POST-MORTEM MECHANICAL INJECTION OF LOW QUALITY BEEF LOINS WITH PORK BACK FAT IMPROVES PALATABILITY ATTRIBUTES

By

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Abstract

An experiment was conducted to examine the palatability attributes of beef striploin steaks mechanically enhanced with pork fat. Beef subprimal strip loins (IMPS 180; n=40 loins) were collected from USDA Standard steer carcasses, vacuum packaged and stored at 2˚C. Loins were longitudinally cut into halves and each half (lateral or medial) was assigned randomly to pork-fat injection (PFI) treatment or to non-injected control (CON). Loin halves assigned to the PFI treatment were injected (12.61 ± 2.45%) using a multi-needle mechanical injector with liquid (60°C) pork fat (subcutaneous loin origin) that had been fully cooked (>71°C). Loin halves from PFI and CON were frozen (-28.9°C) before steaks (2.54 cm) were cut. Steaks for Warner-Bratzler shear force (WBSF), trained and consumer sensory panels and proximate analysis (cooked and uncooked) were thawed at 1°C for 24 h before being cooked in a forced air convection oven set at 177°C until an internal endpoint temperature of 70°C was reached. Eighty pairs of steaks (80 CON/80 PFI) were evaluated for trained sensory panel attributes; panelists scored (1 to 8; scale1= extremely tough/dry/bland/tough/intense, 8= extremely tender/juicy/intense/tender/none) steaks for myofibrillar tenderness, juiciness, beef flavor intensity, overall tenderness, and off-flavor intensity. Seventy pairs of steaks (70 CON/70 PFI) were evaluated at West Texas A&M University by untrained consumers for sensory attributes; panelists scored (1 to 9 scale; 1= dislike extremely, 9=like extremely) samples for tenderness, flavor, juiciness, and texture. In addition, panelists were asked to indicate their overall preference. Steaks allocated to WBSF determinations were chilled for 24h at 1°C before six 1.27 cm cores were removed randomly parallel to the muscle fiber orientation. Cores were immediately sheared using a V-shaped blade on the WBSF machine; peak shear force (kg) values were recorded for each core, and averaged for each steak. Continuous data were analyzed as a completely randomized design using a mixed model; treatment was the fixed effect with random effects of animal and location (lateral or medial half). Ordinal data were analyzed using the Wilcoxon rank-sum test. Overall preference data were analyzed using FREQ procedure and chi-square of SAS.

Uncooked steaks from the PFI treatment had less ($P < 0.01$) moisture (-5.2%) and protein (-1.9%) simultaneous with greater ($P < 0.01$) fat (+7.3%) vs. CON uncooked steaks. Cooked PFI steaks had less ($P < 0.01$) moisture (-1.0%) and more ($P < 0.01$) fat (+1.3%) with no difference ($P = 0.14$) in the protein percentage vs. CON cooked steaks. Trained panelists denoted ($P = 0.02$) an off-flavor for the PFI treatment but were unable to discern other attribute differences. In contrast, untrained consumer panelists denoted ($P = 0.05$) improved juiciness for the PFI treatment and reported preference ($P = 0.01$) for PFI treated steaks. Similarly, WBSF data demonstrated reduced ($P < 0.01$) shear-force values for PFI treated steaks as compared to the CON treatment (2.50 vs. 4.44 kg, respectively). Mechanically injecting low quality beef striploins with pork subcutaneous fat altered proximate analysis and improved palatability. This processing method deserves further investigation and may offer an opportunity for new product development

