EVALUATION OF A HIGH pH SOLUTION AS AN ALTERNATIVE FOR PHOSPHATE MEAT ENHANCEMENT

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CHAPTER I

INTRODUCTION

The beef industry attempts to increase the demand for beef and beef products by producing customized products for different market segments (Boland and others, 1999). Probably because of the consumer time limitation on meal preparation, the inclusion of marinated food products at retail markets became a need (Janz and others, 2005). Usually, enhanced or marinated foods are injected with a brine solution, which commonly contains salt, phosphates, seasonings, and flavorings.

There are many advantages to the use of marinated products such as improving tenderness and juiciness, extending shelf life, controlling pathogenic bacterial growth, and preventing oxidation among others (Foote and others, 2004; McGee and others, 2003; Robbins and others, 2003). As a result, the beef industry is widely producing enhanced meat products to meet consumer demands (Hughes, 2002).

However, at present, the extensive use of phosphates is an issue for some segments of the society, mainly people suffering chronic kidney disease (CKD). During the course of CKD, a decline in renal function leads to phosphate retention. Higher levels of serum phosphate are associated with adverse cardiovascular outcomes, especially in the setting of overt hyperphosphatemia and abnormal serum calcium levels resulting from secondary hyperparathyroidism (Tonelli and others, 2005). Thus, high serum phosphate

levels are commonly associated with death and myocardial infarction in patients with stage 3-4 CKD, a greater prevalence of heart failure, and cardiovascular disease (Kestenbaum and others, 2005; Tonelli and Pfeffer, 2007; Tonelli and others, 2005; Raffaitin and others, 2007). In addition, it is established that, diabetes is the principal leading cause of kidney failure (CDC, 2005). Clinically-based reports and regional studies suggest that, in the United States, diabetes is being diagnosed more frequently. In 2005, 9.6% of people from 20 to 60 years and 20.9% of the people older than 60 years suffered diabetes (CDC, 2005).

In this context, the beef industry needs to find healthier alternatives to phosphate enhancers, while maintaining desirable characteristics. Therefore, the objective of this study was to compare color stability, lipid oxidation, proximate analysis, purge analysis, cook loss, shear force, microbial growth, and sensory attributes of beef subprimal strip loins injected with a high pH-enhancement solution of ammonium hydroxide to those injected with a commercially based phosphate enhancement.

CHAPTER II

REVIEW OF LITERATURE

The American beef industry, consumer attitudes, consumer satisfaction, and beef demand.

The American beef industry is a $175 billion dollar per year industry. There are over 800,000 individual farms and ranches, 2,100 feedlots, and over 250 million domestic consumers (Hughes, 2002). The National Cattlemen's Beef Association (NCBA) affirms that the demand for beef has increased 20% from 1998 (NCBA, 2005), and the United States Department of Agriculture (USDA) reports that beef consumption has grown from 27.9 billion pounds in 2002 to 28 billion pounds in 2006 (USDA, 2007a). Hughes (2002) affirms that the foreign and domestic demand for beef is growing 1.3% per year. Also, the retail equivalent value of the U.S. beef industry has increased from $60 billion in 2002 to $71 billion in 2006 (USDA, 2007b). In addition, beef consumption continues growing because it is the most preferred of the red meats, representing 56 percent of all retail red meat (beef, pork, lamb, and veal) consumed in the United States (NCBA, 2004; Davis and Biin-Hwan, 2005).

In order to increase beef demand, the beef industry has faced many challenges in meeting consumer demands during the last 20 years. Boland and others (1999) used the term "mass customization" to explain how companies are able to produce customized products for different market segments. Starting as far back as the 1960's, some beef

producers evaluated niche markets and, ever since, an increase in product innovation has been evident. The use of USDA quality certification, “natural”, and “organic” programs are just a few examples of niche markets currently being explored. Also, at the retail level, consumers are now offered a variety of processed, ready-to-cook, and ready-to-eat foods within commodity and branded beef. And lately, according to Schuster (2002), director of Montana Beef Council, the NBCA is looking for new ways to create great tasting, cheaper steaks outside of the rib and loin in order to increase beef demand.

Although the beef industry is effectively increasing the demand for beef, there are still factors that affect this industry negatively, such as product safety. According to Centers for Disease Control and Prevention (CDC), each year in the United States, there are approximately 76 million illness, 325,000 hospitalizations, and 5,000 deaths associated with food borne diseases (Mead and others, 1999). Food poisoning can be caused by chemicals, heavy metals, parasites, fungi, viruses, or bacteria. The most commonly recognized bacteria related with beef consumption are *Salmonella* and E*scherichia coli* O157:H7 (Mead and others, 1999). In addition, the same study reported that *E. coli* O157:H7 is responsible for 62,458 illnesses, 1,843 hospitalizations, and 52 deaths while *Salmonella typhi* causes 659 illnesses, 494 hospitalizations, and 3 deaths (Mead and others, 1999). In general, illness outbreaks caused by beef consumption lead to food recalls, which, in turn, have a very large negative impact on the beef industry.

In addition to increasing the amount of research to combat foodborne illness, there is an increase in research to further improve the characteristics desired by consumers. It is well established that tenderness is the most important attribute of beef palatability. Platter and others (2003) concluded that consumers listed tenderness (52%) as the most

important sensory attribute to purchase beef, followed by flavor (32%) and then juiciness (11%). It was also stated that the demand for cuts that have more external and seam fat was lower (Platter and others, 2003). However, a higher level of marbling for loin steaks than chuck roast was found to be more desirable (Unnevehr and Bard, 1993; Platter and others, 2003). Boleman and others (1997) concluded that consumers are able to discriminate among tenderness categories and are willing to pay a premium for tender beef. The Beef Customer Satisfaction Report (National Live Stock and Meat Board, 1995) revealed that the cut of beef makes the greatest impact on customer satisfaction. However, degree of doneness, method of cooking, geographical differences, and marbling levels also influence consumer satisfaction.

Currently, the tendency to consume healthier food is increasing and the demand for natural and organic beef is growing. Boland and others (1999) affirmed that consumers agree to pay more for "natural" beef loin but not for other "natural" cuts like chuck, round, or ground beef. Therefore, it is important to look for new techniques to increase the value of other cuts to compensate higher production costs of natural beef. Givry (2002), in a consumer survey to identify marketing issues for natural beef, concluded that tenderness, leanness, and visual appearance were the key factors influencing consumer purchasing rather than whether products were conventional, organic, and natural food products.

Beef grading system

The USDA quality grade in beef expresses the relative desirability or expected palatability of beef from the carcass. The quality grade is determined by considering the degree of marbling and firmness as observed in the cut surface of the ribeye as well as the

maturity of the carcass. The quality grades available for beef are: Prime, Choice, Select, Standard, Commercial, Utility, Cutter, and Canner (American Meat Science Association-AMSA, 2001).

According to Unnevehr and Bard (1993), the USDA changed the grading nomenclature in 1988 in order to promote "Select" grade beef as a leaner meat than "Choice" grade. In addition, Hughes (2002) affirms that the amount of beef graded Select has increased markedly since 1986, when the name of the existing beef quality grade was changed from Good to Select. According to Hughes (2002), the USDA quality grades played an important role in distributing quality to the marketing chain and providing signals to cattle producers of consumer preferences, since consumers wanted quality information and quality segregation at the retail market. In contrast, Boland and Schroeder (2002) suggested producers should market high yielding animals rather than high quality grade animals, since consumer value leanness over marbling.

George and others (1999), in a study conducted in eight U.S. cities to characterize retail beef loin steaks according to quality grade, post-fabrication aging, and tenderness, concluded that one out every four Select grade strip loin steaks has a tenderness problem. In addition, loin steaks from carcasses grading Select were the least tender and the most variable in tenderness. Thus, he recommended the beef industry continue to investigate new methods for enhancing product quality and consistency in tenderness of Select carcasses (George and others, 1999).

Beef strip loin

On average, the beef strip steak (also known as striploin, shell steak, Delmonico, New York or Kansas City strip steak) weighs approximately 258 g and it is considered one of the highest quality beef steaks on the market (NCBA, 2005).

The Institute Meat Purchase Specifications (IMPS, 1996) describes the beef strip loin boneless (IMPS #180) as follows: "This item is boneless and consists of the anterior section of the loin and contains the 13th rib mark. The hanging tender and tenderloin shall be removed. The rib end shall follow the natural curvature of the 13th rib mark. The sirloin end shall be anterior to the hip cartilage, forming an approximate right angle with the length of the short loin, and exposes the *gluteus medius*. The flank side shall be ventral to, but not more than 3.0 inches (7.5 cm) from the *longissimus dorsi* at the rib end to a point on the sirloin end ventral to, but not more than 2.0 inches (5.0 cm) from the *longissimus dorsi*" (USDA, 1996).

Composition of beef strip loin steaks

According to Huff-Lonergan and Lonergan (2005), lean muscle contains approximately 75% water. The other main components are protein (approximately 20%), lipids or fat (approximately 5%), carbohydrates (approximately 1%) and vitamins, and minerals (approximately 1%; Huff-Lonergan and Lonergan, 2005). The nutritional information of one raw strip loin steak (approximately 258 grams) is presented in Table 2.1 (USDA, 2007a).

Table 2.1: Proximate analysis of beef short loin, top loin (separable lean and fat, trimmed to 1/8" fat, USDA Select, raw) as presented by the USDA National Nutrient Database for Standard Reference

Nutrient	Units	Value per 100 grams	Value in 1 steak 258g
Water	g	63.95	164.99
Energy	kcal	224	578
Protein	g	20.59	53.12
Total lipid (fat)	g	15.04	38.80
Ash	g	0.87	2.24
Carbohydrate, by difference	g	0.00	0.00
Fiber, total dietary	g	0.00	0.00
Sugars, total	g	0.00	0.00

USDA National Nutrient Database for Standard Reference (USDA, 2007a); National Nutrient Database (NDB) No: 13913; Nutrient values and weights are for edible portion

Common treatments to improve meat tenderization

As stated previously, it is well established that tenderness is the number one attribute affecting beef purchases. However, inconsistency in tenderness is an issue for the beef industry. Torrescano and others (2003) affirm that unpredictable variability in beef tenderness is a major problem for the consumer, and consequently, for all the production chain. In addition, Miller and others (2001) concluded that as beef steaks become tougher, flavor and juiciness have a greater effect on consumer satisfaction.

Belew and others (2003) affirm that there are four main factors that influence tenderness in meat, including postmortem proteolysis, intramuscular marbling, connective tissue, and the contractile state of the muscle. These factors also can differentiate tenderness between muscles from the same carcass. Moreover, tenderness can vary among and within individual bovine muscles (Kolle and others, 2004).

Belew and others (2003) ranked 40 bovine muscles according to WBS value and classified muscles as “very tender” (WBS < 3.2 kg), “tender” (3.2 kg < WBS < 3.9 kg), “intermediate” (3.9 kg < WBS < 4.6 kg) or “tough” (WBS > 4.6 kg). According to this

categorization, strip loin steaks are “tender” with a shear force of 3.63 kg (Belew and others, 2003).

Bellew and others (2003) also stated that different beef cuts responded better to different tenderization strategies. Therefore, enhancement practices are very common in the U.S. to decrease variability in tenderness and juiciness and increase weight of salable product (Liu and others, 2006; Hutchison and others, 2007). Some of the most common techniques used to improve tenderness in beef include, mechanical tenderization, electrical stimulation, natural aging, and injection of enhancement solutions.

Mechanical tenderization

Currently, mechanical tenderization (e.g. blade tenderization, needle tenderization) is one of the most successful technologies to enhance tenderness. Mechanical tenderization involves the penetration of the meat with closely spaced thin blades or needles which sever the myofibrillar structure and connective tissue into shorter fragments thus increasing tenderness of the meat (Pietrasik and Shand, 2005; Heller and others, 2007; Schwartz and Mandigo, 1977).

Mechanical tenderization will effectively reduce variability among meat muscles, improve textual characteristics, provide instant tenderization (without additional aging), decrease shear values (15-20% for roast beef), and improve the marketability of certain beef cuts without changing the chemical properties of the meat (Pietrasik and Shand, 2004; Pietrasik and Shand, 2005; Schwartz and Mandigo, 1977; Liu and others, 2006; Heller and others, 2007; Loucks and others, 1984). However, bacterial outbreaks have been associated with mechanical tenderization (USDA, 2005; Gill and others, 2005). A study by Marsden and others (1999) reported that single-pass blade tenderization resulted

in internalization of approximately $3x10^3$ CFU/g of *Salmonella* spp. with surface inoculums of 10^7 CFU/g.

