EVALUATION OF SOLUBILIZED PROTEINS AS AN ALTERNATIVE TO PHOSPHATES FOR MEAT ENHANCEMENT

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FORMAT OF THESIS

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Chapter I

INTRODUCTION

A decline in profit is a problem that has been confronting the beef industry over the last several years (Jeremiah and Gibson 2003). Total beef consumption on a carcass basis, from year 2000 to 2004, has declined by 3.63 kg, whereas poultry consumption has increased by 1.36 kg (USDA 2005). One of the reasons for this decline is the lack of a consistent tender product. Surveys have found that a lack of consistency in tenderness is a major concern for most consumers (Jeremiah 1982; Jeremiah and others 1992; Jeremiah and others 1993). It is projected that inconsistencies in tenderness cost the beef industry $250 million annually (Smith and others 1995). The National Cattlemen's Beef Association set forth a goal of reducing consumer dissatisfaction by 50% by the year 2005 (Tatum and others 1999). It has been reported that 78% of consumers surveyed were willing to pay more for a guaranteed tender product (Miller and others 2001). Neeley and others (1999) in the National Beef Tenderness Survey, went as far to say that tenderness was the primary determinant of a good eating experience.

For the beef industry to maintain the current level of customer acceptance, commercially acceptable or applicable methods must be developed and consistently used to ensure that maximum tenderness of cooked beef is achieved (McMichael 2005). Currently there are numerous applications to enhance tenderness. One of the most common is the use of a phosphate and sodium chloride based enhancement solution. It

is typical to find up to 12% brine solution in meat products that are made up of phosphates, sodium chloride, water, and other functional ingredients (Robbins and others 2002). Vote and others (2000) found that through the incorporation of an enhancement solution containing phosphate, sodium chloride, and an antioxidant all palatability attributes such as tenderness, flavor, and juiciness were improved. By adding phosphates the pH moves toward neutrality, allowing for more charge repulsion and better binding ability of the protein (Trout and Schmidt 1984). However phosphate usage can have its draw backs as it can produce an astringent metallic flavor (Trout and Schmidt 1987) or react with fat during the cooking process to produce a soapy flavor (Dikeman and others 2003). Sodium chloride is added in the solution to help with water binding, inhibit microorganism growth, and to provide flavor (Trout and Schmidt 1987). Sodium chloride has numerous health concerns as it has been linked to hypertension and heart failure (Varagic and Frohlich 2005). With those draw back there is constantly a search to find the next application that will produce a consistent product for the consumer without the unwanted drawbacks.

It is hypothesized that the use of solublized proteins can provide an alternative to the current enhancement applications on the market today. A protein rich solution containing solubilized myofibrillar proteins can be isolated from animal muscle using dilute alkali (pH 10) or acid conditions (pH 3). Solubilized proteins are currently being investigated in the poultry and fish industry for their ability to be used in processed meats to enhance bind and in whole muscle products to increase “juiciness”. However no research has been conducted in the beef industry on the effect of using solubilized protein for an enhancement solution to improve juiciness.

The objective of this study was to determine if a solubilized protein enhancement solution can provide the same water holding ability, tenderness characteristics, and sensory attributes as the current phosphate based enhancement solutions currently utilized on beef subprimals.

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Chapter II

REVIEW OF LITERATURE

Beef Industry

In 2004 there were 94.9 million head of cattle in the United States (http://www.ers.usda.gov/news/BSECoverage.htm). The USDA in 2005 reported that 45.7 grams of beef was consumed per person each day (http://www.nass.usda.gov:8080/QuickStats/cattleconsumption.) Beef makes up 33.5% of the total meat consumed in the United States (Aberle and others 2001).

Romans and others (2001) reported the average dressing percentage of beef to be around 60% on a live basis and 64% on a retail weight basis. Beef carcasses are typically split into two halves for ease of fabrication into retail cuts. The carcass consists of two main areas the forequarter representing 52% of the carcass weight and includes the chuck, rib, plate, brisket, and shank; the hindquarters representing 48% of the carcass and includes the round, sirloin, short loin, flank and rump (Romans and others 2001). The beef carcass can consist of numerous different retail cuts (Kinsman and others 1976). The percent carcass weights for steaks and roasts is around 39%, 25% lean trim, 24% fat trim, and 12% bone for the whole carcass (Romans and others 2001).

Beef Strip Loins

The beef strip loin subprimal weighs approximately 7.26 kg with exterior fat covering and approximately 5.90 kg when trimmed of exterior fat covering. Beef strip

loins have an Institutional Meat Purchase Specifications (IMPS) number from 173-180 depending on how the subprimal is fabricated. "The strip loin is the anterior portion of the loin that is separated from the sirloin by a straight cut, perpendicular to the split surface of the lumbar vertebrae, through a point immediately behind the hip bone, leaving no point of the hip bone in the short loin. The flank shall be removed which is not more than 6 inches from the outer tip of the loin eye muscle through a point on the sirloin end which is not more than 4 inches from the outer tip of the loin eye muscle" (Meat Buyers Guide 1990). The basic composition of a raw beef strip loin separable lean only, trimmed to 0.635 cm fat is:

Table 1. Composition of Raw Beef Select Strip Loin[1,2]

Moisture	Fat	Ash	Protein	Carbohydrate[3]
67.39%	10.73%	0.89%	21.53%	0.00%

[1]Obtained from USDA National Nutrient Database for Standard Reference
[2]Values based per 100 g edible portion.
[3]Carbohydrate determined by difference.

Typically strip loins are grilled or broiled as steaks. Strip loins are most commonly called New York or Kansas City Strips in retail stores and restaurants. In May 2006 beef strip loins brought $4.20/ lb average for select grade subprimals according to USDA Market News (http://www.ams.usda.gov/mnreports/lm_xb452.txt).

Prevalence of Tough Meat in the Industry Today

Belew and others (2003) reported a wide range of tenderness variations among individual beef muscles. The beef carcass as a whole is made up of multiple muscles that differ in physical composition, amount of connective tissue, marbling, and sarcomere

length. Stuby-Souva and others (1994) reported that tenderness differences can occur among carcasses, muscles from the same carcass, and within individual muscles from different carcasses. It has been reported that 15-20% of the steaks sold to consumers are considered to be tough (Miller and others 2001) and 13% of strip loins sold to consumers are considered tough (George and others 1999, Miller and others 2001). Tenderness of beef carcasses can be improved through the incorporation of postmortem treatments or interventions (McMichael 2005).

Tenderness Treatments

Today's industry employs different postmortem treatments to enhance tenderness: including blade tenderization, electrical stimulation, postmortem aging, and phosphate enhancement. This is by no means a comprehensive list of tenderness treatments but a list of ones that are the most prevalent in the industry today.

Mechanical Tenderization

Mechanical tenderization is one of the most commonly applied tenderness treatments in the beef industry with over 50% of food service establishments surveyed in the 1998 National Beef Tenderness Survey reporting some form of mechanical tenderization (Brooks and others 2000). Mechanical tenderization is often referred to as blade tenderization or needling (Davis and others 1977; Glover and others 1977). Mechanical tenderization is commonly used to disrupt the muscle structure, disintegrate external surfaces of meat pieces and to release myofibrillar proteins. Increased tissue disruption through tenderization and tumbling allows increased protein extractability, which results in greater solubilization of muscle proteins and thus may lead to an increase in the cook yield of the products (Motycka and Bechtel 1983; Xargayo and Lagares

1992). Mechanical tenderization involves the penetration of the meat with closely spaced thin blades with sharpen edges, which cut the muscle fibers into shorter segments. Many authors have shown that mechanical tenderization significantly improves the tenderness of less tender cuts of meat and is one of the most effective and efficient technologies currently used to assure tenderness (Mandigo and Olson 1982; Lyon and others 1983; Loucks and others 1984; Flores and others 1986; Jeremiah and others 1999). Jeremiah and others (1999) found that overall tenderness in strip loins were increased by over 15% with the utilization of blade tenderization.

Electrical Stimulation

Electrical stimulation is a tenderness technique that applies electrical current to the carcass shortly before or after harvest by means of an electrical stunner at either low voltage (less 100 volts) or high voltage (greater than 100 volts) for a period typically not longer than 30 seconds (Li 2006). It was found that, electrical stimulation could accelerate post-mortem tenderization (Savell and others 1981) by enhancing the rate of proteolysis stimulated by the release of Ca^{2+} at a higher temperature and also by physically disrupting structure of the muscle fiber (Hwang and others 2003). Troy (1999) reported that the critical time to improve tenderness is before the muscles goes into rigor mortis, or muscle stiffening, that typically occurs 2-4 hours after death. Electrical stimulation accelerates the decline of muscle pH to a level that is critical for the development of cold shortening (Smulders and others 1986). Applying electrical stimulation to the whole carcass does not affect all muscles equally (Olsson and others 1994; Troy 1999).

Postmortem Aging

Postmortem aging is an effective way of enhancing tenderness (Dransfield 1994). It assists in the improvement in palatability that occurs in the muscle by manipulating myofibrillar proteins to increase tenderness (Nishimura and others 1998). There are two different types of postmortem aging, wet or dry aging. Wet aging involves placing individual steaks in a vacuum bag at refrigeration temperature for a period of time. Dry aging exposes the entire carcass or subprimal to air. Miller and others (1997) reported the optimum length to maximize tenderness is 10-14 days. Koohmaraie (1996) reported that for postmortem aging to be effective calpains must actively break down sarcomeres and the myofibrillar structure of the meat. The most effective calpain for postmortem aging is μ-calpain which is activated by calcium ions during the onset of rigor mortis (Miller 1997).