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Approval

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CHAPTER I

INTRODUCTION

Tenderness of meat products always ranks among the top three traits that consumers desire in a meat product (Solomon *et al*., 1997; Killinger, 2004; Choat, 2006). Improving the tenderness of meats has been a research priority for the past 50 years. An AGRICOLA search of the terms "beef tenderness" returns 1,481 hits. The literature includes research on tenderness associations with sarcomere length (Smulders *et al.,* 1990; White *et al.*, 2006; Weaver et al., 2009), collagen quantity (Cross *et al*., 1972, 1973; Berry *et al*., 1974 ; Palka, 2003), collagen quality (Bouton *et al*., 1973; Guignot *et al.*, 1994; Jeremiah *et al*., 1991; Purchas, 1990), proteolytic enzymes (Calkins *et al.*, 1987; Johnson *et al*., 1989; Gerelt *et al*., 2000), aging (Campo *et al*., 1999; DeGeer *et al.*, 2009; Stenstrœm *et al*., 2014), ultimate pH/water holding capacity (Bernthal *et al*., 1989; Pietrasik & Shand, 2005; Sawyer *et al*., 2008), marbling (Koch *et al*., 1993; Berry, 1993; Wheeler *et al*., 1994) and cooking method (Cross *et al*., 1979; Kolle *et al*., 2004; Adhikari *et al.*, 2004).

Pork is the term that describes meat from the porcine species and the primary source of animal protein for the planet. The largest global consumers of pork include China (67.5), Belarus (47.7), European Union (43.0), Japan (36.3), Switzerland (33.1), and the United States (27.3 kg per capita; USDA-FAS, 2011). People eat pork for its nutritive value, availability, tradition, and satiety value. Pork is known to add desirable flavor to other meats including beef (e.g. bacon-wrapped tenderloin).

Today's consumers' prefer to purchase beef and other meats that have satisfactory palatability. For many consumers the price point associated with more desirable middle meats (i.e. tenderloin, ribeye, striploin, sirloin) can be cost prohibitive. Adding tallow to beef has been tested and reported to increase palatability. A technique for injecting liquid edible beef fat into beef carcasses was developed in the early 1960's (Durham *et al.*, 1961). Post-mortem injection of beef fat into beef subprimals has been recently shown to improve tenderness and sensory attributes (Holmes *et al.*, 2013).

The objective of this research was to examine the palatability attributes of low quality beef striploin steaks mechanically enhanced with pork subcutaneous fat.

CHAPTER II

REVIEW OF LITERATURE

2.1 Components of meat palatability

Meat palatability is a desirable attribute consumers continually seek in meat products. Meat palatability can primarily be attributed to the three essential tasting qualities of tenderness, juiciness, and flavor. Rigor mortis is essential to the tenderness attribute; the onset of rigor mortis and its resolution partially determines the tenderness of meat. If meat is immediately chilled to 15°C (59°F) post-mortem, a detrimental effect known as cold shortening occurs, in which the sarcomeres contract up to a third of their original length (Cornforth *et al.,* 1980).

Fat interspersed between the muscle fibers provides meat cuts with an essential component to increase the acceptability of meats. Fat has been associated with improved palatability of meat (Smith *et al*., 1985, 1987; Bowling *et al.,* 1977). O'Quinn (2012) reported decreased consumer acceptability of each palatability trait as fat level decreased.

2.1.1 Tenderness

Tenderness is a measure of how easily meat is broken down during mastication. Meat tenderness is recognized as an important factor in the consumer assessment of meat quality (Bailey, 1979). Tenderness as an attribute of palatability has been extensively researched and published. Wheeler (1997) detailed how one could measure meat tenderness with the Warner-Bratzler Shear Force test. The Warner-Bratzler test uses a mechanized blade forced by a weight to cut through a meat sample. The shear test provides continuous objective information about the toughness and tenderness of meat products.

Shackelford *et al.* (1991) published the first threshold relating Warner-Bratzler shear force values to consumer data. If consumers are unable to discern tenderness of meat products then tenderness improvement is of little to no value. Miller (2001) indicated sensory panelist perceptions of beef flavor and juiciness have a greater impact on consumer overall acceptability. As meat becomes tougher, flavor and juiciness attributes effect the consumer satisfaction of purchase. Consumer sensory evaluation of tenderness shows variation in how beef was prepared or cooked. Boleman (1997) reported 60% sensed pepper, 47.6% herbs or spices, and 45.5% sensed salt. Trained sensory panelists evaluate the palatability attributes of tenderness, flavor, and juiciness; when tenderness is lacking, other attributes determine overall acceptability. Consumer sensory evaluation of overall acceptability is influenced by the methods and preparation of the meat sample. Consumers may prefer seasoning or additives to enhance the acceptability of meat as it correlates with tenderness, flavor, and juiciness. Tenderness research consists of many components such as sarcomere length, collagen quantity, collagen quality, ultimate pH/water holding capacity, proteolytic enzymes, aging, marbling, and cooking method.

