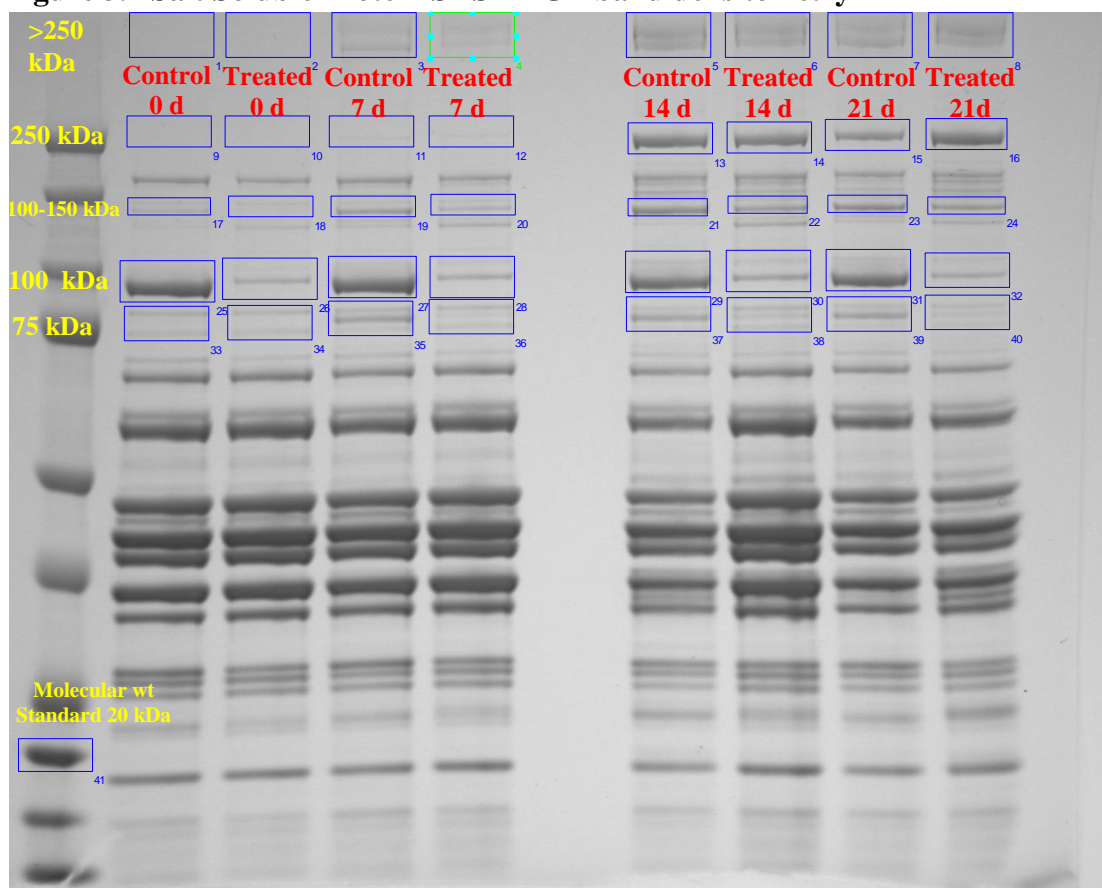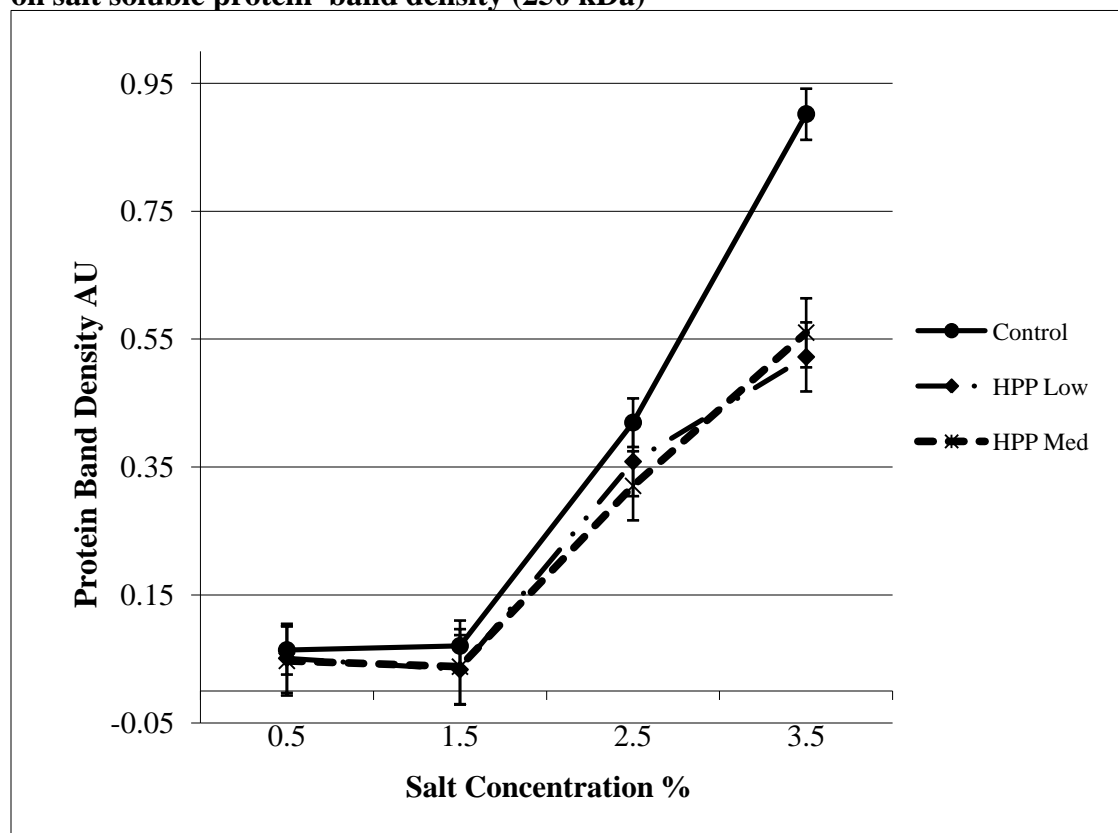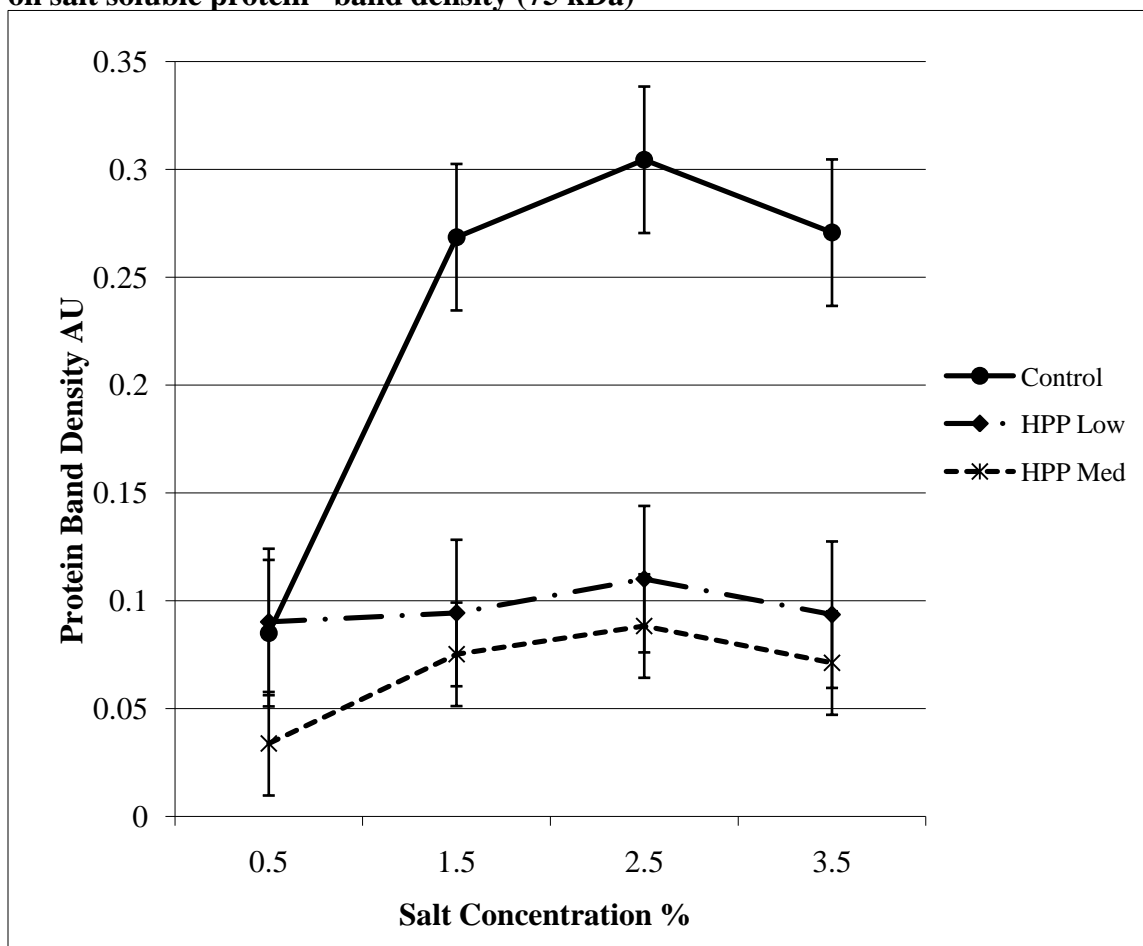**Figure 3.1 Salt Soluble Protein SDS PAGE band densitometry**

Figure 3.2 The effect HPP treatment with varying pressurization liquid temperature on salt soluble protein[*] band density (250 kDa)

[*]Analysis was conducted on *Triceps brachii* muscles

Figure 3.3 The effect HPP treatment with varying pressurization liquid temperature on salt soluble protein* band density (75 kDa)

*Analysis was conducted on *Triceps brachii* muscles