Phosphate Enhancement

Another common tenderness treatment is the use of a phosphate based solution to tenderize the product. It is typical to find up to 12% brine solution in meat products that are made up of phosphates, sodium chloride, water, and other functional ingredients (Robbins and others 2002). This practice is common place in the pork and poultry industry while the beef industry is starting to utilize it to tenderize different cuts of meat. Phosphate enhancing is an acceptable process that often requires minimal processing to achieve the desired increase in tenderness (McMichael 2005). The meat is not only tenderized by solubilization of the protein as a result of injection of the enhancement solution, but the needle itself may act as a mechanical tenderization method to improve tenderness (Xiong 2005). Vote and others (2000) found that through the incorporation of

a solution containing phosphates, sodium chloride, and an antioxidant all palatability attributes such as tenderness, juiciness, and flavor were improved.

The major phosphates utilized in enhancement solutions are: disodium phosphate (DSP), monosodium phosphate (MSP), potassium polymetaphosphate (KMP), sodium hexametaphosphate (HTPP), sodium tripolyphosphate (STPP), and tetrasodium pyrophosphate (TSPP); with HTPP and STPP being the most commonly used forms of phosphates (Cassidy 1977).

Role of Phosphates in Enhancement Solutions

The function of phosphates in myofibrillar solubilization is similar to that of sodium chloride. Phosphates, like sodium chloride, also function to shield charges and open up the protein structure. Unlike sodium chloride, however, phosphates will increase the pH away from the isoelectric point (pH 5.5) to a more neutral pH of 6-6.4. In red meat the final pH that is developed as a result of glycolysis is very close to the isoelectric point of myofibrillar protein, the pH where the protein structure is most "closed" because of lack of charge repulsion. By adding phosphates the pH moves toward neutrality, allowing for more charge repulsion and better binding ability of the protein (Trout and Schmidt 1984). In addition to improved binding ability (Cheng and Ockerman 2003), tenderness is improved more with phosphates than with the use of calcium chloride, sodium chloride, sodium lactate, or water only enhancement solutions (Smith and others 1984; Boles and Swan 1997; Cheng and Ockerman 2003). Reduced oxidation as measured by sensory evaluation of warmed-over flavors (Smith and others 1984) and thiobarbituric reactive substances (Cheng and Ockerman 2003) have also been demonstrated using polyphosphates. Phosphate usage has its drawbacks. From the

highest permitted level, 0.5%, to about 0.3%, phosphates can produce an astringent metallic flavor (Trout and Schmidt 1987). Phosphates can also react with fat during the cooking process to produce a soapy flavor (Dikeman and others 2003).

Health Concerns with Use of Phosphates

The addition of phosphates and sodium chloride in injection brines is the traditional means of enhancing water holding capacity to improve juiciness and solubilize proteins to improve tenderness. However, some people are allergic to increased levels of phosphates in their diet, thus making them unable to consume large quantities of any product that has phosphates in it (Hafer 1998). Since most processed meats are enhanced with a phosphate brine solution it makes it almost impossible for them to consume any meat that has been processed with this solution. Moreover, another adverse reaction to too many phosphates in the diet is abnormally elevated levels of phosphates in the blood stream (Dikeman and others 2003). This comes from too many phosphates coming into the blood stream and not enough of it being used by the body thus elevating the amount of phosphates in the blood stream to dangerous levels. This reaction normally affects organs in the body such as the kidneys and can lead to organ damage or in some cases even organ failure (Dikeman and others 2003). One other problem with too many phosphates in the diet is that it can lead to diarrhea and sometimes hardening of soft tissues and organs (Berner and Shike 1988). High dietary phosphate intake also reduces calcium adsorption, which can lead to osteoporosis in postmenopausal women and the development of brittle bones in renal patients (Anderson 1996). In addition, it can interfere with the body's ability to use iron, calcium, magnesium, and zinc. Finally, it has been noted that increased amounts of phosphates in the diet has a correlation with

Attention Deficit Disorder (ADD) in children, the more phosphates the children with attention deficit disorder consume the greater the incidences of behaviors associated with ADD (Hafer 1998).

Role of Sodium chloride in Enhancement Solutions

Sodium chloride, a generally regarded as safe substance (GRAS), is often added to meat to (1) increase functional properties such as the improvement of water binding ability, (2) reduce microbial growth, and (3) enhance the meaty flavor of meat products (Trout and Schmidt, 1987). It acts to improve functional properties by partially solubilizing myofibrillar proteins. It has been hypothesized that solubilization by sodium chloride occurs because the chloride molecule selectively shields positive charges on the protein molecule causing the isoelectric point to shift to a lower pH than the normal meat muscle after rigor mortis pH of 5.5. This shift allows the meat protein to become partially solubilized (Foegeding and others 1996). Charge repulsion subsequently opens the protein structure allowing it to bind more water. The ability of sodium chloride to induce water binding to the protein from the meat has been demonstrated through increased cook yields and sensory perception of juiciness (Trout and Schmidt 1984; Boles and Swann 1997).

Health Concerns with Use of Sodium chloride

There are, however, some detriments to sodium chloride usage. For consumers susceptible to hypertension, (Dahl 1972; Fries 1976; Law and others 1991a; Law and others 1991b) products with added sodium chloride have limited appeal, thus eliminating a portion of people that consume meat products on a daily basis. Another drawback to using sodium chloride as a tenderizer is that it is linked to increase rates of heart attacks

and strokes (Tuomilehto and others 2001). Therefore, families that have histories of those conditions must also watch their intake of meat enhanced with a sodium chloride solution. It has been reported that the consumption of sodium chloride is more than 6 g/day/person in the United States (WHO 1990). The WHO (1990) recommends the daily intake of sodium chloride to be between 1 and 3 g/day/person. So with all of the health related drawbacks it is easy to see why the meat industry, especially the beef industry, is always looking for a new way to make a product tender by not having the unwanted adverse health conditions while also reducing the cost of enhancement solution.

Acid Solubilization Isoelectric Precipitation

Hultin and Kelleher (1999) stated there was an expanding use of muscle proteins as food because of their functional and nutritional properties. This, in turn, created a need for new ways to obtain protein for human consumption. A new way to obtain protein was achieved by a patent to Hultin and Kelleher in 1999. The patent claimed that a protein rich solution containing myofibrillar proteins and free of membrane lipids can be isolated from animal muscle. Hultin and Kelleher (1999) found that the protein is solubilized below pH 3.5 with the optimal pH range being between 2.5 and 3.5. Muscle tissue is disrupted, by means of grinding or homogenization with water, to solubilize the myofibrillar protein. Protein is separated from the insoluble materials such as fat, connective tissue, collagens, and bones by centrifugation (Hultin and Kelleher 1999). To remove membrane lipids it was suggested that the ratio of meat to aqueous solution be greater than 7:1 preferably around 9:1 and the pH of the solution be adjusted to between 2.5 and 3.5. By utilizing these parameters the protein component of the tissue is dissolved in the aqueous liquid while avoiding gelation. If pH inadvertently drops below

1.0 protein can de denatured and render useless for subsequent gel formation. Kelleher and Hultin (2000) report that during centrifugation three distinct phases are obtained: an upper layer consisting of neutral lipids such as triacylglycerols, a membrane sediment (lower) layer consisting of membrane lipids such as phospholipids, connective tissue, bones, cartilage, collagen, and a middle aqueous layer which contains the solubilized myofibrillar proteins. Centrifugation was conducted at 10,000 x g to help remove the lipids and phospholipids from the solution in order to improve stability of the product (Hultin and Kelleher 1999). It was reported that the solubilization process obtained about 90% of the total myofibrillar protein from muscle tissues. It was found that a second centrifugation after the pH is adjusted to 5.5, the isoelectric point of meat, is required to recover the protein pellet. Kelleher and Hultin (2000) found that only small amounts of protein were found in the supernatant or aqueous phase after the second centrifuge.

Kelleher and Hultin (2000) also studied the effect of acid solubilization on different types of chicken muscles. It was found that protein solubility for chicken breast can be as high as 92.6%. Leg muscle, dark meat, was found to have a lower solubility of 71.1%. The extent of solubilization will determine the amount of protein recovered suggesting that breast meat (white meat) can be solublized better then dark meat of the leg. It was found that breast meat also responded better in all other tests conducted such as yield and functionality than leg and thigh meat.

Stefansson and Hultin (1994) stated that protein solubility was dependent upon pH and sodium chloride concentration. Electrostatic repulsion between cations resulted in increase protein solubility levels. Feng (2000) found that sodium chloride addition increases myosin electronegativity, resulting in a shift of the isoelectric point to a lower

value. The higher the amount of sodium chloride added the lower the amount of solubility suggesting that the lower sodium chloride concentrations will have greater protein solubility. Kelleher and Hultin (1999) found that it was a result of sodium chloride shielding the repulsive forces of the proteins, thus making them less soluble. Using gel electrophoresis, Mireles DeWitt and others (2002) confirmed that protein solubilization decreased with increased sodium chloride addition. In addition, SDS-PAGE confirmed myosin solubilization in red meat was the most effective below a pH of 5 with reduced solubility below 2.5.