2.1.1.1. Sarcomere length

To understand the structure of meat to accurately determine tenderness, scientists study the histology of the muscle. Muscle consists of thousands of muscle fibers bound into bundles by the perimysium connective tissue and the bundles are bound together by the epimysium (Koohmaraie, 1988). Sarcomeres, the contractile unit of muscle, are composed of A-band, H-band, I-band, M-lines, and Z-lines. Sarcomere length (Angus and Angus Cross, 1.50-2.08 µm) is the distance between the two Z-lines (tetrahedral) within a sarcomere; they are very strong structures to withstand the forces that are experienced during contraction (Smulders *et al.*, 1990). Rhee *et al*., (2004) reported the range in muscle means for sarcomere length was 1.3 µm and the length of the sarcomere influences tenderness. Cross et al. (1981) reported when muscles are treated to alter sarcomere length, there is a high, positive correlation between sarcomere length and tenderness. Shorter lengths of sarcomere mean tougher meat and we increase length by electrical stimulation used in industry (McKeith *et al.*, 1980).

2.1.1.2 Collagen quantity

Following sarcomere length, it is important to examine collagen quantity of the animal. Collagen is the main structural protein found in animal connective tissue, yielding gelatin when boiled (Berry et al., 1986). As collagen increases, tenderness decreases and the application of high heat and moisture helps increase tenderness of beef cuts (Boccard *et al.*, 1979). Changes that occur in *semitendinosus* collagen heating were reported by Powell (1997). Collagen denaturation of the insoluble portion of collagen began at 55°C, and maximal denaturation was achieved within 60 minutes. This process resulted in more tender meat as exhibited by lower Warner-Bratzler shear force values (Powell *et al.*, 2000). Collagen can be denatured quicker at higher temperatures 70°C and above but tenderness decreases and myofibrillar hardening occurs (Davey *et al*., 1976; McCrae & Paul, 1974). The correlation between percentage collagen quantity and tenderness is -0.36 for steers (Seideman, 1986)

2.1.1.3 Collagen quality

Post-mortem degradation of collagen plays a major role in providing the desired tenderness of beef and other meat sources. The texture is changed by altering the connective tissue structure and this phenomenon is very important in maintaining consumer acceptability of texture (Nakamura *et al.*, 2010). Degree of collagen crosslinking can greatly influence tenderness and consumer acceptability, and reports of the difference between Bos Taurus and Bos Indicus breeds have been researched extensively (Riley *et al.*, 2005). Such studies suggest that collagen content or quantity does not have much significance when compared with the quality (Savell and Shackelford, 1992). The age of a beef animal has a direct effect on tenderness of the meat it produces. As cattle mature, meat becomes progressively tougher. To evaluate the effects of the maturing process effects on beef tenderness, carcass maturity evaluation is used in determining USDA Quality Grades. There are five maturity groupings, designated as A through E; the following maturity class and age ranges are assumed: A - 9 to 30 months, B - 30 to 42 months, C- 42 to 72 months, D - 72 to 96 months, E - more than 96 months. The link between carcass maturity and collagen quality is as the animal ages, collagen crosslinking increases and tenderness decreases. The amount of collagen present in an A maturity carcass is less than that of an E maturity carcass. As the age of the animal progress the quality grade shifts from Prime, Choice, Select, and Standard for A and B maturity carcasses however, B maturity cattle are not eligible for the Select grade, to C-E maturity carcasses are more likely to be graded Commercial, Utility, and Cutters (Hatem *et al.*, 2003)