The USDA has recommended that meat tenderized via needle or blade tenderization mechanism needs to be cooked to an internal temperature of 68.34°C (155°F), in contrast with in-tact whole muscle beef cuts, which need to be cooked to an internal temperature of 62.78°C (145°F). In addition, USDA urges establishments that produce mechanically tenderized beef products to reassess their Hazard Analysis and Critical Control Points (HACCP) plans to reduce the risk of foodborne illness from meat products (USDA, 2005).

Electrical stimulation

A major cause of meat toughness is the contraction of muscles during chilling. Carcasses are required to be chilled rapidly to prevent microbial growth. However, if a muscle is chilled rapidly before the onset of rigor mortis, the myofibrils contract causing tougher meat (Australian Meat Technology-AMT, 1996).

Electrical stimulation (ES) involves the application of an electrical current to the carcass to improve tenderness. For this process, current can be applied at either low voltage (voltage less than 100 V) or high voltage (voltage greater than 100 V) for 30 seconds. According to Roeber and others (2000), some meat packers in U.S. are using high voltage ES since 1970's. Stiffler and others (1999) demonstrated that, on average, electrical stimulation improves tenderness approximately 23% in beef meat. In addition, Roeber and others (2000) documented that ES-treated steaks with medium voltage, medium duration; medium voltage, long duration; high voltage, medium duration or high

voltage, long duration, reduced shear force by 0.42 kg, 0.39 kg, 0.66 kg, and 0.46 kg, respectively.

Post-mortem aging

According to Schwartz and Mandigo (1977), aging is the most common method used to increase meat tenderness. Aging reduces the strength of the myofibrillar structure but does not impact connective tissue. During this process, meat is stored at refrigerated temperatures for extended periods of time, on average, between 7 to 30 days. Hutchison and others (2007) documented that for top sirloin steaks, seven days of aging was sufficient to improve tenderness.

There are two types of postmortem aging, wet or dry aging. During the wet process, cuts of meat are vacuum packaged and refrigerated. During the dry process, subprimals or entire carcasses are exposed to air (Campbell and others, 2001).

Dry aging is not widely utilized because it results in dehydration of cuts or carcasses, sometimes losing up to 10% of the original weight (Campbell and others, 2001). In addition, there are some concerns about the flavor profile of dry aged beef such as stronger beefy and/or brown-roasted flavor as compared to wet aged or un-aged beef (Campbell and others, 2001). However, dry aging adds economic value and provides distinctive palatability profiles for high quality markets, which is not obtainable with vacuum aging (Boland and others, 1999).

Campbell and others (2001) reported that steaks dry aged for 21 days had 0.4 kg lower Instron shear force compared with the control. In addition, Gruber and others (2006) observed a reduction in Warner-Bratzler shear force (WBSF) for USDA Select strip loin steaks from 6.64 kg to 5.02 kg after 14 days of aging (Gruber and others, 2006).

Injection enhancement

Enhanced meat products are any meat product which has been injected with a solution. Typically, enhanced beef is injected with a water solution including salt, phosphate, sodium lactate, seasonings, and flavorings to improve texture, flavor, tenderness, and consistency. An enhancement solution injected at 6-10% may also help to decrease the lipid oxidation process (Seyfert and others, 2005). Moreover, the addition of rosemary extract in enhancement solutions contributes to longer shelf-life (Morgan, 2003).

In 2003, Morgan concluded that enhanced beef steaks are more tender than non-injected steaks of similar USDA grade. McGee and others (2003) indicated that injection of sodium tripolyphosphate, sodium chloride, and sodium lactate helps traditionally less tender beef cuts meet consumer expectations of a higher quality product. In addition to increasing tenderness and juiciness, enhancement allows product from a lower quality grade to be cooked to a higher degree of doneness without sacrificing consumer satisfaction (Robbins and others, 2003).

Sheard and Tali (2004) documented that improvements in juiciness and tenderness are better for marinated products compared with those that can be achieved by production or processing factors when evaluated by a sensory panel. In addition, in a study conducted by Bagley (2006), steaks enhanced with salt, phosphates, and papain solution were significantly more tender and juicier compared with those that were bladed tenderized or left untreated. Moreover, it is also stated that marinated pork products can reduce shear force as much as 50% when compared to non-marinated meats (Sheard and

Tali, 2004). This reduction is mainly due the retention of water by the myofibrillar structures (Sheard and Tali, 2004).

Furthermore, Murphy and Zerby (2004) concluded that combining NaCl, dextrose and phosphate result in improved tenderness in lamb. These compounds appear to interact resulting in a synergistic effect on tenderness due the effect of increased ultimate pH and decreased cook loss. However, these additives individually did not provide significant improvements in tenderness.

Use of phosphates as enhancement

Boles and Swan (1997) have shown that pre-rigor injection of salt, lactate and phosphate improved tenderness. However, the injection of phosphate has the greatest effect on tenderness due the improvement of water holding capacity by rising meat pH and solubilizing myofibrillar proteins. In addition, Lawrence and others (2004) concluded that the injection of phosphates and salt to beef steaks provided higher yields, increased water holding capacity of muscle, and resulted in higher sensory panel scores panels for tenderness than enhanced steaks treated with a calcium lactate solution.

In addition, Robbins and others (2003) mentioned that beef steaks are more tender and juicier when enhanced with a phosphate/salt-containing solution. However, the enhancement also had detrimental effects on color during retail display (Robbins and others, 2003).

It is well documented that phosphates enhance water holding capacity by increasing the pH from its isoelectric point (pH 5.5) to a more neutral pH (between 6 and 7), and by raising the ion strength to ~0.6. The effect of phosphates in increasing water holding capacity is also due to its ability to sequester divalent metal ions and to dissociate

actomyosin. Therefore, for maximum water binding a pH between 6 and 7 and ion strength of 0.6 are required (Shahidi and Synowiecki, 1997).

The maximum amount of food-grade phosphates permitted, by the USDA for incorporation in meat products, is 0.5%. In addition, USDA has approved, for use in curing brines, the addition of sodium tripolyphosphate, sodium hexametaphosphate, sodium acid pyrophosphate, sodium pyrophosphate, monosodium phosphate and disodium phosphate (USDA, 1982).

Health concerns with use of phosphates

According to Higdon (2007), the average phosphorus intake by the an average American has increased 10% to 15% over the past 20 years. This increment can be attributed to phosphoric acid in soft drinks and phosphate additives in processed foods. Serum phosphate levels can rise slightly with a high phosphorus diet, especially after meals. High phosphate levels in the blood reduce the formation of the active form of vitamin D (calcitriol) in the kidneys, reduce blood calcium, and lead to increased parathyroid hormone (PTH) release by the parathyroid glands.

In addition, elevated phosphate levels have been implicated with vascular morbidity and mortality among dialysis patients. A decline in renal function leads to phosphate retention; however, serum phosphate levels appear high until relatively late in the course of chronic kidney disease (CKD). Thus, high serum phosphate levels are associated with death and myocardial infarction in patients with stage 3-4 CKD. Additionally, higher serum phosphate levels also are associated with a greater prevalence of heart failure, cardiovascular disease, and cardiovascular medication use (Kestenbaum and others, 2005; Tonelli and Pfeffer, 2007; Tonelli and others, 2005). Furthermore, Caring for

Australians with Renal Impairment (CARI, 2006) indicates that in stage 5 kidney disease, a combination of high calcium, high phosphates, and low parathyroid hormone level is associated with the worst effect. Therefore, CARI suggests to patients with kidney failure (stage 5 or end-stage) and those treated with haemodialysis or peritoneal dialysis, to maintain the serum levels between 3.5 to 5.5 mg/dL (CARI, 2006).

In addition, diabetes is the most common cause of end-stage renal disease (Raffaitin and others, 2007). According to CDC (2005), diabetes is the leading cause of kidney failure, accounting for approximately 44% of new cases in 2002. The same year, 153,730 people with end-stage kidney disease due to diabetes were living on chronic dialysis or with a kidney transplant in US (CDC, 2005).

It is also known that high consumption of phosphates can cause allergies, diarrhea, hardening of soft tissues or organs, and interferes with adsorption of iron, calcium, magnesium, and zinc (Shahidi and Synowiecki, 1997; Waterhouse, 2000; Fine and others, 1998).

Role of sodium chloride in enhancement solutions

The inclusion of salt improves yield and palatability characteristics, meat color, and extends shelf life. Moreover, consumer acceptability of beef steaks increases when meat is enhanced with salt brine solution (Baublits and others, 2006b; Robbins and others, 2003).

Boles and Swan (1997) reported that a sodium chloride (NaCl) solution increased cooking yields, decreased post mortem pH decline, and increased water-binding. In addition, Judge and Aberle (1980) found that the infusion of sodium chloride into pre-rigor meat increased the water-holding capacity due to the expansion of the myofibrillar

lattice. In a recent study, Baublits and others (2005) concluded that the inclusion of sodium chloride in the enhancing solutions potentially helps to prevent off-flavor differences with varying phosphates and phosphate concentrations.

The myofibrillar proteins of muscle are insoluble in low-salt concentrations; however, they solublize in concentrated salt solutions (300 to 600 mM). This property is important to give proper texture for certain products. In addition, salt enhances sensory properties, and effects preservation. Salt can also reduce the growth rate of spoilage bacterial by reducing the water activity which is influenced by the level of salt in the aqueous phase in a product. Thus, salt plays several important roles in the meat processing industry (Mathews and Strong, 2005).

Health concerns with use of sodium chloride

Currently, the food industry is under pressure from consumers and government to deliver reductions in sodium content in products due to its relationship with hypertension. Several researches have shown that an excess in sodium in the diet is the primary cause of high blood pressure in the United States (USDA, 2006).

Bashyam (2007) indicated that hypertension is common in societies with a high intake of salt. However, there are some individuals who consume excess salt but do not develop hypertension. Thus, the development of hypertension depends both of the environmental factors (e.g. salt) and the individual's genetic background (Bashyam, 2007). In addition, an increase of sodium intake has also been associated with altered structure and function of large arteries and cardiovascular disease (Varagic and others, 2006; Cailar du and others, 2002).

The Food Safety and Inspection Service (FSIS) established that individual meat or poultry products that use the term "health" or any other derivative on the label can not contain more than 480 mg of sodium per labeled serving size and 600 mg of sodium for a meal-type product per serving size (USDA, 2006). The FDA currently recommends maintaining sodium consumption below 2,300 mg per day.

Therefore, potassium chloride has been used to substitute sodium chloride up to a 40% level. The use, in a ratio of 40:60, reduces sodium up to 34-35% (Mathews and Strong, 2005).

Ammonium hydroxide

Ammonium hydroxide is Generally Recognized as Safe (GRAS) by FDA (21 CRF 184.1139) when used in accordance with Good Manufacturing Practices (GMP). Ammonium hydroxide can be used as a leavening agent, pH control agent, surface-finishing agent, boiler water additive, or feed additive. The pH and the relative density vary with the concentration; as concentration increases, pH will increase to 13.5 at 30% concentration. Oral exposure and ingestion of ammonium hydroxide are linked to liver damage and hepatic coma (Organic Materials Review Institute, 2001).

Ammonium hydroxide as antimicrobial agent

Ammonium hydroxide has been used to prevent pathogenic bacteria growth for the food industry. Gupta and others (1988) concluded that adding ammonium hydroxide to ground beef, at 0.134 to 0.67M, was effective in reducing total viable aerobic count and Gram-negative bacteria at -20°C, 4°C, or 37°C. It is also reported by Gupta and others (1988) that this antibacterial activity is due to the toxicity of ammonia rather than the rise in the pH.

Additionally, Himathongkham and others (2001) concluded that fumigation with ammonia is an effective treatment for reduction of *Escherichia coli* 0157:H7 and *Salmonella* in alfalfa seed and mung beans. Stopforth and others (2005) evaluated the effectiveness of applying ammonium hydroxide on beef contaminated with *E. coli* O157:H7 and *S. Typhimurium*. They concluded that ammonium hydroxide is more effective controlling Gram-negative than Gram-positive bacteria since ammonium hydroxide readily solubilizes the outer membrane of Gram-negative walls resulting in damage to the wall and cytoplasmic membrane (Stopforth and others, 2005). However, it is also established that for ammonium hydroxide to have an effective bactericidal effect, meat pH must be greater than 9.0 (Stopforth and others, 2005).

Ammonium hydroxide as meat enhancer

Gupta and others (1988) concluded that ground beef treated with ammonia hydroxide improved in water holding capacity and reduced protein loss and cook loss in ground beef. Moreover, samples treated with ammonium hydroxide were more pinkish in color than untreated samples (Gupta and others, 1988).

In addition, Hamling and Calkins (2006) evaluated beef chuck and round muscles enhanced with ammonium hydroxide and salt. They reported that ammonium hydroxide improved tenderness due to the increase in the pH of the muscles treated (Hamling and Calkins, 2006). Furthermore, Hamling and others (2006) documented that trained tasted panels found improved tenderness, juiciness, and overall acceptability, and reduction in connective tissue in steaks treated with 20% pump solution containing ammonium hydroxide versus untreated samples.