Composition of Acid Solubilized Protein

The composition of protein recovered from acid solubilization processes was reported to have significantly lower amounts of fat, ash, cholesterol, and collagen than nonsolublized protein. Fat in beef hearts was reduced from 1.69% ± 0.31 in control to 0.15 % ± 0.16. Ash was reduced from 1.04 % ± 0.04 to 0.25% ± 0.03. Cholesterol was reduced from 123.7 mg/100g ± 12.2 to 37.1 mg/100g ± 3.5. Collagen was reduced from 2.21 mg/g ± 0.33 to 0.21 mg/g ± 0.01 (Mireles Dewitt and others 2002). James and Mireles Dewitt (2004) also reported that protein recovery was higher from an acid solubilization than from a traditional surimi process. It was also found that fat content was lower in acid solubilized protein than surimi extracted protein. Collagen was approximately four times as high in the surimi, and cholesterol was double in surimi when compared to acid solubilized protein (James and Mireles Dewitt 2004).

The cooked composition of acid solubilization manufactured products from beef hearts also had significantly less fat and collagen then surimi manufactured products from beef hearts (James and Mireles Dewitt 2004).

Current and Future Research Areas

Because myofibrillar proteins are solubilized by sodium chloride, they are typically regarded as "salt soluble" proteins as opposed to "water soluble" proteins. However, recent studies have demonstrated that myofibrillar proteins can be solubilized without the aid of sodium chloride by using a low or high pH and a low ionic strength solution (Stanley and others 1984; Hennigar and others 1989; Vareltzis and others 1989; Stefansson and Hultin 1994; Krishnamurthy 1996; Feng and Hultin 1997; Mireles DeWitt and others 2002). It was reported that under very low fat conditions acid solubilized protein produced a protein gel with excellent cook yield, high water holding ability, and much improved gel texture characteristics (James and Mireles Dewitt 2004). Protein gels without the aid of sodium chloride preformed as well or better then gels made with the addition of sodium chloride (Kristinsson and Hulton 2003; James and Mireles Dewitt 2004,), thus suggesting that sodium chloride is not necessarily needed to get a good protein gel interaction.

The enhanced functionality of solubilized proteins is attributed to the changes in myofibrillar protein (especially myosin). During solubilization myosin unfolds and only partially re-folds when recovered by isoelectric precipitation. The result is a pre-solubilized protein that is much more functional with respect to binding of water and proteins. Solubilized proteins are currently being investigated in the poultry and fish industry for their ability to be used in processed meats to enhance bind and in whole muscle products to increase "juiciness". Work to date has focused on injecting same source solubilized proteins into chicken breasts and fish filets. In both cases, injected proteins were able to increase the water-holding capacity and juiciness of the injected

meat product. Validation of this research has yet to be published but it is expected to be published soon. Once the research is published it can be utilized commercially in both the poultry and fish industry.

Currently there is no research on beef subprimals utilizing acid solubilized protein for an enhancement solution. The purpose of this study is to determine if a solubilized protein based enhancement solution can provide the same water holding ability, tenderness characteristics, and sensory attributes as the current phosphate base enhancement solution on beef subprimals.

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CHAPTER III

EVALUATION OF SOLUBILIZED PROTEINS AS AN ALTERNATIVE TO PHOSPHATES FOR MEAT ENHANCEMENT

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ABSTRACT

Select grade strip loins (n=10) were enhanced 10% with either a target phosphate based or an acid solublized protein based solution. Color score, aerobic plate count, lipid oxidation, purge loss, cook yield, Warner-Bratzler shear force, and sensory analysis were measured to characterize storage quality. The phosphate based solution consisted of 4.5% phosphate, 3.6% sodium chloride, 90.9% water and 1% Herbalox seasoning. The protein based solution was prepared in two solutions: one consisting of 1:9 protein to water ratio and the other an aqueous solution of 1% Herbalox seasoning and 3.6% sodium chloride. Enhancement solutions were injected twice at 5% to create a 10% total injection. It was found that the protein enhanced steaks out performed the phosphate enhanced steaks for percent discoloration and overall acceptability. The phosphate enhanced steaks performed better than the protein enhanced steaks for lean color, fat color, aerobic plate count, lipid oxidation, percent purge, cook yield, and Warner Bratzler shear force. It should be noted that for protein enhanced steaks lean color and cook yield although significantly different did perform similar to phosphate enhanced steaks.

INTRODUCTION

Total beef consumption on a carcass basis has been declining for several years. From 2000 to 2004 beef demand declined by over 3.5 kg/ person (USDA 2005). An important factor in this decline in consumption is the industry's inability to consistently produce a tender product. Several authors have reported a wide range of tenderness variation between carcasses, individual muscles from the same carcasses, and within individual muscles from different carcasses (Stuby-Souva and others 1994; Miller and others 2001; Belew and others 2003). Miller and others (2001) reported that 15-20% of all steaks sold to consumers are considered to be "tough" and 13% of strip loins are considered tough by consumers (George and others 1999).

Today's beef industry employs several postmortem treatments to help manage the amount of tough products sold to consumers. Some of the most common tenderness treatments are mechanical tenderization, electrical stimulation, postmortem aging and phosphate enhancement. Mechanical tenderization is commonly used to disrupt muscle structure, disintegrate external meat surface, and increase solubilization of muscle proteins to improve tenderness (Motycka and Bechtel 1983; Xargayo and Lagares 1992). Electrical stimulation helps to tenderize the product by enhancing the rate of proteolysis and by physically disrupting the muscle fibers (Savell and others 1981; Troy 1999; Hwang and others 2003). Postmortem aging assists in the improvement of palatability by manipulating myofibrillar proteins by degradation of intercellular membranes that release calcium to activate calpains to increase tenderness (Koohmaraie 1996; Miller 1997, Nishimura and others 1998). For phosphate enhancement the meat is tenderized by partial solubilization of the proteins (Vote and others 2000; Xiong 2005). All current

postmortem treatments have drawbacks such as health or cost effects, so there is always a search to find a new tenderness treatment.

It is hypothesized that the use of solublized proteins can provide an alternative to current enhancement solutions on the market today. A protein solution containing solublized myofibrillar proteins can be isolated from muscle using dilute alkali (pH 10) or acid conditions (pH 3). The solubilized proteins retain their functionality and can still be recovered and utilized to form gels (Hultin and Kelleher 1999). Solubilized proteins are currently being investigated in the poultry and fish industry for their ability to be used in processed meats to enhance binding and in whole muscle products to increase "juiciness". However no research has been conducted on the effect of using solubilized protein for an enhancement solution to improve juiciness and tenderness of beef.
The objective of this study was to compare physical and chemical attributes of the solubilized protein enhancement solution to a phosphate based enhancement solutions currently utilized on beef subprimals.

MATERIAL AND METHODS

Sample Collection

Paired beef strip loins (n=10 pairs; IMP #180) were individually identified and obtained from randomly selected USDA Select quality grade carcasses aged for two days at a beef fabrication facility in Dodge City, KS, and transported to the Food and Agricultural Products Center at Oklahoma State University after carcass disassembly. At the meat packing plant subprimals from each side of the carcass were tagged with duplicate numbers containing an L or R (1L, 1R) to identify from which side of the carcass the subprimal was obtained. Subprimals were then vacuumed packaged (Cyrovac

B-2620 bag, Duncan, SC; water vapor transmission rate 0.5-0.6 gm and oxygen transmission 3-6cc m^2) and put into boxes and transported to the Food and Agriculture Product Center at Oklahoma State University in a refrigerated vehicle. Subprimals where then stored overnight at 4°C until further analysis could be conducted.

Sample Enhancement

The next day the initial (green) weight of each subprimal was recorded (Mettler Toledo Model SW Mettler Toledo Co. Columbus, OH). The left or right subprimal from each pair was randomly selected to be injected with either the control solution (phosphate) or the treatment solution (solubilized protein). Enhancement was conducted at 4ºC using a stitch pump enhancer consisting of 20 single needles with an interior bore size of 25 mm run at 45 strokes per minute at 2 PSI (Formaca Reiser Model FGM 2020S Food Machine Co. Millbrae, CA) calibrated to inject 105% of the recorded green weight. Calibration was performed each time enhancement solution was changed. The subprimals were loaded onto a conveyor belt with the single stitch needles going into the top of the subprimal to deliver the enhancement solution needles penetrated to 2 inches from the bottom of the subprimal.

Solubilized Protein Solution

One subprimal was randomly selected from each pair (n=10) for solubilized protein enhancement. An aqueous solution containing, 1% Herbalox seasoning Type HT-W (Kalsec Kalamazoo, MI) and 3.6% sodium chloride (w/w) was injected at 5% into the subprimals. A second injection was made using solubilized protein prepared according to James and Mireles DeWitt (2005). Briefly, beef trim was obtained from carcass fabrication and adipose tissue was removed to obtain a mostly lean sample. Lean

beef trim was then placed in a commercial bowl copper (Seydelman Model #80075-1 Stuttgart, German) with one part water and chopped for two minutes until a homogenous mixture was obtained at 4°C. The slurry was further diluted with additional 8 parts water. Final ratio of beef to water was 1:9. The pH of the diluted slurry was then lowered to 2.5 using 50% food grade phosphoric acid (Fisher, Fair Lawn, NJ). The slurry was then passed though cheese cloth (Vertec Folded 4 PLY Simpsonville, SC) to remove large fat and connective tissue particles. The protein solution was then injected into the subprimals at 5% for a final product enhancement at 110% green weight. Separate injections were conducted to prevent loss of solubilized proteins by sodium chloride. Previous research has demonstrated (Kelleher and Hultin 2000) that the myofibrillar fraction of muscle foods can be solubilized using low or high pH conditions only in very dilute ionic conditions.

Phosphate Solution

One subprimal randomly selected from each pair (n=10) was enhanced at 110% of its green weight with a phosphate solution (control group). The phosphate based solution consisted of 4.5% phosphate, 3.6% sodium chloride, 90.9% water and 1% Herbalox Seasoning Type HT-W. Two injections at 5% were conducted to mimic the double injection utilized with the solubilized protein treated samples.