2.1.1.4 Ultimate pH and water holding capacity

An increase in the ultimate pH of meat, as a result of depleted glycogen reserves prior to slaughter, affects meat quality greatly (Silva *et al.*, 1999). Beef with increased ultimate pH is dark, firm, and dry (DFD); it is more susceptible to bacterial spoilage and bland flavor. The muscle cuts of DFD cattle are more tender than normal muscle meat cut. As pH increase the negative ions present in the muscle increase the muscle cell and more water binds inside the muscle. This increase of water improves tenderness of the meat and reduces water loss. The relationship between ultimate pH and tenderness has been reported to have a negative linear relationship (Bouton *et al.,* 1973; Guignot *et al.,* 1994). However, other researchers (Jeremiah *et al*., 1991; Purchas, 1990; Purchas & Aungsupakorn, 1993) reported a curvilinear relationship, with minimum tenderness between 5.8 and 6.2 pH values. Evaluation of the ultimate pH and tenderness correlation in beef and the time length of aging could produce more tender meat. Meat & Livestock Australia uses pH in their grading system of beef carcasses. Each carcass is identified with a carcass ticket and pH with weight, sex, hanging method etc. is recorded in the Data Capture Unit. AUS-MEAT Standards for grading requires using a pH meter carcasses and meet a standard that must be below 5.71, and temperature should be below 12˚C (Perry et al., 2001; Polkinghorne *et al.*, 2008; Watson *et al.*, 2008)

2.1.1.5 Proteolytic enzymes

The primary mechanism of postmortem improvement in tenderness of meat is through the degradation of the native structure of the muscle. The most common method identified as having a positive effect on tenderness, is postmortem cooler aging. The use of proteolytic enzymes is another method for postmortem meat tenderization and how we introduce them to the meat cut (Gerelt *et al.,* 2000). The calpain system is responsible for the aging process, and is composed of μ-calpain, m-calpain and calpastatin. Calpains and their inhibitor (calpastatin) are calcium-dependent (Goll *et al.,* 2003). Calpain proteolysis of myofibrillar proteins occurs through the degradation of the z -line proteins primarily desmin and titin of the sarcomere structure. The use of proteolytic enzymes to improve tenderness can be achieved by introducing the meat to brine containing the enzymes that allow the meat to absorb the enzymes (Gerelt *et al.,* 2000).

2.1.1.6 Aging

There are two types of aging methods used to improve beef tenderness. Dry-aging is a process used to produce uniquely flavored beef. It has a distinct beefy, brown roasted flavor and is considered more desirable by some consumers (Smith *et al.,* 1978). The dry-aging process changes beef by two methods, moisture is evaporated from the muscle and the oxidation from introduction of air to the fat creates a greater concentration of beef flavor and taste. Then beef's natural enzymes (calpains) break down the myofibrillar proteins in the muscle, which increases tenderness of beef. Dry-aging takes approximately 28 to 35 days at a humidity level of 80 to 85%. Dry-aging uses a continuous air flow at temperatures of -0.5 to 1 ˚C (Campbell *et al.*, 2001). The second process is wet aging used by 95% of beef producers (DeGeer *et al.,* 2009). Wet–aged beef usually has a sour and strong bloody/serumy flavor. It also assures less moisture loss and greater retention in product weight (DeGeer *et al.*, 2009). Wet-aged beef has typically been aged in a vacuum-sealed bag to retain its moisture. It has been researched and reported that using vacuum packaging for aging beef, is actually not aging the cut but preserving it, and may account for improve tenderness only (DeGeer *et al.,* 2009).

2.1.1.7 Marbling

Marbling refers to the white flecks and streaks of fat within the lean sections of meat. Also called intramuscular fat, marbling adds flavor and is one of the main criteria for judging the quality of cuts of meat. In general, the more marbling it contains, the higher quality cut of meat is. Marbling also acts as a solvent for the volatile compounds that develop during production, handling, and thermal processing of beef (Moody *et al.*, 1970). As intramuscular fat increases, the fat flavor improves consumer acceptability (Miller *et al.,* 2000). Marbling improves palatability attributes of beef, and the lack of marbling decreases the tenderness of the cut (Thompson *et al.,* 2002). Research over the last 30 years indicates that marbling fat has a low relationship to palatability and explains only about 5 to 10% of the variation in tenderness of the loin muscle (Wheeler *et al*., 1993).