Relationship between pH and tenderness

It is well established that the increase in ultimate meat pH leads to improvements in meat tenderization through changes in proteolitic activity, such as calcium-activated proteases, which have an optimum activity close to pH 7.0. Moreover, it has been suggested that there is a negative relationship between pH and sarcomere length. Meat has lower shear force values at higher pH because the stretching of sarcomeres avoids toughness (Purchas, 1990). In addition, Offer and Trinick (1983) suggest that most of the water present in meat is situated in the myofibrils, between the thick and thin filament spaces. It has been estimated that nearly 85% of the water in muscle cells is held within the myofibrils (Kołczak and others, 2007). Thus, an increase of the pH to a pH greater than 5.0 leads in a negative charge of both thin and thick filaments, followed by a repulsive force between filaments, which tends to enlarge the lattice, causing changes in the volume of the myofibrils, and, consequently, increases the water holding capacity.

Conclusion

The extensive use of phosphates as food additives by the beef industry is a health concern for certain segment of consumers, mainly those suffering from CKD. Therefore, the beef industry needs to find new alternatives to the use of phosphates, while maintaining desirable characteristics. There are few studies that have evaluated the injection of ammonium hydroxide as an enhancement solution in beef. These studies provided evidence that ammonium hydroxide effectively can reduce bacterial growth, raise final meat pH, enhance water holding capacity, increase tenderness and juiciness, and reduce connective tissue (Hamling and others, 2006; Hamling and Calkins, 2006; Gupta and others, 1988). Thus, the subsequent study was conducted to evaluate the

injection of an ammonium hydroxide, pH 10, solution as an alternative for meat enhancement.

CHAPTER III

EVALUATION OF A HIGH pH SOLUTION AS AN ALTERNATIVE TO PHOSPHATE FOR MEAT ENHANCEMENT

ABSTRACT

Paired USDA Select beef strip loins, aged for 2 days, were enhanced to 110% of original weight with either a high pH solution containing 3.6% sodium chloride, 1% Herbalox seasoning and adjusted to pH 10 with ammonium hydroxide (~0.1%, FFC grade); or a phosphate based solution prepared using 3.6% sodium chloride, 1% Herbalox seasoning, and 4.5% sodium tripolyphosphate. In order to evaluate beef quality, sample pH, proximate analysis, microbial growth, lipid oxidation, color score, purge loss, cook loss, Warner-Bratzler shear force, and sensory panel were measured during 14 days of storage under retail conditions. Composition of enhanced steaks differed only in protein content ($P < 0.05$). Phosphate enhanced steaks were nearly 2% lower in protein content. The lower protein content was attributed to the higher purge observed in ammonium hydroxide enhanced steaks. Overall, phosphate enhanced steaks performed better than ammonium hydroxide treatment in all quality parameters measured except for controlling microbial growth. The ammonium hydroxide treatment had significantly lower ($P < 0.05$) aerobic and anaerobic plate counts. The initial aerobic count for the ammonium hydroxide treatment was 5.2×10^3 cfu/g and for phosphate treatment was 2.7×10^4 cfu/g. However, after 14 days of storage at 34°F (~1°C), the final aerobic counts were 2.7×10^5

cfu/g and 2.6 x 10^7 cfu/g for the ammonium hydroxide and phosphate treatments, respectively. Similar observations were made for total anaerobic counts. Although quality improvements were not as good as phosphate, these data still demonstrated that adjusting the enhancement solution to pH 10 with ammonia hydroxide is effective in controlling bacterial growth, generating higher yields, increasing water holding capacity, and improving tenderness and juiciness in beef steaks compared with untreated steaks (based on data from previous studies).

Keywords: Ammonium hydroxide, phosphate, pH, enhancement, meat

INTRODUCTION

It is important for the beef industry to meet consumer and retail market demands. Retail demands include an ever increasing desire for improving low value cuts and carcass value. As a result, value-added approaches such as novel fabrication techniques have been used to satisfy consumer demands (Robbins and others, 2003). Also, solution enhancement has been widely used to improve palatability in order to increase the acceptance of lower value cuts of meat (Morgan and others, 1991). Thus, currently enhanced meat products are extensively produced by the meat industry. There are many advantages to using meat enhancers such as improving tenderness, moisture, and flavor; extending shelf life; increasing food safety; improving appearance; developing new products; consumer convenience; reducing rancidity; and increasing profitability (Foote and others, 2004). In most cases, a combination of phosphates, salt, nitrites, antioxidants, sugar, and/or flavorings are added or injected into meats as an enhancement solution to achieve these advantages. However, at present, the extensive use of phosphates presents

two concerns for the industry. Phosphates are chemical additives, and therefore, can be perceived by the consumer as ingredients that are not natural to the product; a concern because some consumers demand "natural" beef products (Perez-Rocha and Varsi, 2003). In addition, phosphates are a health concern for certain segments of society. People suffering from kidney disease, impaired renal function or perfusion, dehydration, or uncorrected electrolyte abnormalities must avoid foods containing high levels of phosphates (Block and others, 1998; Goodman and others, 2004; Ibels and others, 1978; Tonelli and others, 2005; Vann and Mireles DeWitt, 2007). Consequently, it is important for the meat industry to find alternatives to decrease the utilization of phosphates in enhancement solutions in order to better serve all consumers. Therefore, the objective of this study was to compare color stability, lipid oxidation, proximate analysis, purge analysis, cook loss, shear force, microbial growth, and sensory attributes of beef subprimal strips loins injected with a high pH ammonium hydroxide enhancement solution to those injected with a commercial used phosphate enhancement solution.

MATERIALS AND METHODS

Sample collection

Paired USDA Select beef strip loins were randomly identified and collected at a beef fabrication facility. Strip loins were labeled, vacuum packaged, transported to the Food and Agricultural Products Research and Technology Center (FAPC) on the campus of Oklahoma State University (OSU), and stored at 4ºC.

Sample enhancement

Strip loins were aged at 4ºC for 2 days. Initial weight or green weight of each strip loin was recorded. Each paired strip loin was randomly assigned to be injected with either a phosphate or the ammonium hydroxide based solution at 4ºC using a stitch pump enhancer calibrated to inject at 110% of the recorded green weight.

Enhancement solutions

The ammonium hydroxide solution was an aqueous solution containing 1% Herbalox seasoning type HTW (Kalsec, Kalamazoo, Mich., U.S.A.) and 3.6% sodium chloride (w/w) adjusted to pH 10 using food grade ~0.1% ammonium hydroxide (Fisher Scientific, Fair Lawn, New Jersey, U.S.A.). The phosphate solution was prepared with 4.5% sodium tripolyphosphate, 3.6% sodium chloride, and 1% Herbalox seasoning type HTW. The pH of the phosphate solution was 8.45.

Fabrication of strip loins

After injection, strip loins were held for 30 min at 4ºC. To equilibrate, the weight of each strip loin was recorded prior to fabrication into 2.54 cm steaks using a standard band-saw. Individual steak weights were recorded. Steaks were placed into plastic trays with absorbent pads and packaged under a high-oxygen (80% oxygen, 20% carbon dioxide) modified atmosphere packing (MAP) using a MAP machine (G. Mondini S.p.a., Type CV/VG-S, Brescia, Italy). Packaged steaks were placed in dark storage at 4ºC for 4 days in order to simulate transportation to retail stores. After 4 days dark storage, steaks were placed in a retail case at 4ºC under cool white fluorescent lights, with a continuous intensity of 75 foot-candles for 14 days.

Day 5 to 19 sampling

Three packaged steaks were randomly selected from each treatment on days 5 (day 0 of retail display), 12 (day 7 of retail display), and 19 (day 14 of retail display). One packaged steak was used to measure steak purge, HunterLab color, cook loss, and shear force analysis. A second steak was used for steak purge, HunterLab color, cook loss, and sensory analysis. The third steak was dedicated for completion of aerobic plate count (APC), anaerobic plate count, proximate analysis, and 2-thiobarbituric acid reactive substances (TBARs) analysis (Appendix A).

Proximate analysis

The steaks selected for proximate analysis and TBARs analysis were sampled first for microbiological assay and then frozen. Steaks were thawed and sampled for TBARs analysis. The remainder of the steak was powdered using liquid nitrogen and a frozen waring blender in a cold room at 4ºC. Powdered samples were measured for moisture (Association of Official Analytical Chemists [AOAC], method number 950.46), crude fat (AOAC, method number 960.39), ash (AOAC, method number 920.153), and protein (AOAC, method number 928.08).

Microbiological analysis

Aerobic and anaerobic plate counts were conducted in accordance with the official methods of analysis of AOAC international by FoodProtech® (Stillwater, Okla., U.S.A.).

Lipid oxidation

Samples from day 5, 12, and 19 were packaged in Whirl-Pak® bags, and frozen at -20ºC until analyzed. A 10 g sample was taken from the surface (0.5 to 0.7 cm) of the

steak and analyzed according to a modified method published by Buege and Aust (1978). Results were reported as mg malondialdehyde (MDA) equivalents per kg of fresh meat.

Color score

Steaks were analyzed for color stability according to the Guidelines for Meat Color Evaluation (AMSA, 1991) by a trained, six member panel. Panelists scored steaks twice a day (am and pm) for lean color (8 = Bright Cherry-Red, and 1 = Extremely Dark Brown), fat color (8 = Creamy White, and 1 = Dark Brown or Green), percent discoloration (7 = None [0%], and 1 = Complete [100%]), and overall acceptability (7 = Extremely desirable, and 1 = Extremely undesirable). The average of the six evaluations was used for each steak. Also, the average of the am and pm scores was the used for each day. Color scores were taken on steaks randomly selected for day 19 analysis.

HunterLab color score

Quantitative evaluation of color was measured using a MiniScan^{TM} XE Plus (HunterLab, Reston, VA). Three readings were taken on each steak, avoiding any seam fat, prior to cooking and the average of the three readings was documented for each steak. For each treatment, two steaks were measured (sensory evaluation and shear force) from each subprimal (n = 10) on day 5 and day 12. Lightness (L* = 0 indicates black, and L* = 100 indicates white), a* (negative values indicate green, and positive values indicate red), and b* (negative values indicate blue, and positive values indicate yellow) values were measured on days 5, 12, and 19.

Purge analysis

The amount of liquid, or purge lost during the retail storage of the steak was recorded by subtracting stored steak weight from initial steak weight.

$$\%\, purge = \frac{\left(weight_{priortopackage}\right) - \left(weight_{afterstorage}\right)}{\left(weight_{priortopackage}\right)} x100$$

Shear force

The steaks selected for shear force were cooked to an internal temperature of 70ºC (medium degree of doneness), allowed to cool at room temperature (21ºC), and then measured according to Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat (AMSA, 1995). Shear force was determined using the Universal Instron testing Machine with a Warner-Bratzler shear head attachment. Six cores were taken from each steak, parallel to the muscle fiber orientation, sheared, and then averaged for the steak.

Cook loss

Prior to cooking, raw steak weight was recorded. Then, steaks were cooked as outlined in the section for shear force. The amount of moisture lost through cooking steaks was calculated:

$$cookloss = \frac{\left(weight_{priortocook}\right) - \left(weight_{cooked}\right)}{\left(weight_{priortocook}\right)} x100$$

Sensory panel

Sensory evaluation was conducted following the methodology in the Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Fresh Meat (AMSA, 1995). An experienced group of panelist (n = 20) was asked to individually evaluate two cooked steak cubes (2.54 cm x 2.54 cm) for tenderness (8 = Extremely tender, and 1 = Extremely tough), juiciness (8 = Extremely juicy, and 1 = Extremely dry), connective tissue (4 = None, and 1 = Extremely abundant), and overall acceptance (7 = Extremely desirable, and 1 = Extremely undesirable). Each panelist was

randomly assigned samples from three paired strip loins to evaluate. Samples from paired strip loins (phosphate vs. ammonium hydroxide treated) were evaluated six times by six different panelists.

Statistical analysis

Averages were calculated using Microsoft Excel. Data were analyzed using Proc Mixed of SAS (SAS Inst. Inc., Cary, N.C., U.S.A.) as a 2 x 3 factorial in a randomized block design using $\alpha = 0.05$. Sample ID was the random variable; treatment and day were fixed variables. When appropriate, means were separated using the Duncan test.

RESULTS AND DISCUSSION

Enhancement

The target percent pump weight was 110% of the initial weight for the enhancement solutions. Ammonium hydroxide enhanced strip loins were actually enhanced at 11.50% ± 2.09% while phosphate enhanced strip loins were enhanced at 13.58% ± 1.61% pumping rate (Appendix B).