Fabrication of Subprimals into Steaks

Treated subprimals were fabricated thirty minutes after injection at the Food and Agriculture Products Center into 2.54 cm thick steaks using a standard band-saw (Biro Model 3334 The Biro MFG. Co. Marblehead, OH). Steaks were pre-weighed and individually placed into plastic trays (Cyrovac CS977 Duncan, SC) with absorbent pads

(Cryovac DRI-LOC AC 50 Duncan, SC). Steaks were then packaged in modified atmosphere at 80% O_2 and 20% CO_2 with a seal time of 0.80 second, vacuum time of 1.65 seconds, compression time of 1.00 second, gas time of 1.75 seconds at 85 PSI with a flow rate of 24NL/cycle at 6 atm, lid bobbin time of 0.10 seconds, lid unload time of 3.00 seconds and a time-out/piston of 4.50 seconds (G. Mondini CV/VGS Brescia, Italy). Steaks were sealed with oxygen barrier film (Cryovac Lid 1050/550 Lidstock Duncan, SC) with less than 20.0 cc m^2, at 40°F at 100% relative humidity, oxygen transmission rate and a moisture vapor transmission rate of 0.10 grams for 24 hours, 100in^2 at 40°F, 100% relative humidity consisting of 1.0 mils nominal total gauge. Steaks were placed in boxes and put in a dark room at 4°C to simulate transportation to retail stores for four days. Steaks were then transferred to a retail case at 4°C with cool white fluorescent lights (1600-1900 lux) for the remainder of the study day 5-11.

Day 5-11 Sampling

Once in the retail display case, steaks were color scored each day (8 AM and 5 PM) until day 10. Two steaks were randomly selected from each treatment on days 5, 7, 9, and 11. One steak was used to measure retail case purge, cook yield, and shear force analysis. The other steak was used for sensory analysis. On days 5 and 9 a third steak was randomly selected for aerobic plate count and 2-thiobarbaturic acid reactive substances (TBARS) analysis.

Proximate Analysis (AOAC 2000)

Three raw and three cooked steaks were randomly selected from each treatment on days 5, 7, and 9. Moisture analysis was conducted in accordance with method number 950.46. Fat analysis was conducted in accordance with method number 960.39. Ash

analysis was conducted in accordance with method number 920.153. Protein analysis was conducted in accordance with method number 992.15.

Color Score

Steaks were color scored (days 5-10) by a trained panel (n= 5) according to the Guidelines for Meat Color Evaluation (AMSA 1991) using a scale of 1-7. Lean color was measured using 7 = bright cherry red to 1 = extremely dark brown color. Fat color was measured on the scale of 7 = creamy white to 1 = dark brown or green. Percent discoloration was measured as 7 = no discoloration to 1 = complete discoloration. Overall appearance was measured as 7 = extremely desirable to 1 = extremely undesirable. Each day results for lean color, fat color, percent discoloration, and overall appearance were averaged for the five panelists to create a mean value for each time period.

Aerobic Plate Count

Aerobic plate count (APC) was conducted by Food Protech Inc. (Stillwater, OK) using standard aerobic plate count petrifilm (3M St. Paul, MN).

Lipid Oxidation

On days 5 and 9 samples were removed from the retail display case, packaged in whirl-pak bags and frozen at -20°C until further analyzed. Analyses were performed using procedures described by Buege and Aust (1978) with the following modifications: Since lipid oxidation is a surface phenomenon a 10 g sample was obtained from the surface of the steak (approximately 10 mm thick) and homogenized with 30 mL deionized water in a Waring Commercial Blender for approximately 30 secs (Model 51BL32 Turrington, CN). Homogenates were centrifuged at 1850 x g for 10 minutes at

4°C (Beckman Model J-6M Houston, TX). Two mLs of homogenate were added to TBA reagent (Fsiher, Fair Lawn, NJ) and heated in a boiling water bath for 15 minutes. After cooling samples were centrifuged at 1850 x g for 10 minutes at 25°C. Absorbance of the supernatant was read at 531 nm using a spectrophotometer (Beckman Model DU 7500). Results were reported as Thiobarbituric acid reactive substances (TBARs) representing mg malondialdehyde (MDA) equivalents per kg of fresh meat.

Purge Analysis

Purge analysis was conducted by measuring the amount of moisture lost during the storage of the steak. The weight of the steak after it was removed from the package in the retail case was subtracted from the initial weight of the steak at day zero of the study and then divided by the initial weight of the steak and multiplied by 100 to get the percent purge of the steak. Analysis was conducted on Days 5, 7, 9, and 11.

Cook Yield

Cook yield was measured by subtracting the weight after cooking from weight before cooking and then dividing by the weight before cooking. That number was then be multiplied by 100 to get a percent cook yield of each steak. Analysis was conducted on days 5, 7, 9, and 11.

Shear Force

Shear force was measured using a Universal Instron Testing machine with a Warner-Bratzler shear head attachment (Model 4502 Canton, MS). Shear force analysis was conducted on steaks that were cooked to an internal temperature of 70ºC (medium degree of doneness) using an impingement oven (Lincoln Model 1022 Lincoln Food Service Products Inc. Fort Wayne, IN). Steaks were allowed to cool to room temperature

for 1 hour before coring. Six cores were removed parallel to the muscle fiber orientation with a 3D Black and Decker Drill (Townson, MD) modified to have a 1.27 cm diameter bore size with a maximum RPM of 2000. A crosshead speed between 200-250 mm/min was used during analysis. Individual shear force values for each steak were averaged and the mean was used in statistical analysis (Wheeler 1997). The analyses were conducted on days 5, 7, 9, and 11.

Sensory Panel

Sensory analysis was conducted according to the Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Fresh Meat (AMSA, 1995). The steaks were cooked to a medium degree of doneness (70°C) using an impingement oven. Steaks were transported to the test kitchen to be cut into 2.54 cm cubes for serving. Panelist (n=20) received three paired randomly assigned steaks to evaluate (Table 2).

Each animal pair was seen six times by six different panelists. Analysis was conducted on days 5, 7, and 9. Each panelist had a ballot containing four different categories juiciness, tenderness, amount of connective tissue and overall acceptability. The juiciness had eight different categories ranging from 8= extremely juicy to 1= extremely dry. Tenderness ranging from 8=extremely tender to 1=extremely tough. The amount of connective tissue ranging from 1=none to 8=abundant. The overall flavor category ranging from 8=extremely intense to 1=extremely bland. All of the results from the panelist were averaged to get a mean for each category.

Statistical Analysis

All results were analyzed using generalized least squares (PROC Mixed SAS Inst., Inc., Cary, NC). Data for color score, purge analysis, cook loss, and shear force was analyzed as a split plot randomized block design with animal id as the block, treatment as the main unit treatment, day as the subunit treatment, error A was animal id by treatment and error B was the error term. Data for aerobic plate count and lipid oxidation was analyzed as a 2 x 2 factorial in a randomized block design with the block being animal id, factor A treatment and factor B day. Data for sensory panel was analyzed as a paired experiment with combination blocks of animal id and panel id. Mean separation was accomplished using Least Significant Difference. All tests were conducted at the nominal significance level of 0.05.

RESULTS AND DISCUSSION

Percent Enhancement

For phosphate and protein enhancement a target percent pump weight was set at 110% of the green weight. The actual mean value for the phosphate enhanced subprimals was 10.64% with a standard deviation of 0.98 (Appendix B) creating an enhancement slightly above the target. The actual mean value for the protein enhanced subprimals was 9.96% with a standard deviation of 0.99. This slight variation between the two treatment types might also explain some of the variation found between the two enhancement solutions in the sections following.

Proximate Analysis

Proximate analysis was conducted on the three randomly selected samples for each treatment time period (Table 3). The unenhanced steak values obtained were

comparable to the USDA National Nutrient Database for Standard Reference for protein and ash. This study's results were lower in percent fat suggesting that our samples were trimmed of more fat during the project. The values for moisture also varied from the USDA values by about five percentage points suggesting our product had more moisture than the standards used by the USDA due to increase values of protein and decreased levels of fat present in our product. For the protein and phosphate enhanced steaks it was found that percent moisture did increase as expected with the enhancement with the phosphate enhancement having the most moisture. The protein values were higher in the protein enhanced steaks than the phosphate enhanced steaks however the protein values were relatively the same for both the unenhanced steaks and protein enhanced steaks. The fat values were slightly lower in both enhanced treatments than the unenhanced steak samples. Ash values were slightly higher in the phosphate enhanced steak which is to be expected since the mineral phosphate is added for the enhancement solution.

In the cooked steak values it was shown that the moisture values did decrease by about five percent due to cooking with the phosphate enhanced samples always having around 2 percent more moisture than the protein enhanced samples. Initially protein values were virtually the same for both phosphate and protein enhanced samples however at day 7 there was a five percent difference with the protein enhanced samples being higher and again at day 9 there was a two percent difference in protein. Fat and ash values stayed consistent with the raw enhanced values.

Color Score

Color score panelists evaluated the color of samples based on four different categories (Appendix C). Each animal and treatment type combination was observed by

five different panelists. The five individual values were averaged to create a mean value for each animal for both the phosphate and protein enhanced steaks. The mean for each animal was then combined with all of the other like treatment types (n=10) to create a mean value for each treatment type for each day sampled (Days 5, 6, 7, 8, 9, 10) for all four different category.