2.1.1.8 Cooking Method

Meat tenderness is recognized as an important factor in consumer assessment of meat quality (Bailey, 1972). Changes in the connective tissue and myofibrillar proteins result from the change in meat tenderness due to cooking meat. It is well known and reported that connective tissues, such a collagen, degrade at high temperatures of cooking. Research has been focused on the effects of low-temperature and extended time of cooking to improve tenderness and decrease cooking loss (Bramblett *et al.,* 1959). Bramblett *et al.*, (1959) reported that holding meat at an internal temperature of 57 to 60 °C may also significantly improve tenderness. Cooking methods including broiling, grilling, boiling, and roasting can improve tenderness and other palatability attributes of beef as cooking denatures collagen crosslinking and collagen provides juices and flavor to the cut.

2.1.2 Juiciness

Meat juiciness is the perceived quantity of juices experienced during the act of mastication. Juiciness is an important palatability attribute consumers seek when chewing meat. Consumers can distinguish if meat is dry or juicy and they typically associate dry with toughness and juicy with tenderness (Rhodes & Nute, 1980).

2.1.2.1 Ultimate pH/Water Holding Capacity

Huff-Lonergan and Lonergan (2005) defined water-holding capacity of meat as the ability of the postmortem muscle to retain water when external pressures (e.g. gravity, heating) are applied to it. The quantity of free water in meat varies from 30 to 50 % of the total content depending on the type of meat and aging process (Carpenter *et al.*, 1961; Dhanda, 1999). As sarcomeres shorten, less available space remains for water to bind to the myofibrillar proteins. Previous research studies have found the benefits of increased ultimate pH. Increased pH influences water-holding capacity of meat above the iso-electric point of myofibrillar proteins (Gault, 1985). This increase of water-holding capacity improves tenderness as the proteins have moisture to protect the myofibrillar structure during cooking. Minimal post-mortem contraction is essential to a juicier cut of meat for the consumer (Huff- Lonergan & Lonergan, 2005).

2.1.2.2 Fat content

Lipid content of the meat is an important component of meat juiciness. Fernandez et al. (1999) examined the influence of intramuscular fat content on the sensory attributes and consumer acceptability of pork and reported that increased intermuscular fat was associated with significantly higher juiciness scores. Intramuscular fat is essential and has an imperative role in the development of the sensory features of meat products whether they are fresh, cured, or dried (Ruiz et al., 2000; Ventanas et al., 2007). Pork fat was considered more palatable and gave consumers a euphoric experience during the sensory evaluation over beef and lamb lipid inclusion into meat products (Melton, 1987). Lipids are essential to the flavor profiling of red meat. The density of fat is less than heat-denatured meat proteins, and thus meats with higher fat contents are more tender. The lubrication factors of lipids provide less resistance during chewing of meat. The insurance of fat suggests that lipids protect against heat-induced toughening of muscle fibers during cooking. The deposits of fat within meat cuts, weakens connective tissue by increasing strain on the connective tissue and thus improves meat tenderness (Blumer, 1959). These theories do not work independently of each other; however, meat higher in fat content tends to be more tender, juicier, and flavorful than very lean meats.

2.1.2.3 Cooking endpoint

Cooking meat causes shrinkage and loss of fluid from the meat and evaporation from the meat surface. Drip loss is caused by the shrinkage of the myofibrils, endomysium, and perimysium. Aldrich and Lowe (1954) reported that beef round shrank 16.6% of their original volume when cooked to 90°C and additional 1 h holding at 90°C increased shrinkage by 50%. Dunlavy and Lamkey (1994) reported that as the endpoint temperature increased, juiciness of beef roasts decreased, without negative descriptions of other attributes. Prestat *et al.,* (2002) reported endpoint temperature, pump level, and cooking method interactions occurred for juiciness. Un-pumped pork chops were juicier when grilled. As endpoint temperature increased, juiciness decreased in un-pumped chops while it remained constant in pumped chops.