Sample pH

Treatment, day, and the day by treatment interaction for pH were not significant ($P > 0.05$). The ammonium hydroxide enhanced steaks had a lower numerical pH than the phosphate enhanced steaks (5.73 ± 0.10 vs. 5.99 ± 0.12, respectively; Figure 3.1). The final pH of meat is important with respect to maintaining color, holding water, and improving tenderness. A higher final meat pH is typically attributed to the improvement of these properties. Smith and others (1984), reported a final pH of 5.97 in phosphate enhanced beef round roast and a final pH of 5.46 for untreated beef round roast. In

addition, Sheard and Tali (2004), reported a final pH of 5.94 for salt and phosphates enhanced pork loin and 5.45 for untreated steaks. Baublits and others (2006a) observed a final pH of 5.51 and 5.47 in beef *biceps femoris* injected with 2.0% sodium chloride at 12% and 18% pump rate, respectively. It can be stated that ammonium hydroxide pH 10 solution has effectively raised the final meat pH. These data are in consistency with Hamling and Calkins (2008) that reported, pH values increased from around 5.60 to 6.14 or higher of triceps brachii, biceps femoris, and rectus femoris injected with 1% sodium chloride and sufficient ammonium hydroxide to adjust the pH of the brine solution to 11.4.

Proximate analysis

No significant differences for fat, ash, or moisture content between treatments were observed (Table 3.1). Protein content was not significant over days; however, there was a significant difference among treatments ($P < 0.05$). The steaks enhanced with ammonium hydroxide solution had higher protein content (20.18 ± 0.80%) than steaks enhanced with phosphate (18.64 ± 0.65%). The higher percent of protein in ammonium hydroxide enhanced steaks was attributed to a higher purge loss. According to the USDA National Nutrient Database for Standard Reference (USDA, 2007a), the percent of moisture of USDA Select strip loin steak is 63.95%, thus ammonium hydroxide pH 10 solution, did improve percent of moisture of enhanced steaks (74.49%).

Microbiological analysis

Aerobic and anaerobic microbial growth was significant between treatment day and their interaction ($P < 0.05$). Microbial populations were lower in steaks enhanced with ammonium hydroxide. Microbial populations of ammonium hydroxide steaks at the

end of the study (14 d retail of display) were essentially the same as the phosphate injected steaks at 0 d retail display (day 5 of study; Fig. 3.2). These finding are in agreement with Gupta and others (1988) that reported ammonium hydroxide caused a decrease in the total aerobic bacteria and Gram-negative bacteria in ground beef stored at 37ºC, 4ºC and -20ºC.

Lipid oxidation

Steaks enhanced with phosphate had lower ($P < 0.05$) TBARs value (mg maldonaldehyde eq/kg) than ammonium hydroxide enhanced steaks (Fig. 3.3). Phosphate enhanced steaks had 0.15 ± 0.02 mg maldonaldehyde (MDA) per kg of fresh meat compared to 0.33 ± 0.02 mg MDA/kg of fresh meat for ammonium hydroxide steaks. Lipid oxidation was different between days ($P < 0.05$); however, all values were below 0.5 mg MDA/kg of fresh meat suggested as the critical borderline level for the detection of off-flavors by taste panels for the duration of the study (Jensen and others, 1998; Lauzurica and others, 2005; Lanari and others, 1995).

Subjective color

The interaction for day and treatment was significantly different ($P < 0.05$) for lean color, fat color, percent of discoloration, and overall acceptability (Fig. 3.4 and 3.5). The phosphate treatment was more effective at maintaining color stability up to day 18, at which time steaks from both treatments became completely dark brown. Phosphate enhanced steaks performed better with respect to lean color than the ammonium hydroxide treatment ($P < 0.05$; 4.97 ± 0.10 vs. 4.29 ± 0.91, respectively). Fat color scores were also different ($P < 0.05$) between treatments. The phosphate treatment showed less fat color deterioration than ammonium hydroxide treatment (6.20 ± 1.19 vs. 5.93 ± 1.30,

respectively). Also, steak discoloration was higher ($P < 0.05$) in phosphate (5.77 ± 1.14) than ammonium hydroxide (5.21 ± 1.11) enhanced steaks. Overall acceptability was higher ($P < 0.05$) for phosphate (4.66 ± 1.41) than ammonium hydroxide (3.81 ± 1.21) treatments.

Objective color

The objective color value was different between treatments ($P < 0.05$) but was not significant ($P > 0.05$) by day. Ammonium hydroxide treated samples were lighter (44.83 ± 3.0) than phosphate (40.18 ± 2.2) enhanced steaks (Fig. 3.6). These results are similar to findings by Baublits and others (2006a) that concluded L* values are lower for steaks treated with phosphate and salt than untreated steaks. In addition, several studies have reported a darker color in phosphate-injected steaks and suggested the color was a result of increased pH (Janz and others, 2005; Robbins and others, 2002). Miller (2007), affirms that due to high pH, lean meat surfaces act similarly to a dry sponge resulting in increased water binding capacity within the muscle. Therefore, the muscle appears dark because of higher intracellular water, which reflects less light (Miller, 2007). In addition, King and White (2006) affirm that meat with a pH above 6.2 has tightly water-retaining fibers that block oxygen transfer favoring the formation of deoxymyoglobin rather than oxymyoglobin. The purple-red myoglobin and the closed structure of the muscle absorbs light, making the meat appear dark (King and White, 2006). Regarding the redness, or a* value, there was a significant day by treatment interaction ($P < 0.05$). Redness of the steaks decreased over time (Fig. 3.7). Phosphate enhanced steaks were redder (20.97 ± 4.39; $P < 0.05$) than ammonium hydroxide enhanced steaks (15.62 ± 3.57). This is also in agreement with previous studies that reported phosphates are effective retaining redness

and increasing vividness (Baublits and others, 2006a; 2006b; Robbins and others, 2002). The b* value, or "yellowness", of steaks decreased over time for each treatment; however, there was not a significant difference between treatments (Fig. 3.7; $P = 0.73$). Baublits and others (2006a and 2006b) reported that steaks at 7 days of display were less yellow than those at 5 days of display.

Purge analysis

The percent of purge was different between treatments (Fig. 3.8; $P < 0.05$). Purge was 3.5% less for phosphate enhanced steaks (2.09 ± 2.29%) as compared to ammonium hydroxide enhanced steaks (5.40 ± 0.97%). Purge also increased over time ($P < 0.05$). However, the day by treatment interaction was not significant ($P = 0.64$). Higher protein content in ammonium hydroxide steaks was likely a result of increased purge. Baublits and others (2006c), reported a purge of 10.54% in beef *triceps brachii* injected with tap water at 12% pump rate, thus, it can be stated that ammonium hydroxide, ph 10 solution, has decreased the percent purge of enhanced beef strip loin steaks.

Cook loss

There was less cooking loss in the phosphate treatment than ammonium hydroxide treatment (20.53 ± 3.06 vs. 26.69 ± 2.17, respectively; Fig. 3.9). However, day and the day by treatment interaction did not have an effect on cook loss ($P = 0.32$ and $P = 0.86$, respectively). These finding are in agreement with Robbins and others (2002) that concluded phosphate enhancement decrease cooking loss, holding > 6% of the injected solution. In addition, Lawrence and others (2004) reported a cook loss of 23.43% in steaks enhanced with phosphate and salt solution at 11.5% by weight. Furthermore, Baublits and others (2006b) reported 38.95% cook loss in beef *triceps brachii* muscles

injected with tap water. In addition, Hayes and others (2005) reported a 31.4 % of purge in pork loin injected with 5.5% salt and 3.3% β-lactoglobulin enriched fraction. Therefore, these reports agree with the current data indicating high cook loss when steaks are enhanced.

Shear force

Phosphate enhanced steaks were significantly ($P < 0.05$) more tender than ammonium hydroxide enhanced steaks (2.58 ± 0.46 vs. 3.37 ± 0.90, respectively; Fig. 3.10). Day and day by treatment interaction did not affect tenderness ($P = 0.85$ and $P = 0.76$, respectively). The Warner-Bratzler shear (WBS) force value for phosphate enhanced steaks from this study (2.58 kg) is similar to the one (2.69 kg) reported by Lawrence and others (2004). Based on the value reported by Belew (2003) for untreated top sirloin steaks (3.63 kg), ammonium hydroxide pH 10 solution has improved tenderness (3.37 kg). In addition, Hamling and Calkins (2006) reported that shear force values in triceps brachii, biceps femoris, and rectus femoris decreased from 4.07 to 3.58, 4.02 to 2.67, and 4.35 to 3.35, respectively, when 15% pump injection containing ammonium hydroxide was applied. According to the classification of shear force by Belew and others (2003; see page 8), phosphate enhanced steaks correspond to the "very tender" category, while ammonium hydroxide enhanced steaks are "tender".

Sensory panel

Panelists documented that phosphate enhanced steaks were more tender (Table 3.2), juicier, and had less connective tissue ($P < 0.05$) than steaks enhanced with ammonium hydroxide. The average panel scores for tenderness and juiciness were 6.58 ± 0.61 and 6.03 ± 0.72 for phosphate enhanced steaks, respectively, while they were 4.98 ±

0.82 and 4.48 ± 0.78 for ammonium hydroxide enhanced steaks, respectively. These observations are similar to Lawrence and others (2004) that reported, enhanced steaks with phosphate and salt were tender, with a score of 6.4 for tenderness and 5.7 for juiciness. In addition, Vote and others (2000) reported 4.48 and 4.39 for tenderness and juiciness, respectively, of Select beef strip loins steaks injected with distilled water at 10% pumping rate. Regarding connective tissue, the overall mean for phosphate enhanced steaks was 3.48 ± 0.34 and 3.1 ± 0.37 for ammonium hydroxide enhanced steaks. Finally, overall acceptability was significantly higher ($P < 0.05$) for phosphate enhanced steaks than for ammonium hydroxide enhanced steaks (5.4 ± 0.49 vs. 4.13 ± 0.81, respectively). However, in previous study Hamling and Calkins (2006), documented that overall acceptability of enhanced steaks with ammonium hydroxide and salt were rated 1.31, 1.63, and 1.67 points higher than un-treated steaks from triceps brachii, biceps femoris, and rectus femoris, respectively. In addition, none of the traits analyzed for sensory panel were significantly different with regard to day or the day by treatment interaction except for connective tissue, which had a day effect ($P < 0.05$) only.

CONCLUSION

Although enhancement of USDA Select strip loins with an ammonium hydroxide solution at pH 10 was not as effective as the industry based phosphate injection solution, these data suggests that adjusting the enhancement solution to pH 10 using ammonium hydroxide can raise the final pH, control bacterial growth, generate higher yields, increase water holding capacity, and improve sensory attributes of beef steaks compared with un-treated steaks (based on data from previous studies). In addition, an ammonium

hydroxide solution did outperform the phosphate treatment in both aerobic and anaerobic microbial populations. However, it appears that the pH 10 solution did not sufficiently raise the final meat pH as phosphate based solution. Therefore future research should be conducted to determine if higher levels of ammonium hydroxide can sufficiently change the final meat pH to enhance color stability, water holding ability, and tenderness, while controlling microbial growth.

Table 3.1. Proximate analysis of injected strip loin steaks enhanced with ammonium hydroxide solution or phosphate based solution stratified by days and treatments.

Day	Treatment	% Moisture[a]	% Fat[a]	% Ash[a]	%Protein
5	Ammonium Hydroxide	74.49±1.55	4.79±1.15	1.39±0.08	20.26±0.81[b]
	Phosphate	75.47±1.05	4.60±1.46	2.12±0.19	18.53±0.57[c]
12	Ammonium Hydroxide	74.15±1.04	4.33±1.28	1.47±0.42	20.27±0.86[b]
	Phosphate	75.22±1.70	4.36±1.64	2.10±0.10	18.68±0.64[c]
19	Ammonium Hydroxide	74.98±1.56	4.83±1.92	1.37±0.09	20.02±0.79[b]
	Phosphate	75.13±1.52	4.88±1.59	2.03±0.11	18.70±0.46[c]

[a] Treatment and day effects for moisture, fat, and ash were not significant.
[b,c] Means appearing in the same column with different superscripts are significantly within day different ($P < 0.05$).

Table 3.2. Sensory evaluation of strip loin steaks enhanced with ammonium hydroxide solution or phosphate based solution.

Day	Treatment	Tenderness [1]	Juiciness [2]	Connective Tissue [3]	Overall Acceptability [4]
5	Ammonium Hydroxide	5.05[a] ± 0.85	4.72[a] ± 0.71	3.15[a] ± 0.34	4.50[a] ± 0.87
	Phosphate	6.67[b] ± 0.56	5.83[b]± 0.61	3.45[b] ± 0.38	5.32[b] ± 0.37
12	Ammonium Hydroxide	4.92[a] ± 0.82	4.23[a] ± 0.80	3.05[a] ± 0.41	3.75[a] ± 0.57
	Phosphate	6.50[b] ± 0.68	6.23[b] ± 0.79	3.52[b] ± 0.31	5.48[b] ± 0.60

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

[1] Tenderness scale were: 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] Juiciness scale were: 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] Connective tissue scale were: 4=None; 3=Slightly abundant; 2=Moderately abundant; 1=Extremely abundant.