For lean color results it was found that there was a significant treatment by storage time interaction. On days 5, 6, 7, and 8, morning (AM) treatment times were significantly different. All other times performed in a similar manner between treatment types (Table 4). It should be noted that the phosphate enhanced steaks had a lean color score value of 7.88$\pm$ 0.19 on day five and 7.32$\pm$ 0.25 for protein and both rated as moderately bright cherry red. On the final day of the study the mean values for phosphate enhanced steaks was 5.20$\pm$ 0.98 compared to 5.14$\pm$ 1.17 both rated as a slightly dark red which would still be considered acceptable to the consumer. The mean values for lean color were numerically higher for phosphate at all time periods except for day 10 AM where the protein enhanced streaks were 0.02 higher. The overall average of 6.47 for the storage period is comparable to Lawrence and others (2004) where strip loins were injected with a 12% phosphate solution and stored for five days in a commercial retail case. The overall average of 6.13 for protein enhanced steaks is slightly lower than the phosphate values but not significantly different. Half the amounts of sodium chloride and Herbalox Seasoning Type HT-W were added to the protein solution injected product to reduce the likelihood of reducing the solubility of proteins, subsequently this reduction in antioxidant could explain the difference in values associated for fat and lean color score values.

For fat color it was found that there was a significant treatment by storage time interaction. After day 6 PM time period it was found that phosphate enhanced steaks were significantly different than protein enhanced steaks with the phosphate enhanced maintaining the higher values which correlates to a more favorable fat color (Table 5). At day five the fat color for both samples were rated as mostly creamy white in color where at day 10 or the end of the color study it was found that the phosphate enhanced steaks still rated as mostly creamy white and for protein enhanced steaks it was found that it was rated as slightly tan in color. The overall average of 7.29 for phosphate enhanced steaks is significantly better than the 6.93 of protein enhanced steaks. Differences in fat color averages between phosphate and protein enhanced steaks could be associated with the lower amount of herbalox injected into the protein enhanced steaks.

For percent discoloration it was found there was no significant treatment main effect or a significant treatment by storage period interaction. However it was found that there was a significant storage period main effect which is expected as meat ages it will change color due to oxidation of the myoglobin changing the color of the meat from red to a brown color (Table 6). It was found that the overall mean for percent discoloration on phosphate enhanced steaks was 6.37 compared to 6.50 showing that the protein enhanced steaks were able to retain the red color as well as the phosphate enhanced steaks although there was not a significant treatment effect. The overall average for phosphate enhanced steaks is slightly better than Lawrence and others (2004) at 11-25% discoloration.

For overall appearance of the steak, in regards to color score, it was found that there was neither a significant interaction between treatment and storage period nor a significant main unit treatment effect. It was found there was a significant day main unit effect. It is shown in Table 7 that over time during the study the overall acceptance of each treatment decreased with a mean value of 6.96 or close to extremely desirable at day five and a mean score of 4.27 for day 10 slightly above the acceptable rating. These overall color ratings showed that the protein enhanced steaks compared favorably to the phosphate enhanced steaks through out the study. The effect of a very low pH (2.5) injection solution on meat color was a significant concern in this study. At low pH myoglobin is altered to metmyoglobin and, in fact, the color of the injection solution was a very dark brown. However, it was demonstrated that the low pH protein enhanced steaks did not have a negative effect on color over the course of the study.

Aerobic Plate Count

For aerobic plate count (APC) there was a significant treatment and day main effect. For this study the interaction between treatment and storage period resulted in a p-value of 0.0553. Since the p-value was so close to the 0.05 significant cut-off for this study it was determined to go ahead and call it a significant interaction effect of treatment by storage period. At day five levels of APC bacteria were not significantly different and bacterial loads were approximately 4 logs. Several authors have reported an increase in microbial load due to the injecting of products because of the increased area for microorganisms to grow (Cannon and others 1993; Robbins and others 2002) thus suggesting that increased APC counts in injected meat is probably caused by this effect. By day 9, there was a 2 log difference between phosphate treated and protein treated

steaks (Table 8). All values are within the acceptable limit of bacteria for steaks as reported by Morris and others (1997). It was determined that the phosphate treatment did a better job at inhibiting the growth of aerobic bacteria during the course of the study. Some factors that might contributed to lower aerobic plate counts in the phosphate enhancement solution is the higher amounts of sodium chloride found in the solution at .36% compared to .18% injection found in the protein enhanced steaks. Additionally, evaluated levels of APC could be contributed to the additional protein that was injected back into the protein enhanced steaks. Since all processing steps were conducted by hand increase time for the protein enhancement to be made could have also increased the exposure to bacteria during the processing steps of blending, chopping, and solubilization. Automation of the protein solubilization process may help decrease contamination. The values reported for phosphate enhancement treatments are comparable to those reported by Pietrasik and others (2006) at day 5 of approximately 4 logs. However, the day 9 levels for phosphate enhanced steaks were well below the approximate 7 logs reported by Pietrasik and others (2006), suggesting that the phosphate treatment performed better than in previous studies.

Lipid Oxidation

According to Faustman and others (1989) the accumulation of carbonyl compounds by oxidation of unsaturated fatty acids and meat phospholipids is correlated with myoglobin oxidation in fresh beef. Highly unsaturated fatty acids and their proximity to myoglobin in meat will cause the micosomal lipid oxidizing system to be a potentially important inducer of oxidation of myoglobin (Lin and Hultin 1977). It was found that lipid oxidation and pigment oxidation in fresh meat were closely coupled

(Greene 1971; Renerre 1990). In turn implying delaying lipid oxidation should result in the delay of meat discoloration. The main indicator of lipid oxidation in meat is the presence of thiobarbituric acid reactive substances (TBARS). Many researchers have characterized meat samples having TBARS level of 1.0 mg/kg as having oxidative flavors that could be detected by trained consumer panels.

Case ready strip loins did not show a treatment by storage period interaction nor a main storage period effect on lipid oxidation. However, a significant main treatment effect was observed with the phosphate treatment performing better than the protein enhanced treatment (Table 9). The phosphate treatment average for both days five and nine were below the 1.0 mg/kg. The protein enhancement at day five and nine were already over the detectible limit thus indicating that the protein enhancement which included lower amounts of the antioxidant herbalox or rosemary was unable to retard lipid oxidation as well as the traditional phosphate enhancement that included a higher amount of rosemary solution. One possible reason why lipid oxidation was higher was the lower amount of antioxidant added to the protein solution. When adding rosemary to the protein solution it was added at a lower concentration to allow for ease of flow through the injection machine. The TBAR values for the phosphate treatment were comparable to the 0.61 reported by Pietrasik and others (2006).

Purge Analysis

For percent purge there was a significant treatment main effect. There was neither a significant storage period main effect nor a treatment by storage period interaction effect. At day five the initial purge loss was significantly different in the phosphate enhanced steaks and the protein enhanced steaks with the protein displaying

significantly higher amounts of purge (Table 10). This trend continued throughout the remainder of the study. Elevated purge levels in the protein could possibly be associated with the protein not binding to the meat due to there not being enough protein in the solution to create a good matrix for binding. It has been suggested that a higher protein ratio in the protein solution may improve water binding. In addition, use of a higher salt concentration in the protein injected steaks may not have the negative effect originally envisioned with the protein based injection solution. A lower salt concentration was selected because it was originally felt that salt may decrease solubility of the proteins in the solubilized protein solution and therefore decrease their functionality. However, the high purge levels experienced by the protein injected steaks may have resulted due to insufficient levels of salt. Solubilization of meat proteins by salt may enable them to interact better with the solubilized proteins being injected into the meat. Phosphate purge levels for the strip loin steaks are in agreement with Pietrasik and others (2006) of 2.61% compared 2.71% for phosphates over all days. Purge levels are approximately half of the values reported by Lawrence and others (2003) of 4.5-5%.

Cook Yield

Cook yield is a measurement of how much water is lost during cooking. With that being said, the lower the percentage the better the product, as it will be juicier because the moisture is retained in the steak. One factor that a phosphate base enhancement solutions helps with is the juiciness because the moisture is aided by the addition of phosphate in the meat to bind to the protein present in meat. For cook yield percentage, there were a significant treatment and storage period main effects. There was

not a treatment by storage period interaction effect. For day five, phosphate and protein varied by approximately two percent at 26 and 28% respectively (Table 11). This trend stayed constant throughout the study with phosphate enhanced streaks always having the lower or better percent cook yield. Our findings for phosphate enhanced steaks were slightly higher than Rhee (2004) of 19-23% cook yield in the strip loin. Lawrence and others (2003) reported a cook yield ranging from 19-25% comparable to our results. The moisture in the protein enhanced steaks was not bound as well to the myofibrillar protein as the phosphate enhanced steaks thus increasing their cook yield percentage. Cook yield may also be improved with increased amount of protein added to the enhancing solution.

Warner-Bratzler Shear Force

Each steak was sampled six times, those six samples were then averaged to create a mean value for each steak (n=10 phosphate, protein; Days 5, 7, 9, 11). It was determined there was a significant treatment main effect however there was neither a significant storage period main effect nor a treatment by storage period interaction effect. The values for phosphate enhanced steaks performed better than the protein enhanced steaks (Table 12). It should be noted that both samples fell into the tenderness category of "tender", Warner-Bratzler Shear Force <4.5 kg (Bratzler 1949). Miller and others (2001) said that if a WBSF value of 4.0 kg or less can be achieve then 94% of consumer satisfaction is achieved and less than 4.3 kg 86% consumer satisfaction. For the phosphate enhanced steaks the lowest WBSF values were recorded on day 9 of the study at 2.99 kg of force. The lowest WBSF values for the protein enhanced steaks were achieved on day 5 of the study at 3.91 kg of force. The difference in WBSF values can be associated with the phosphate enhancement solution doing a better job of tenderizing

the product over time by solublizing the myofibrillar protein better than the protein enhanced solution. A higher moisture content in the phosphate enhanced steaks also could of played a role in the lower values reported for the phosphate enhanced steaks. The phosphate enhanced steaks were lower in shear force values throughout the study than those values reported of 3.79 kg of Vote and others (2000) and McMichael (2005) of 3.62 kg.

Sensory Panel

Sensory panelists evaluated samples based on four different categories (Appendix D). Each animal and treatment type combination was observed by six different panelists. The six individual values were averaged to create a mean value for each animal for both the phosphate and protein enhanced steaks. The mean for each animal was then combined with all of the other like treatment types (n=10) to create a mean value for each treatment type for each day sampled (Day 5, 7, 9). There was a significant treatment effects on all four different categories: tenderness, juiciness, connective tissue, and overall acceptability. There were no significant storage period main effects or treatment by storage period interactions on any of the four sensory categories.

For tenderness it was found that the phosphate enhance steaks performed significantly better than the protein enhanced streaks over all (Table 13). These results for phosphates are slightly lower than values reported by Lawrence and others (2004) at a tenderness value of 6.4 on a scale of 1-8 with eight being extremely tender and one being extremely tough. The difference in tenderness values between phosphate enhanced steaks and protein enhanced streaks could be associated with the phosphate based

solution's ability to solubilize the myofibrillar proteins better than the protein based solution creating what appeared to be a more tender product to the sensory panelists.

For juiciness it was found that the phosphate enhanced steaks performed significantly better than the protein enhanced steaks overall. The juiciness values for overall phosphate enhanced steaks was 5.7 on an eight point scale (8=extremely juicy, 1=extremely dry) where as the protein enhanced steaks had a value of 5.08 which gives both treatments a ranking of slightly juicy (Table 13). However, the phosphate treatment is closer to the moderately juicy category and significantly different than the protein treated streaks. The values for phosphate base steaks again agree with Lawrence and others (2004) and are slightly lower than values of 6.07 reported by Vote and others (2000). The obvious reasons for difference in juiciness are the differences reported in purge analysis and cook loss for the different treatments. When more moisture is retained in the steaks during retail storage and not lost during cooking the steak will seem juicier to the sensory panel.

Connective tissue values were significantly different for the phosphate treated steaks and the protein treated streaks. It was reported that the phosphate treated steaks had lower amounts of connective tissue detected by the sensory panel, reported at the slightly abundant level range (3.44) where the protein enhanced steaks had a value of (2.91) moderately to slightly abundant (Table 13). The phosphate based enhancement solution values differ slightly than those report by Lawrence and others (2004) of having practically none to no connective tissue present in their sensory panel. The reasons for increased connective tissue scores could be attributed to lower values of juiciness

reported since when there is more moisture involved in the sample it can reduce the amount of connective tissue observed (Morris and others 1997).

For overall acceptability of treated steaks it was found that phosphate enhanced steaks performed better than protein enhanced steaks. Phosphate enhanced steaks received an overall score of slightly desirable where the protein enhanced steaks received a score of acceptable. The higher values for phosphate enhanced steaks can probably be attributed to the steaks being rated higher for tenderness and juiciness as they are generally correlated to higher acceptability.

CONCLUSION

It was found that the phosphate enhanced steaks performed better than the protein enhanced steaks in all areas evaluated except for percent discoloration and overall acceptability color score where they perform significantly the same. At this time it is not feasible to suggest a switch to protein enhancing of meat in the beef industry. Future research needs to be conducted to determine if a protein enhancement can be developed to perform comparably to a phosphate enhancement. One suggestion is to reduce the amount of water used in the protein enhancement and go to a 1:5 protein to water ratio from a 1:9 ratio. At some point in time if the water holding ability of the protein enhancement can be improved it would help to create a market for a new enhancement solution in the beef industry and help create a healthier product and in turn reducing another ingredient from enhanced beef.

Table 2. Statistical Plan for Sensory Panel[1]

Block Panelist Number	Panel Number 1	Panel Number 2
	Animal # paired[2]	Animal # paired[2]
1	1,2,3	6,7,8
2	1,2,5	6,7,10
3	1,4,5	6,9,10
4	2,3,4	7,8,9
5	3,4,5	8,9,10
6	1,2,4	6,7,9
7	1,3,4	6,8,9
8	1,3,5	6,8,10
9	2,3,5	7,8,10
10	2,4,5	7,9,10

[1]Plan 11.1a Obtain from Cochran 1968.
[2] Each animal sampled for both phosphate and protein enhanced samples

Table 3. Proximate analysis values for different time periods throughout the enhancement study.

Treatment	Moisture%	Protein%	Fat%	Ash%	CHO%[1]
USL[2]	72.26 + 1.66[a]	21.84 + 0.62 [a]	5.61 + 1.77 [a]	1.04 + 0.04 [a]	0.00[a]
PhER[3]	75.85 + 1.39[b]	20.38 + 0.43 [a]	4.70 + 0.95 [b]	1.60 + 0.18 [b]	0.00[a]
PrER[4]	73.35 + 1.82[c]	21.86 + 0.70 [a]	4.39 + 1.23 [b]	1.13 + 0.05 [a]	0.00[a]
PhCD5[5]	66.51 + 2.64[d]	26.58 + 0.94 [b]	4.57 + 2.62 [b]	1.58 + 0.27 [b]	0.00[a]
PrCD5[6]	64.47 + 2.37 [e]	26.77 + 1.47 [b]	5.12 + 1.32 [b]	1.67 + 0.31 [b]	0.00[a]
PhCD7[7]	69.88 + 2.57 [f]	23.79 + 0.97 [c]	4.87 + 1.72 [b]	1.80 + 0.24 [b]	0.00[a]
PrCD7[8]	67.14 + 0.43 [f]	28.34 + 1.14 [d]	4.28 + 0.80 [b]	1.22 + 0.07 [a]	0.00[a]
PhCD9[9]	68.89 + 0.99 [f]	25.97 + 1.08 [b]	4.00 + 1.81 [c]	1.71 + 0.14 [b]	0.00[a]
PrCD9[10]	66.44 + 0.76 [d]	27.68 + 1.94 [d]	4.91 + 0.66 [b]	1.10 + 0.05 [a]	0.00[a]

[1]Carbohydrate determined by difference.
[2] USL = Unenhanced Strip Loin Raw
[3] PhER = Phosphate Enhanced Strip Loin Raw
[4] PrER = Protein Enhanced Strip Loin Raw
[5] PhCD5 = Phosphate Enhanced Strip Loin Cooked Storage Day 5
[6] PrCD5 = Protein Enhanced Strip Loin Cooked Storage Day 5
[7] PhCD7 = Phosphate Enhanced Strip Loin Cooked Storage Day 7
[8] PrCD7 = Protein Enhanced Strip Loin Cooked Storage Day 7
[9] PhCD9 = Phosphate Enhanced Strip Loin Cooked Storage Day 9
[10] PrCD9 = Protein Enhanced Strip Loin Cooked Storage Day 9

[a,-f] Means appearing in the same column with different superscripts are significantly different ($P \leq 0.05$).

Table 4. The effect of treatment type and storage period for lean color score of strip loin steaks.

Day	Phosphate[1]	Protein[1]
Day 5 AM	7.88 ± 0.19 [a]	7.32 ± 0.25[b]
Day 5 PM	7.68 ± 0.34 [a]	7.28 ± 0.47[a]
Day 6 AM	7.62 ± 0.24 [a]	6.90 ± 0.38[b]
Day 6 PM	7.00 ± 0.32 [a]	6.60 ± 0.53[a]
Day 7 AM	6.74 ± 0.38 [a]	6.20 ± 0.63[b]
Day 7 PM	6.52 ± 0.58[a]	6.22 ± 0.69[a]
Day 8 AM	6.34 ± 0.49[a]	5.82 ± 0.81[b]
Day 8 PM	5.88 ± 0.59[a]	5.72 ± 0.88[a]
Day 9 AM	5.66 ± 0.75 [a]	5.58 ± 0.97[a]
Day 9 PM	5.72 ± 0.66 [a]	5.36 ± 1.32[a]
Day 10 AM	5.44 ± 0.85 [a]	5.46 ± 1.14[a]
Day 10 PM	5.20 ± 0.98 [a]	5.14 ± 1.17[a]

[a,b] Means appearing in the same row with different superscripts are significantly different ($P \leq 0.05$).

[1] 8 = Bright cherry red, 7 = Moderately Bright Color Red, 6 = Cherry Red, 5 = Slightly Dark Red, 4 = Moderately Dark Red or Brown, 3 = Dark Red or Brown, 2 = Very Dark Brown, 1 = Extremely Dark Brown

Table 5. The effect of treatment type and storage period for fat color score of strip loin steaks.