[4] Overall acceptability scale were: 7=Extremely desirable; 6=Desirable; 5=Slightly desirable; 4=Acceptable; 3=Slightly undesirable; 2=Undesirable; 1=Extremely undesirable.

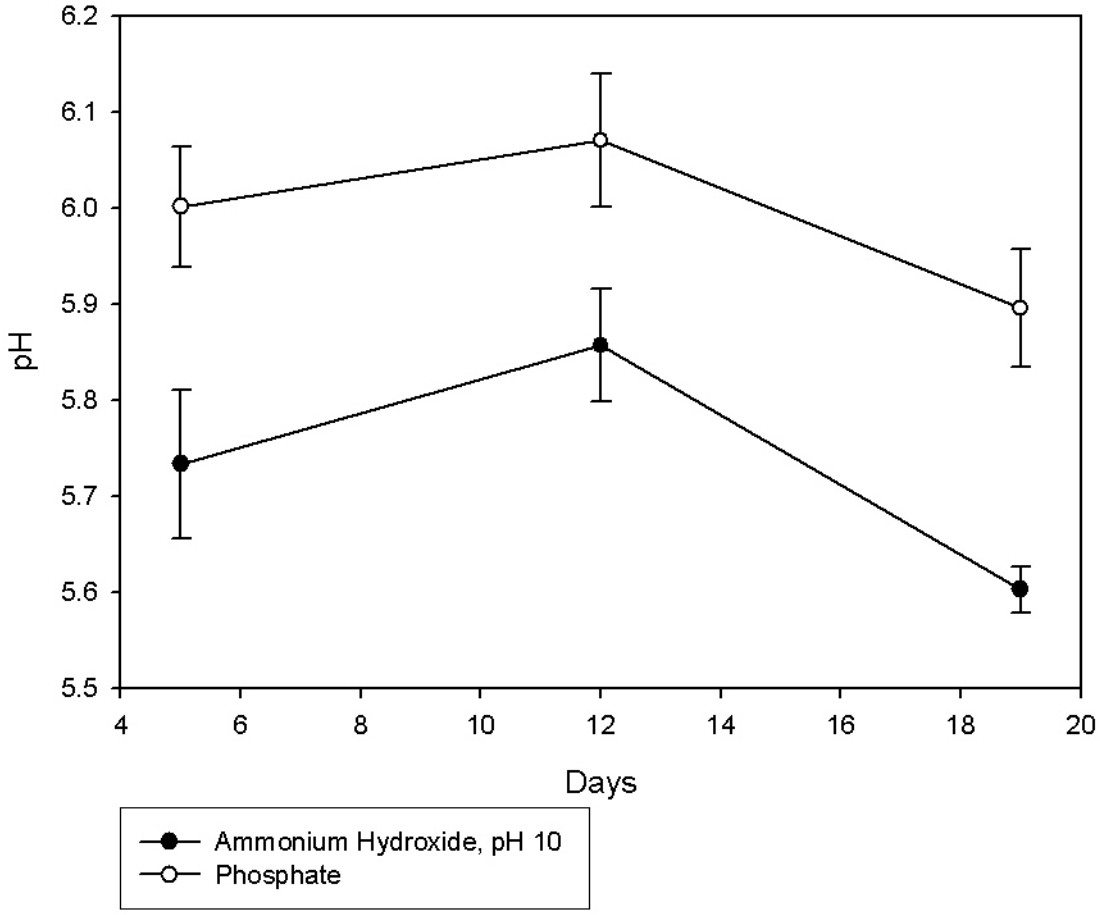

Figure 3.1. Strip loin steak pH after injection to 110% green weight with phosphate (4.5% sodium tripolyphosphate, 3.6% sodium chloride, 1% Herbalox) or ammonium hydroxide (3.6% sodium chloride, 1% Herbalox, pH 10).

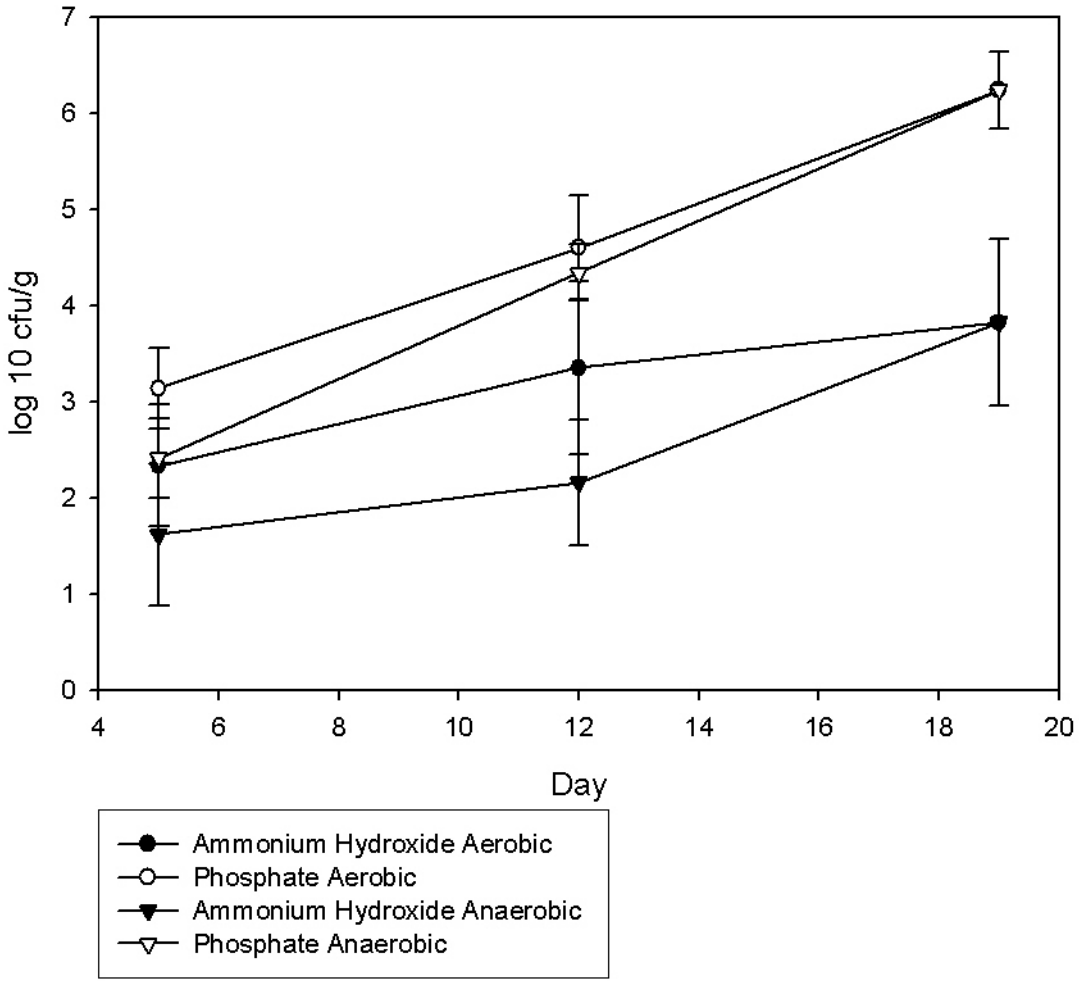

Figure 3.2. Aerobic and anaerobic microbial populations of strip loin steaks after injection to 110% green weight with phosphate (4.5% sodium tripolyphosphate, 3.6% sodium chloride, 1% Herbalox) or ammonium hydroxide (3.6% sodium chloride, 1% Herbalox, pH 10).

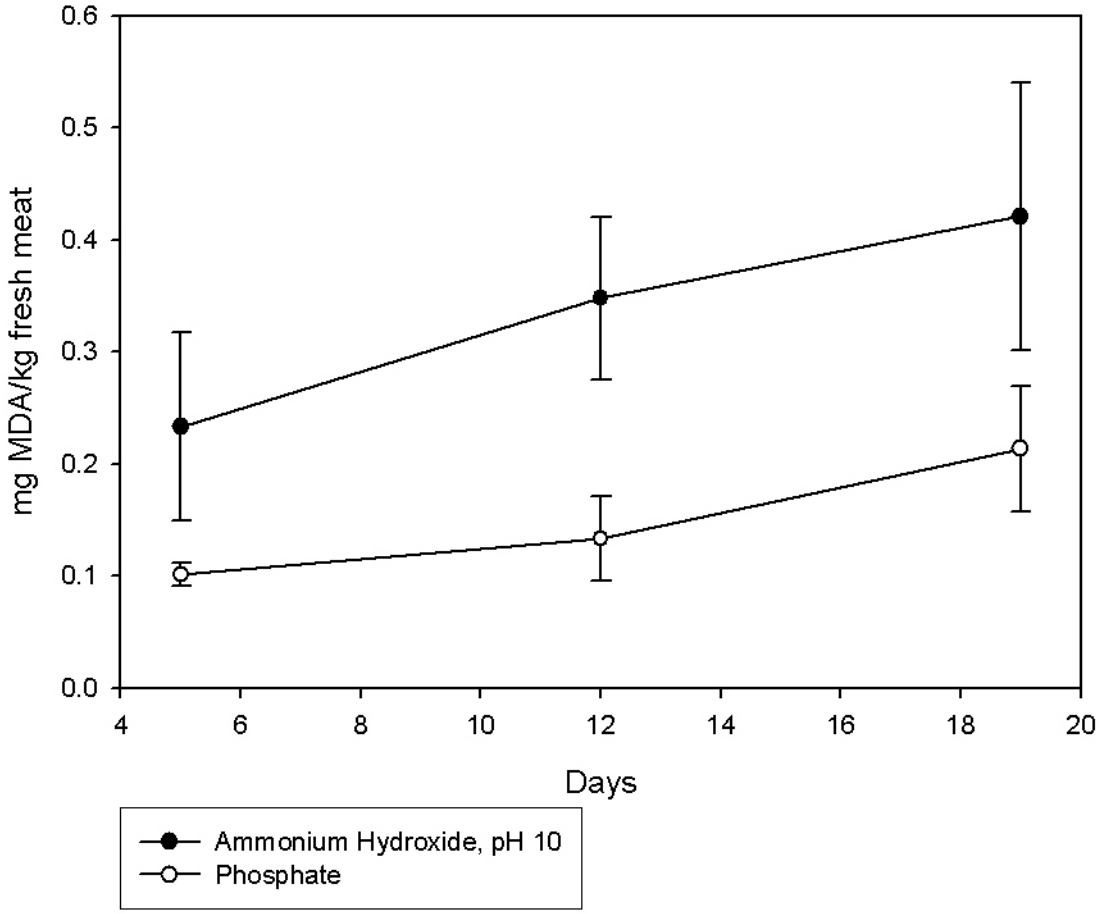

Figure 3.3. Lipid oxidation of strip loin steaks after injection to 110% green weight with phosphate (4.5% sodium tripolyphosphate, 3.6% sodium chloride, 1% Herbalox) or ammonium hydroxide (3.6% sodium chloride, 1% Herbalox, pH 10).