Day	Phosphate[1]	Protein[1]
Day 5 AM	7.80 ± 0.00^{a}	7.80 ± 0.00^{b}
Day 5 PM	7.80 ± 0.00^{a}	7.72 ± 0.10^{a}
Day 6 AM	7.60 ± 0.00^{a}	7.58 ± 0.06^{a}
Day 6 PM	7.60 ± 0.00^{a}	7.52 ± 0.25^{a}
Day 7 AM	7.54 ± 0.21^{a}	7.10 ± 0.25^{b}
Day 7 PM	7.48 ± 0.19^{a}	7.06 ± 0.27^{b}
Day 8 AM	7.44 ± 0.18^{a}	6.93 ± 0.33^{b}
Day 8 PM	7.08 ± 0.19^{a}	6.64 ± 0.42^{b}
Day 9 AM	6.48 ± 0.29^{a}	6.04 ± 0.39^{b}
Day 9 PM	7.14 ± 0.27^{a}	6.42 ± 0.46^{b}
Day 10 AM	6.68 ± 0.38^{a}	6.28 ± 0.42^{b}
Day 10 PM	6.92 ± 0.27^{a}	6.12 ± 0.36^{b}

[a,b] Means appearing in the same row with different superscripts are significantly different ($P \leq 0.05$).
[1] 8 = Creamy White, 7 = Mostly Creamy White, 6 = Slightly Tan, 5 = Tan, 4 = Slightly Brown
3 = Moderately Brown, 2 = Brown or Slightly Green, 1 = Dark Brown or Green

Table 6. The effect of storage period for percent discoloration of strip loin steaks.

Day	Day Means[1]
Day 5 AM	6.96± 0.14[a,b]
Day 5 PM	6.95± 0.18 [b,c]
Day 6 AM	6.93± 0.23 [b,d,e]
Day 6 PM	6.91± 0.27 [b,e,f]
Day 7 AM	6.76± 0.34[b,e,g]
Day 7 PM	6.67± 0.53[e,h,i]
Day 8 AM	6.48± 0.71[j]
Day 8 PM	6.23± 1.10[i,j,k]
Day 9 AM	6.11± 1.25 [k]
Day 9 PM	6.00± 1.35[k]
Day 10 AM	5.70± 1.56[l,m]
Day 10 PM	5.57± 1.35[m,n]

[a-n] Means appearing in the same column with different superscripts are significantly different ($P \leq 0.05$).
[1] 7 = None, 6 = 1 – 10%, 5 = 11 – 25%, 4 = 26 – 50%, 3 = 51 – 75%, 2 = 76 – 99%, 1 = Complete

Table 7. The effect of storage period for overall appearance color score of strip loin steaks.

Day	Day Means[1]
Day 5 AM	6.96± 0.04[a,b]
Day 5 PM	6.95± 0.08b[a,c,d]
Day 6 AM	6.93± 0.23 [b,d,e]
Day 6 PM	6.91± 0.23[b.f]
Day 7 AM	6.76± 063[g]
Day 7 PM	6.67± 0.76[b,h]
Day 8 AM	6.48± 0.87[i]
Day 8 PM	6.23± 1.04[j]
Day 9 AM	5.02± 1.27[j]
Day 9 PM	4.70± 1.25[k,l]
Day 10 AM	4.45± 1.32[l,m]
Day 10 PM	4.27± 1.34[j,k]

[a-m] Means appearing in the same column with different superscripts are significantly different ($P \leq 0.05$).
[1] 7= Extremely Desirable, 6= Desirable, 5= Slightly Desirable, 4= Acceptable,
3= Slightly Undesirable, 2= Undesirable, 1= Extremely Undesirable

Table 8. The effect of treatment type and storage period for aerobic plate counts (log_{10} cfu/g) of strip loin steaks.

Treatment	Day 5	Day 9
Phosphate	4.24 ± 0.42^{a}	5.86 ± 0.50^{b}
Protein	4.60 ± 0.44^{a}	7.08 ± 1.11^{c}

[a,b,c] Means appearing in the same row with different superscripts are significantly different ($P \leq 0.05$).
[a,b,c] Means appearing in the same column with different superscripts are significantly different ($P \leq 0.05$).

Table 9. The effect of treatment type on lipid oxidation (mg/kg) of strip loin steaks.

Treatment	Day 5	Day 9
Phosphate	0.68 ± 0.33^{a}	0.76 ± 0.54^{a}
Protein	1.48 ± 0.84^{b}	1.54 ± 0.72^{b}

[a,b] Means appearing in the same column with different superscripts are significantly different ($P \leq 0.05$).

Table 10. The effect of treatment type on percent purge of strip loin steaks.

Treatment	Day 5	Day 7	Day 9	Day 11
Phosphate	2.29± 1.10[a]	2.52± 1.04[a]	2.51± 0.62[a]	3.49± 1.90[a]
Protein	8.93± 5.19[b]	7.29± 3.24[b]	6.38± 2.55[b]	9.49± 5.01[b]

[a,b] Means appearing in the same column with different superscripts are significantly different ($P \leq 0.05$).

Table 11. The effect of treatment type and storage period for percent cook loss of strip loin steaks.

Treatment	Day 5	Day 7	Day 9	Day 11
Phosphate	26.24± 3.76[a]	24.67± 3.00[c]	26.20± 3.07[a]	24.67± 2.67[c]
Protein	28.98± 3.05[b,d]	28.08± 2.77[b,e]	28.71± 3.05[b,d]	26.62± 2.81[b,f]

[a,b,c] Means appearing in the same column with different superscripts are significantly different ($P < 0.05$).
[a,b,c,d,e,f] Means appearing in the same row with different superscripts are significantly different ($P \leq 0.05$).

Table 12. The effect of treatment type on Warner-Bratzler shear force values (kg) of strip loin steaks.

Treatment	Day 5	Day 7	Day 9	Day 11
Phosphate	3.31± 0.84[a]	3.00± 0.75[a]	2.99± 0.76[a]	3.09± 0.49[a]
Protein	3.91± 0.59[b]	4.00± 0.50[b]	4.19± 0.63[b]	3.94± 0.70[b]

[a,b] Means appearing in the same column with different superscripts are significantly different ($P \leq 0.05$)

Table 13. The effect of treatment type on overall mean values of known sensory categories for strip loin steaks.

Treatment	Tenderness[1]	Juiciness[2]	Connective Tissue[3]	Overall Acceptability[4]
Phosphate	6.28 ± 1.34^{a}	5.77 ± 1.32^{a}	3.44 ± 0.70^{a}	5.13 ± 1.38^{a}
Protein	4.77 ± 1.51^{b}	5.08 ± 1.34^{b}	2.91 ± 0.80^{b}	4.16 ± 1.35^{b}

[a,b] Means appearing in the same column with different superscripts are significantly different ($P \leq 0.05$).
[1] 8 = Extremely Tender, 7 = Very Tender, 6 = Moderately Tender, 5 = Slightly Tender, 4 = Slightly Tough, 3 = Moderately Tough, 2 = Very Tough, 1 = Extremely Tough
[2] 8 = Extremely Juicy, 7 = Very Juicy, 6 = Moderately Juicy, 5 = Slightly Juicy, 4 = Slightly Dry, 3 = Moderately Dry, 2 = Very Dry, 1 = Extremely Dry
[3] 4 = None, 3 = Slightly Abundant, 2 = Moderately Abundant, 1 = Extremely Abundant
[4] 7 = Extremely Desirable, 6 = Desirable, 5 = Slightly Desirable, 4 = Acceptable, 3 = Slightly Undesirable, 2 = Undesirable, 1 = Extremely Undesirable

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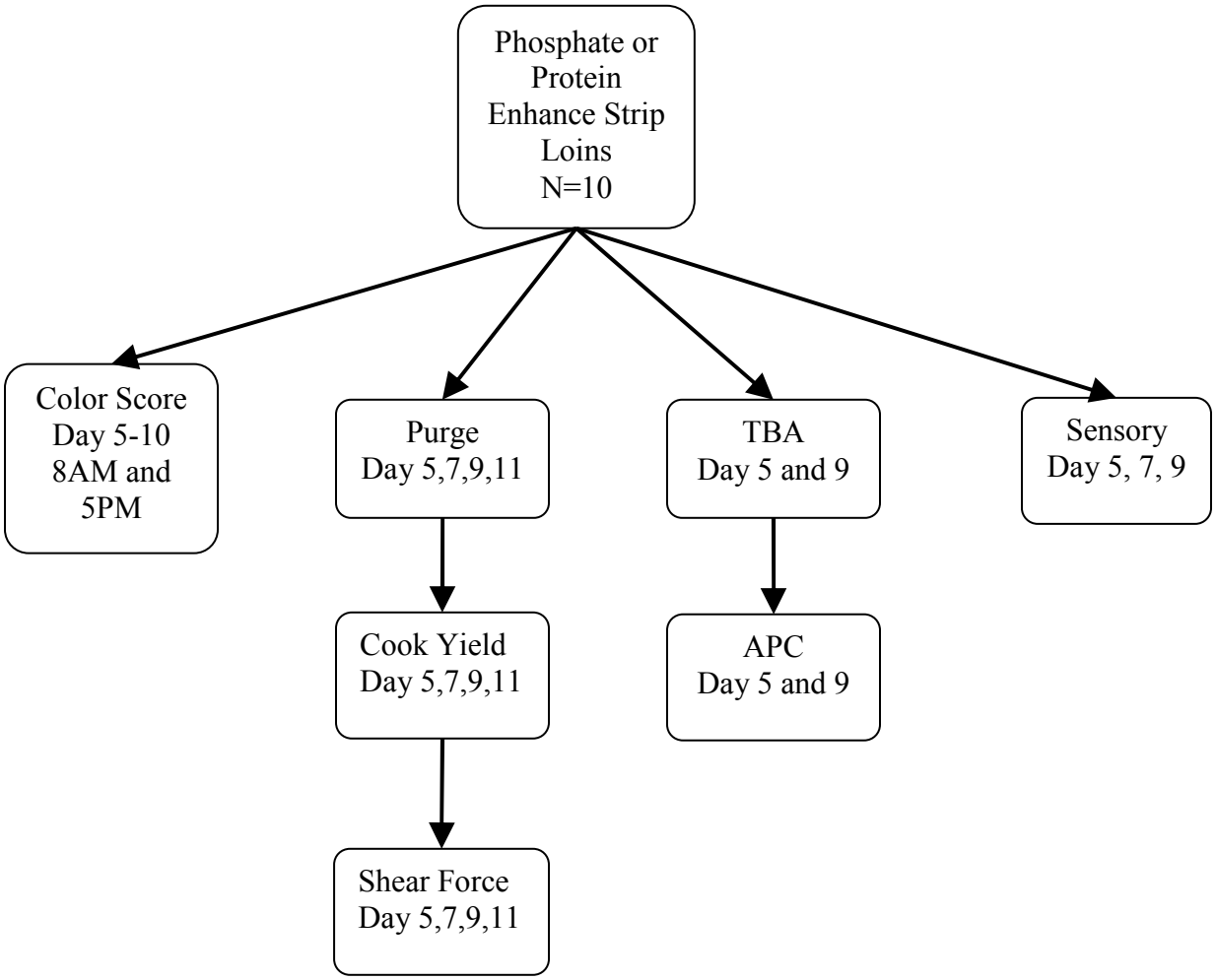
CHAPTER IV