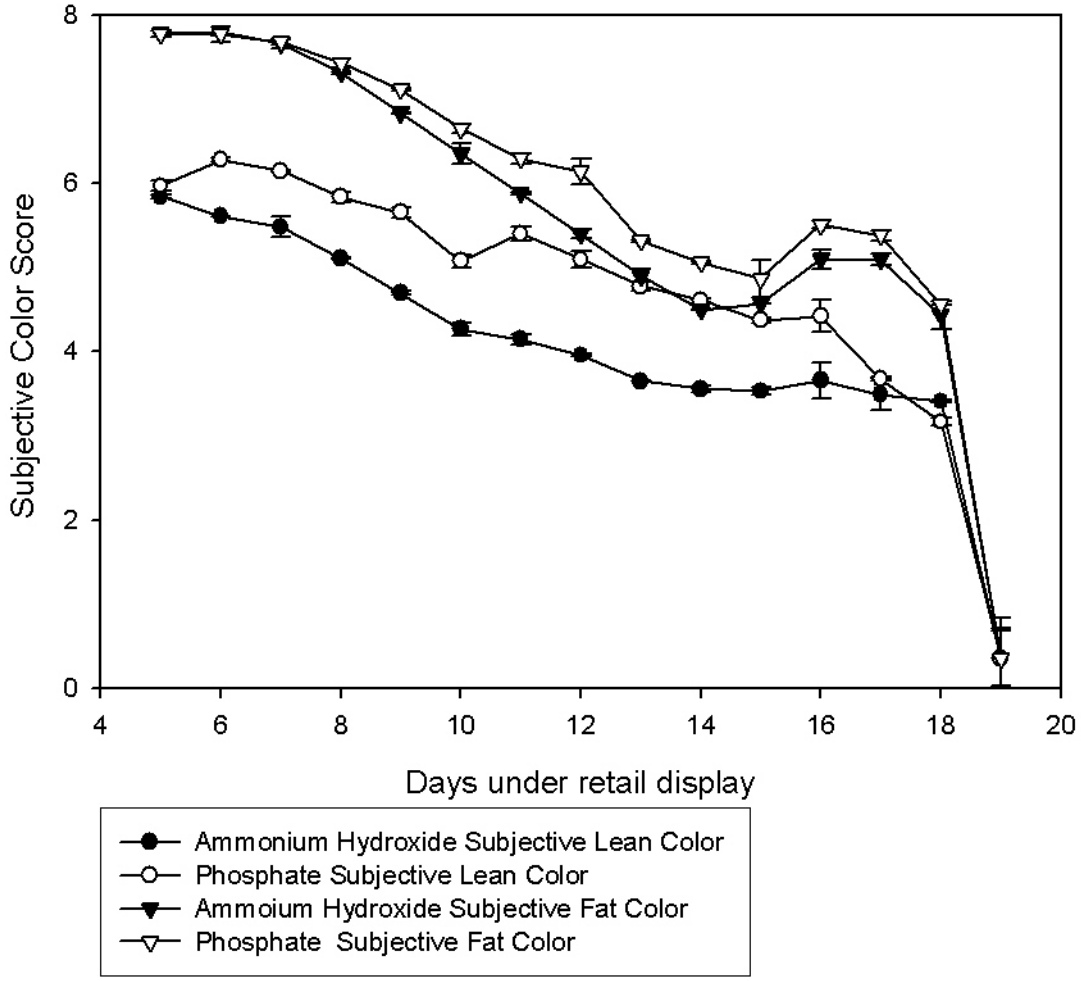

Figure 3.4. Subjective color evaluation of strip loin steaks after injection to 110% green weight with phosphate (4.5% sodium tripolyphosphate, 3.6% sodium chloride, 1% Herbalox) or ammonium hydroxide (3.6% sodium chloride, 1% Herbalox, pH 10). Lean scale: 8=Bright Cherry-Red, and 1=Extremely Dark Brown. Fat scale: 8=Creamy White, and 1=Dark Brown or Green.

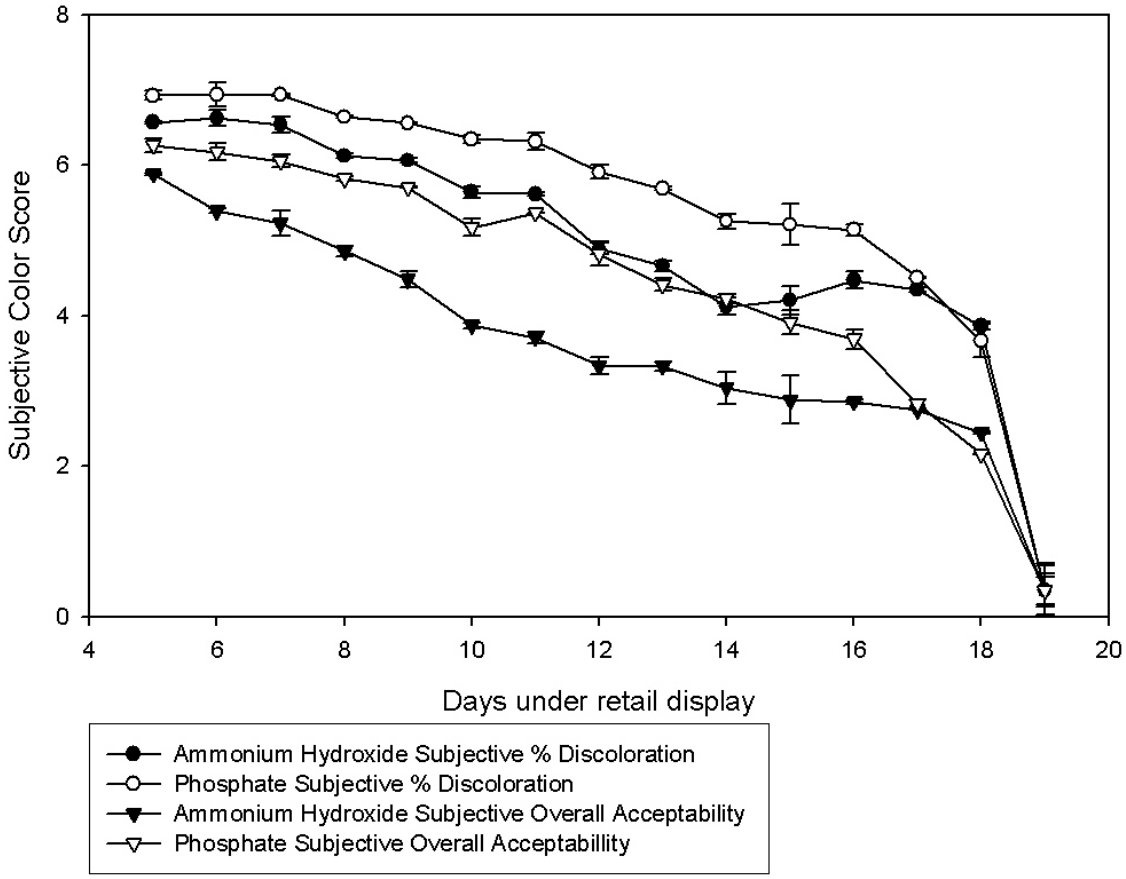

Figure 3.5. Subjective color evaluation of strip loin steaks after injection to 110% green weight with phosphate (4.5% sodium tripolyphosphate, 3.6% sodium chloride, 1% Herbalox) or ammonium hydroxide (3.6% sodium chloride, 1% Herbalox, pH 10). % of discoloration scale: 7=None (0%), and 1=Complete (100%). Overall acceptability scale: 7=Extremely desirable, and 1=Extremely undesirable.

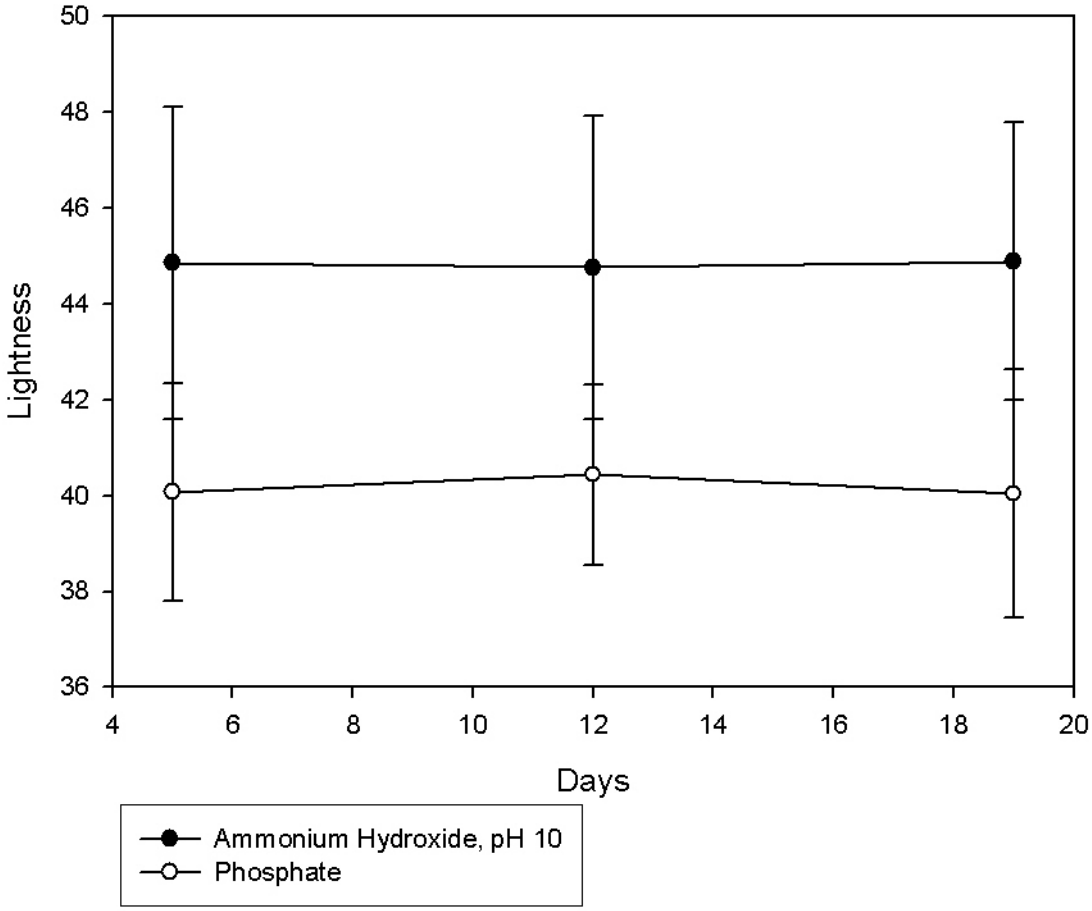

Figure 3.6. Lightness as measured by HunterLab L* (white = 100, black = 0) of strip loin steaks after injection to 110% green weight with phosphate (4.5% sodium tripolyphosphate, 3.6% sodium chloride, 1% Herbalox) or ammonium hydroxide (3.6% sodium chloride, 1% Herbalox, pH 10).

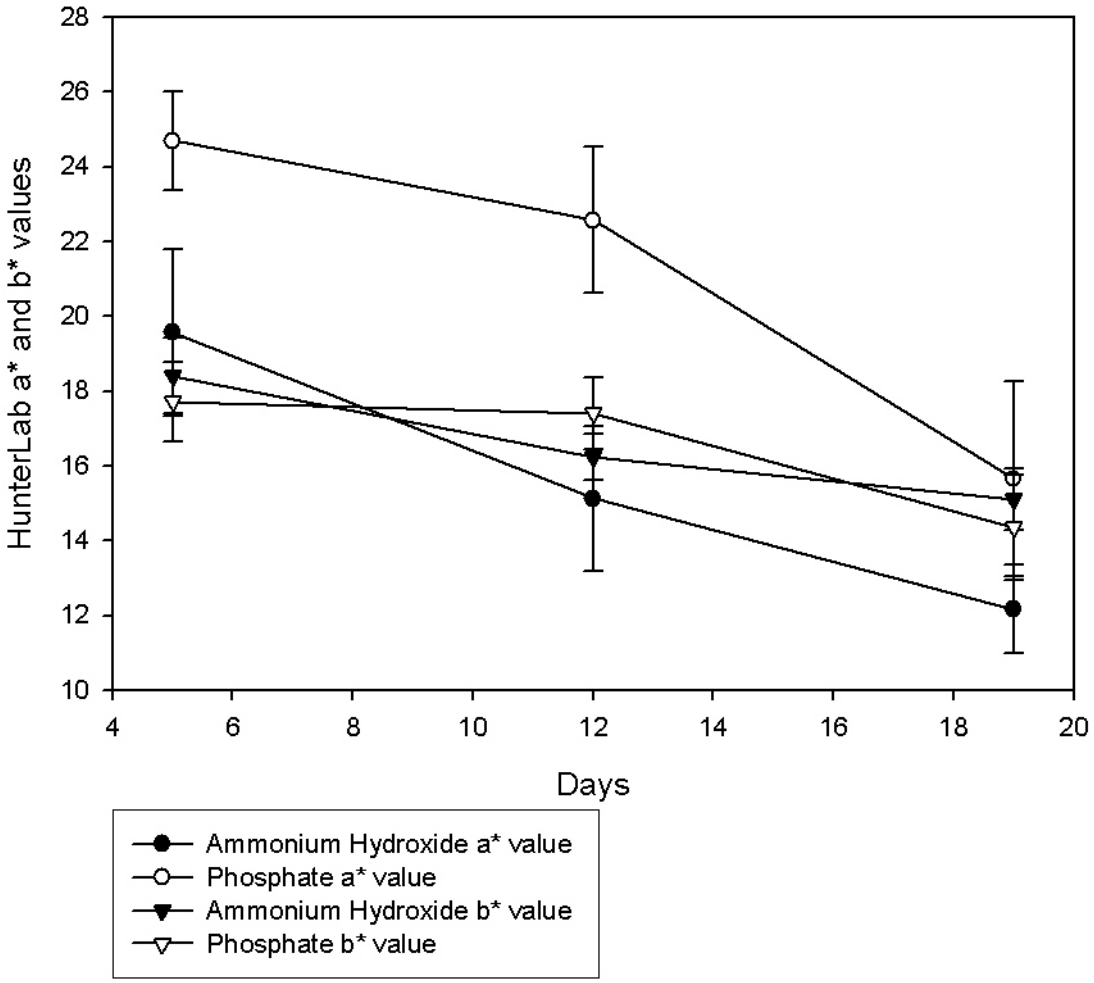

Figure 3.7. Objective redness as measured by HunterLab a* (negative values indicate green and positive values indicate red) and yellowness as measured by HunterLab b* (negative values indicate blue while positive values indicate yellow) of strip loin steaks after injection to 110% green weight with phosphate (4.5% sodium tripolyphosphate, 3.6% sodium chloride, 1% Herbalox) or ammonium hydroxide (3.6% sodium chloride, 1% Herbalox, pH 10).

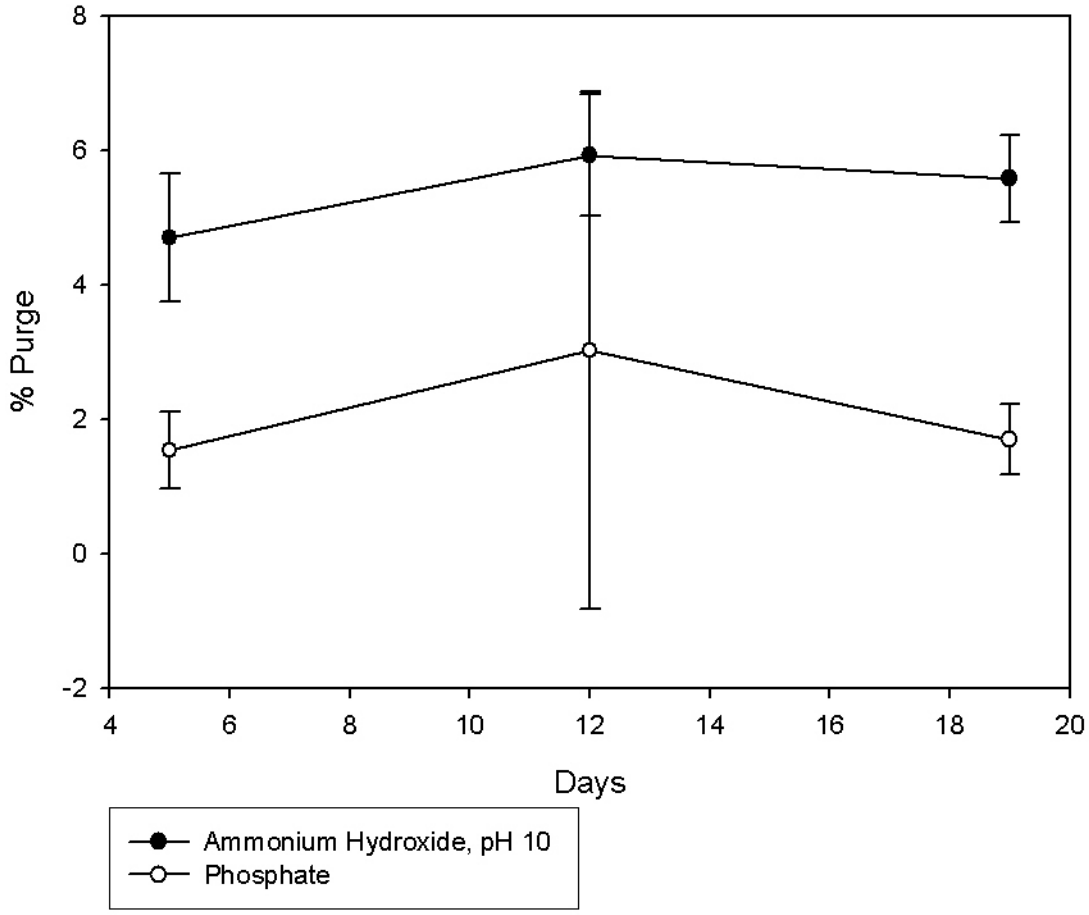

Figure 3.8. Purge of strip loin steaks after injection to 110% green weight with phosphate (4.5% sodium tripolyphosphate, 3.6% sodium chloride, 1% Herbalox) or ammonium hydroxide (3.6% sodium chloride, 1% Herbalox, pH 10).

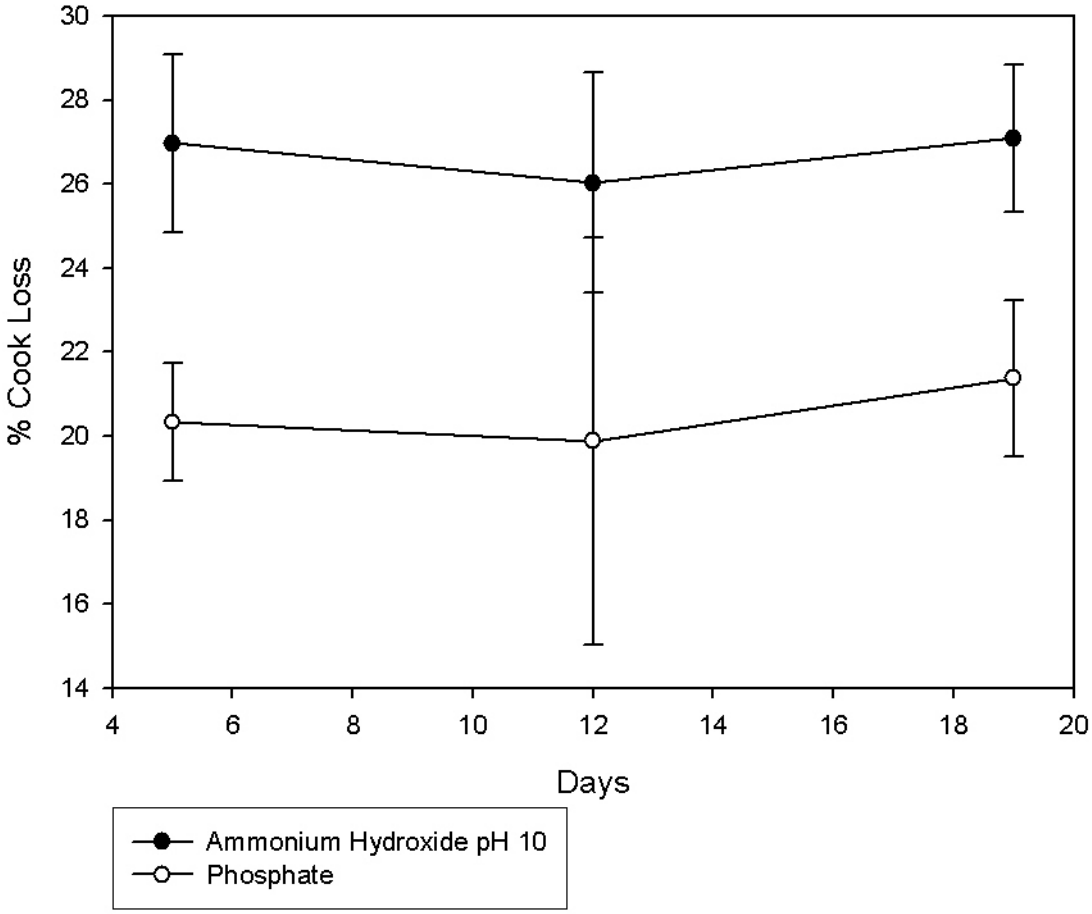

Figure 3.9. Cook loss of strip loin steaks after injection to 110% green weight with phosphate (4.5% sodium tripolyphosphate, 3.6% sodium chloride, 1% Herbalox) or ammonium hydroxide (3.6% sodium chloride, 1% Herbalox, pH 10).

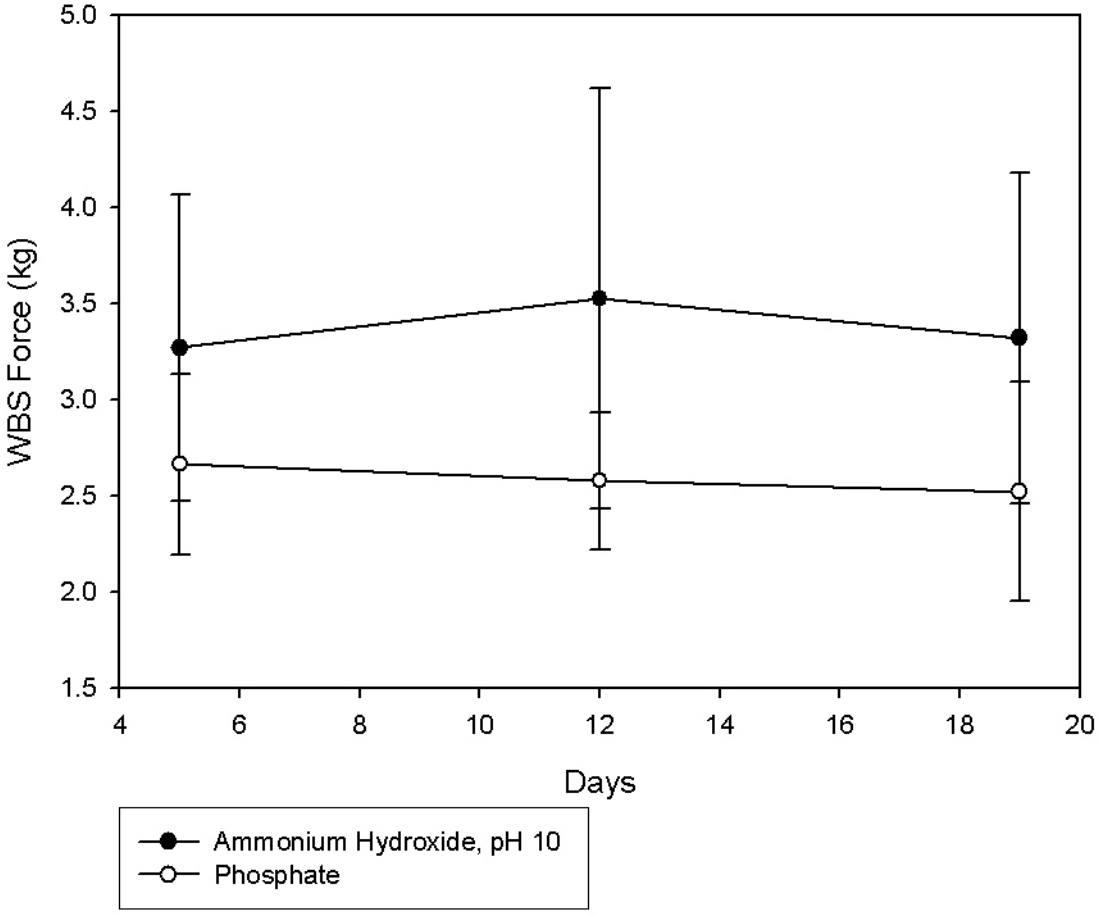

Figure 3.10. Warner Braztler Sheer (WBS) Force (kg) on strip loin steaks after injection to 110% green weight with phosphate (4.5% sodium tripolyphosphate, 3.6% sodium chloride, 1% Herbalox) or ammonium hydroxide (3.6% sodium chloride, 1% Herbalox, pH 10).