APPENDIX

APPENDIX A

SCHEMATIC OF EXPERIMENTAL DESIGN



APPENDIX B

PERCENT ENHANCEMENT STRIP LOIN SUBPRIMALS

		Protein	Enhanced		
Sample ID	**Initial wt lbs.**	**First Pump 5% wt lbs.**	**Second Pump Initial wt lbs**	**Final wt**	**Total % Enhanced**
1	9.9	10.4	10.4	10.9	10.10
2	11.5	12.3	12.3	12.8	11.30
3	10.5	11.2	11.1	11.6	10.48
4	11.9	12.5	12.4	13	9.24
5	9.5	10.3	10.2	10.6	11.58
6	12.1	12.5	12.5	13.2	9.09
7	12.4	13	13	13.5	8.87
8	10.7	11.3	11.2	11.7	9.35
9	10	10.5	10.5	10.9	9.00
10	10.4	10.9	10.8	11.5	10.58
Average					**9.96**
STD					**0.99**

		Phosphate	Enhanced		
Sample ID	**Initial wt lbs.**	**First Pump 5% wt lbs.**	**Second Pump Initial wt lbs**	**Final wt**	**Total % Enhanced**
1	11.1	11.9	11.8	12.4	11.71
2	12.2	13	13	13.7	12.30
3	11.9	12.6	12.5	13.2	10.92
4	12.3	12.9	12.9	13.6	10.57
5	11.3	12.2	12.1	12.6	11.50
6	11.9	12.3	12.3	13	9.24
7	11.8	12.4	12.3	13	10.17
8	10.6	11.1	11.1	11.6	9.43
9	9.9	10.4	10.4	10.9	10.10
10	14.3	15.3	15.2	15.8	10.49
Average					**10.64**
STD					**0.98**

APPENDIX C

COLOR SCORE EVALUATION SHEET

Name:_____ **Date:**_____**Time:**_____**Day**_____

ID	LEAN COLOR	FAT COLOR	% DISCOLOR	OVERALL
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

Lean Color

8. Bright Cherry-Red
7. Moderately Bright Color Red
6. Cherry Red
5. Slightly Dark Red
4. Moderately Dark Red or Brown
3. Dark Red or Brown
2. Very Dark Brown
1. Extremely Dark Brown

Fat Color

8. Creamy White
7. Mostly Creamy White
6. Slightly Tan
5. Tan
4. Slightly Brown
3. Moderately Brown
2. Brown or Slightly Green
1. Dark Brown or Green

% Discoloration or Browning

7. None
6. -1 – 10%
5. 11 – 25%
4. 26 – 50%
3. 51 – 75%
2. 76 – 99%
1. Complete

Overall Appearance

7. Extremely Desirable
6. Desirable
5. Slightly Desirable
4. Acceptable
3. Slightly Undesirable
2. Undesirable
1. Extremely Undesirable

APPENDIX D

SENSORY PANEL BALLOT

Oklahoma State University
Food Science

Panelist ID _____ Booth #_____ Date_____

Session 2:00 / 2:30

Sample ID	Tenderness	Juiciness	Connective Tissue	Overall Acceptability	Comments

Tenderness	Juiciness	Overall Acceptability
8 Extremely Tender 7 Very Tender 6 Moderately Tender 5 Slightly Tender 4 Slightly Tough 3 Moderately Tough 2 Very Tough 1 Extremely Tough	8 Extremely Juicy 7 Very Juicy 6 Moderately Juicy 5 Slightly Juicy 4 Slightly Dry 3 Moderately Dry 2 Very Dry 1 Extremely Dry	7 Extremely Desirable 6 Desirable 5 Slightly Desirable 4 Acceptable 3 Slightly Undesirable 2 Undesirable 1 Extremely Undesirable
Connective Tissue		
4 None 3 Slightly Abundant 2 Moderately Abundant 1 Extremely Abundant		

APPENDIX E

SENSORY PANEL COMMENTS

Animal ID	Treatment	Day	Comments
1	Protein	5	Not very tasty
1	Phosphate	5	More tasty than 1-Protein
1	Phosphate	5	Really good
2	Phosphate	5	Not very tasty
3	Protein	5	Good at first, then feels like goes dry
3	Phosphate	5	Softer than 1-phos but less tasty
4	Protein	5	Light hint of saltiness
4	Phosphate	5	Salty to the taste
5	Phosphate	5	Tastes similar to 3-Protein
7	Phosphate	5	Could taste rosemary
9	Protein	5	Salty
10	Protein	5	Off-flavor
10	Phosphate	5	Slight off-flavor
10	Phosphate	5	Slight salt flavor, but good
2	Protein	7	Strange flavor
3	Protein	7	Stringy
5	Phosphate	7	Very salty
5	Phosphate	7	Best Sample
6	Protein	7	Tough a lot of connective tissue
6	Phosphate	7	Tasted “hammered”
10	Phosphate	7	Tasted a little bland/almost paper
1	Phosphate	9	Salty
2	Phosphate	9	Very salty
2	Phosphate	9	Odd taste
2	Phosphate	9	Had rubber taste, but was good
3	Protein	9	Liver flavor
3	Protein	9	Strange consistency
3	Protein	9	Ok flavor, little juicy
3	Phosphate	9	2 thumbs up-best sample
4	Phosphate	9	Great!!
5	Protein	9	Good flavor
8	Phosphate	9	Salty

Oklahoma State University Institutional Review Board

Date:	Wednesday, June 29, 2005
IRB Application No	AG0555
Proposal Title:	Formulation of Processed Meats Using Acid-Solubilization Isoelectric Precipitated Protein
Reviewed and Processed as:	Exempt

Status Recommended by Reviewer(s): Approved Protocol Expires: 6/28/2006

Principal Investigator(s

Christina Mireles DeWitt
104E Animal Science
Stillwater, OK 74078

Dustin Vann
122 Animal Science
Stillwater, OK 74078

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

☑ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval.
2. Submit a request for continuation if the study extends beyond the approval period of one calendar year. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of this research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact Beth McTernan in 415 Whitehurst (phone: 405-744-5700, beth.mcternan@okstate.edu).

Sincerely,

Sue C. Jacobs, Chair
Institutional Review Board

VITA

Dustin Glen Vann

Candidate for the Degree of

Master of Science

Thesis: Evaluation of solubilized proteins as an alternative to phosphates for meat enhancement.

Major Field: Food Science

Biographical:

Personal Data: Born in Enid, Oklahoma, April 6, 1982, the son of Richard and Kathleen Vann

Education: Graduated from Billings High School, Billings, Oklahoma, May 2000; Received Bachelor of Science Degree in Animal Science from Oklahoma State University, Stillwater, Oklahoma, May 2004;
Completed the Requirements for the Master of Science degree with a major in Food Science at Oklahoma State University in July 2006.

Experience: Raised in Billings, Oklahoma; Employed by Oklahoma State University Animal Science Department as an undergraduate, 2000-2004; summer intern at Hiland Dairy in 2002; summer intern at Cornell University in 2003; summer intern at Schreiber Foods in 2004 graduate research assistant & teaching assistant, 2004-2006.

Professional Organizations: Institute of Food Technology; American Meat Science Association; American Dairy Science Association

Name: Dustin Glen Vann Date of Degree: July, 2006

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EVALUATION OF SOLUBILIZED PROTEINS AS AN ALTERNATIVE TO PHOSPHATES FOR MEAT ENHANCEMENT

Pages in Study: 66 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study: Select grade strip loins (n=10) were enhanced with a 10% target phosphate based or an acid solublized protein solution. Color score, aerobic plate count, lipid oxidation, purge loss, cook yield, Warner-Bratzler shear force, and sensory analysis were measured to characterize storage quality. The phosphate based solution consisted of 4.5% phosphate, 3.6% sodium chloride , 90.9% water and 1% Herbalox seasoning. The protein based solution was prepared in two solutions one consisting of 1:9 protein to water ratio and the other an aqueous solution of 1%Herbalox seasoning and 3.6% sodium chloride. Enhancement solutions were injected twice at 5% to create a 10% total injection

Findings and Conclusions: Protein enhanced steaks out performed the phosphate enhanced steaks for percent discoloration and overall acceptability. The phosphate enhanced steaks performed better than the protein enhanced steaks for lean color, fat color, aerobic plate count, lipid oxidation, percent purge, cook yield, and Warner Bratzler shear force. It should be noted that for protein enhanced steaks lean color and cook yield although significantly different did perform similar to phosphate enhanced steaks

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ADVISER'S APPROVAL: Dr. Christina DeWitt