CHAPTER IV

REFERENCES

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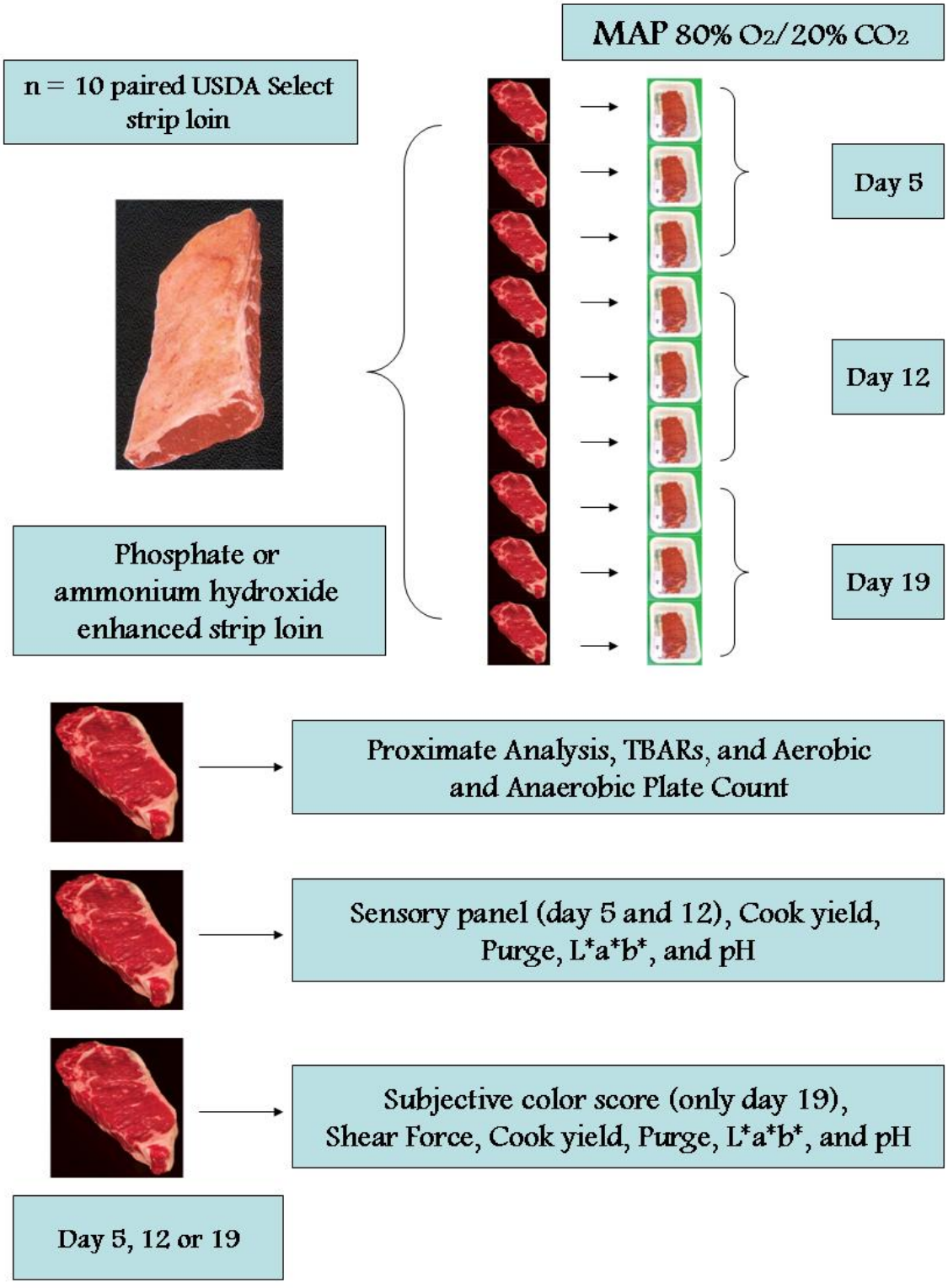
APPENDIX A
SCHEMATIC OF EXPERIMENTAL DESIGN



APPENDIX B
PERCENT ENHANCEMENT STRIP LOIN SUBPRIMALS

Treatment **PHOSPHATE**

Strip loins	Initial Weight	Pump Weight (10%)	% of pumping (original WT)	Equilibration weight	% of pumping (equilibration)
4L	12.10	14.30	18.18	13.75	13.64
8L	15.75	18.25	15.87	17.80	13.02
3L	17.25	20.65	19.71	18.95	9.86
6L	14.55	17.05	17.18	16.55	13.75
7L	13.55	16.10	18.82	15.55	14.76
1R	11.60	13.80	18.97	13.25	14.22
2R	8.95	10.80	20.67	10.35	15.64
5R	10.15	12.20	20.20	11.65	14.78
9R	17.05	19.70	15.54	19.15	12.32
10R	15.20	18.05	18.75	17.30	13.82
% Pumping Average			**18.39**		**13.58**

Treatment **AMMONIUM HYDROXIDE**

Strip loins	Initial Weight	Pump Weight (10%)	% of pumping (original WT)	Equilibration weight	% of pumping
1L	12.20	13.20	8.20	13.10	7.38
2L	11.80	13.45	13.98	13.05	10.59
5L	10.60	12.55	18.40	11.90	12.26
9L	17.35	19.95	14.99	19.25	10.95
10L	18.85	22.10	17.24	20.55	9.02
4R	11.50	13.50	17.39	12.90	12.17
8R	16.15	19.10	18.27	18.35	13.62
3R	11.90	13.90	16.81	13.35	12.18
6R	16.10	18.85	17.08	18.10	12.42
7R	13.20	15.95	20.83	15.10	14.39
% Pumping Average			**16.32**		**11.50**

APPENDIX C
SENSORY PANEL BALLOT

Oklahoma State University
Food Science

Evaluation of a high pH solution as an alternative to phosphate for meat enhancement

Panelist ID:_____ Date/Time:_____

Session 11:00AM/11:30AM

Sample	Tenderness	Juiciness	Connective Tissue	Overall Acceptability	Comments/Observations
1					
2					
3					
4					
5					
6					

Tenderness	**Juiciness**	**Connective Tissue**	**Overall Acceptability**
8 Extremely tender	8 Extremely juicy	4 None	7 Extremely desirable
7 Very tender	7 Very juicy	3 Slightly abundant	6 Desirable
6 Moderately tender	6 Moderately juicy	2 Moderately abundant	5 Slightly desirable
5 Slightly tender	5 Slightly juicy	1 Extremely abundant	4 Acceptable
4 Slightly tough	4 Slightly dry		3 Slightly Undesirable
3 Moderately tough	3 Moderately dry		2 Undesirable
2 Very tough	2 Very dry		1 Extremely undesirable
1 Extremely tough	1 Extremely dry		

APPENDIX D
COLOR SCORE EVALUATION SHEET

Evaluation of a high pH solution as an alternative to phosphate for meat enhancement

Name:_____ Date_____ Time_____ Day_____

Sample ID	Lean Color	Fat Color	% Discolor	Overall
18				
25				
38				
44				
57				
63				
72				
85				
93				
105				
117				
124				
132				
145				
157				
167				
172				
185				
193				
208				

Lean Color	Fat Color	% Discoloration or Browning	Overall Acceptability
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	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	7 None 6 1-10% 5 11-25% 4 26-50% 3 51-75% 2 76-99% 1 Complete	7 Extremely desirable 6 Desirable 5 Slightly desirable 4 Acceptable 3 Slightly Undesirable 2 Undesirable 1 Extremely undesirable

APPENDIX E
SENSORY PANEL COMMENTS

Day	Treatment	Comments
5	Phosphate	Salty, rosemary taste
5	Phosphate	A touch salty
5	Phosphate	Little off-flavor
5	Phosphate	Funny taste
5	Phosphate	Not strong beef taste
5	Phosphate	Off-flavor detected
5	Phosphate	Very good
5	Phosphate	The best, this is great!
5	Phosphate	Heavy off-flavor
5	Phosphate	Salty
5	Phosphate	Very dry
5	Phosphate	Salty/off-flavor
5	Phosphate	Has a distinctive flavor
5	Ammonium Hydroxide	Decent flavor
5	Ammonium Hydroxide	Slight off-flavor
5	Ammonium Hydroxide	No flavor
5	Ammonium Hydroxide	Little off-flavor
5	Ammonium Hydroxide	Very good flavor
5	Ammonium Hydroxide	Not strong beef taste
5	Ammonium Hydroxide	Off-flavor
5	Ammonium Hydroxide	Dry
5	Ammonium Hydroxide	Salty, non beef flavor
5	Ammonium Hydroxide	Good flavor, not very juicy
12	Phosphate	Little off-flavor
12	Phosphate	Very good
12	Phosphate	Just little salty
12	Phosphate	Good flavor
12	Phosphate	Off-flavor
12	Phosphate	Slightly off-flavor
12	Phosphate	Muggy/soft like tofu
12	Phosphate	Poor beef flavor
12	Phosphate	Good beef flavor
12	Phosphate	Chewy
12	Ammonium Hydroxide	Very good
12	Ammonium Hydroxide	Flavorless
12	Ammonium Hydroxide	Off-flavor
12	Ammonium Hydroxide	Slightly off-flavor
12	Ammonium Hydroxide	Too salty, does taste like beef
12	Ammonium Hydroxide	Dry, tough, funny flavor
12	Ammonium Hydroxide	Beef flavor

Oklahoma State University Institutional Review Board

Date:	Thursday, March 22, 2007
IRB Application No	AG077
Proposal Title:	Evaluation of a High pH Solution as an Alternative to Phosphate for Meat Enhancement
Reviewed and Processed as:	Exempt

Status Recommended by Reviewer(s): Approved Protocol Expires: 3/21/2008

Principal Investigator(s

Claudia A. Cerruto Noya
107 Animal Science
Stillwater, OK 74078

Christina Mireles DeWitt
104E 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 219 Cordell North (phone: 405-744-5700, beth.mcternan@okstate.edu).

Sincerely,

Sue C. Jacobs, Chair
Institutional Review Board

Title of Project: Evaluation of a high pH solution as an alternative to phosphate for meat enhancement

INFORMATIONAL FORM

The following document contains important information concerning your participation on this research study. Please read all the information carefully. Your participation in this study is voluntary and you may at anytime stop participating.

1. This research is being conducted for the Department of Animal Science at Oklahoma State University and will take place at Oklahoma State University.
2. The purpose of this research is to evaluate processed beef meat formulated with ammonium hydroxide.
3. The cuts of beef meat have been further processed using salt, phosphate and rosemary at levels approved by the FDA and USDA.
4. The length of this study is about one month and you will be asked to participate in 3 sessions of 30 minutes each one.
5. Beef meats will be served to you, and you will be expected to evaluate these meats and mark a ballot with your impression of the characteristics listed on the ballot.
6. The investigators consider your participation in this study to be minimal risk as defined in 45 CFR 46 "minimal risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during performance or routine physical or physiological examinations or test".
7. Your participation is completely voluntary and you may quit at any time.
8. You are encouraged to ask any questions concerning meat processing and test procedures.
9. You will not be asked to make any identifying marks on the ballot and efforts are being made to maintain the confidentiality of your response. The anonymous data will be entered in the computer and will be accessible by the research investigators.
10. In the event of physical illness or injury result from research procedures, there will be no financial compensation or free treatment offered to you.
11. You have not requested to waive or release Oklahoma State University of liability for the negligence of its sponsor, agents or employees.

This research has been review and approved by the Institutional Review Board for Human Subjects in Research at Oklahoma State University. If you have questions about your rights as a researcher volunteer, you may contact Dr. Sue C. Jacobs, IRB chair, 219 Cordell North, Stillwater, OK 74078, 405-744-1676 or irb@okstate.edu.

For additional questions about procedures contact:
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Stillwater. OK 74078
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VITA

Claudia Alejandra Cerruto Noya

Candidate for the Degree of

Master of Science

Thesis: EVALUATION OF A HIGH PH SOLUTION AS AN ALTERNATIVE TO PHOSPHATE FOR MEAT ENHANCEMENT

Major Field: Food Science

Biographical:

Personal Data:

Born in Coroico-Nor Yunga, La Paz, Bolivia in September, 1976. Daughter of H. Edith Noya de Cerruto and Freddy Cerruto F.

Education:

Graduated as Veterinarian and Zootechnic from the Catholic Bolivian University, La Paz-Bolivia in December, 2000. Completed the specialization-training in Studies on Protozoan Diseases at the National Research Centre for Protozoan Diseases (NRCPD), Obihiro, Japan in October, 2003. Specialization in Higher Education at Catholic Bolivian University, La Paz-Bolivia in May, 2005. Completed the requirements for the Master of Science at Oklahoma State University, Stillwater, Oklahoma in May, 2008.

Experience:

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Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EVALUATION OF A HIGH pH SOLUTION AS AN ALTERNATIVE TO PHOSPHATE FOR MEAT ENHANCEMENT

Pages in Study: 62 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study: Paired USDA Select beef strip loins, aged for 2 days, were enhanced to 110% of original weight with either a high pH solution containing 3.6% sodium chloride, 1% Herbalox seasoning and adjusted to pH 10 with ammonium hydroxide (~0.1%, FFC grade); or a phosphate based solution prepared using 3.6% sodium chloride, 1% Herbalox seasoning, and 4.5% sodium tripolyphosphate. In order to evaluate beef quality, sample pH, proximate analysis, microbial growth, lipid oxidation, color score, purge loss, cook loss, Warner-Bratzler shear force, and sensory panel were measured over 14 days under retail conditions.

Findings and Conclusions: Although enhancement of USDA Select strip loins with an ammonium hydroxide solution at pH 10 was not as effective as the industry based phosphate injection solution, these data suggests that adjusting the enhancement solution to pH 10 using ammonium hydroxide can raise the final pH, control bacterial growth, generate higher yields, increase water holding capacity, and improve sensory attributes of beef steaks compared with un-treated steaks (based on data from previous studies). In addition, an ammonium hydroxide solution did outperform the phosphate treatment in both aerobic and anaerobic microbial populations. However, it appears that the pH 10 solution did not sufficiently raise the final meat pH as phosphate based solution. Therefore future research should be conducted to determine if higher levels of ammonium hydroxide can sufficiently change the final meat pH to enhance color stability, water holding ability, and tenderness, while controlling microbial growth.

ADVISER'S APPROVAL: Dr. Deb VanOverbeke