2.1.3 Flavor

Maillard reactions are a series of events while cooking involving the aldehydes, amines, and ultimate development of meat flavor and dark pigments (Hodge, 1967). Calkins and Hodgen (2007) reported that hundreds of compounds contribute to the flavor and aroma of meat and the interactions between those compounds influence the perception of flavor. Ration ingredients may alter meat flavor; inclusion of linseed or flaxseed in ruminant and non-ruminant diets may result in a detrimental oily iron flavor. Swine often taste like what they eat; pigs fed corn have a flavor is that similar to nutty, in contrast to those fed a diet of sugar refinery waste which produce sweeter flavors. Spanier et al. (1996) reported that post-mortem aging effects meat flavor quality; as aging progressed, desirable flavors such a beefy, brothy, browned-caramel and sweet declined whereas off-flavors of bitter and sour increased.

2.1.3.1 Lipid profile and quantity

Despite the common reference to animal fats as saturated, less than half of all fatty acids in meat are saturated. Lean beef contains more monounsaturated fatty acids than saturated fatty acids and a small amount of polyunsaturated fatty acids (Scollan *et al.*, 2006). Approximately one-third of beef's total saturated fatty acid content is stearic acid. The lipid profile of grass-fed beef is different from that of grain-fed beef, which effects aroma and flavor. These attributes are linked to the chemical makeup of the meat. In a study comparing the flavor compounds between cooked grass-fed and grain-fed beef, the grass-fed beef contained higher concentrations of diterpenoids, derivatives of chlorophyll. Flavor of lipids are increased by the dry-aging process which changes beef by two means. Moisture is evaporated from the muscle and fat is oxidized and creates a greater concentration of beef flavor and taste. Beef's natural enzymes break down the connective tissue in the muscle, producing more tender beef. Dry-aging takes place for about 28 to 35 days humidity level of 80 to 85% with continuous air flow with a temperature of -0.5 to 1˚C at this point meat can reach a potential balance between tenderness, taste, and juiciness (Levis and Chambers, 2001). Wet-aged beef has typically been aged in a vacuum-sealed bag to retain its moisture. Wet-aging is popular because it takes less time: typically only a few days. Moisture accumulates while in the vacuum bag and its amount depends on the timing of aging and there is little weight loss. The lipid profile of wet aged beef is that of less beefy and as time of aging progresses the fat aroma is expressed as a sour scent (DeGeer *et al*., 2009).

2.1.3.2 Cooking method & flavor

An AGRICOLA search of the terms “beef flavor” and “cooking method” returns 334 hits. Flavor and intensity of meat flavor are important to both consumers and the meat industry; however it is not extensively researched and not well defined how cooking affects flavor. Beef cuts and the different cooking methods impact the overall beef flavor (Aguirre *et al.*, 2015). There have been may reviews on meat flavor but the effects of cooking methods and the development of meat flavor are not well understood (Chang and Peterson, 1977). A flavor lexicon is a set of words to describe the flavor of a product. A lexicon is applied or practiced using descriptive sensory analysis techniques (Drake and Civille, 2003). Maughan and Martini (2012) reported the identity and quantity of flavor attributes of beef and other protein sources. Five mixtures of beef and chicken were prepared using dry heat cooking on electric griddles, mixtures included 100% beef (100:0), 75% beef and 25% chicken (75:25), 50% beef and 50% chicken (50:50), 25% beef and 75% chicken (25:75), and 100% chicken (0:100). The investigators reported significant differences between the samples in astringent, brothy, fatty, gamey, grassy, juicy, metallic, oxidized, salty, sweet, and umami flavor. As the amount of chicken increased in the sample; astringent and fatty attributes decreased in intensity; whereas juicy, brothy, salty, sweet, and umami increased (Maughan and Martini, 2012). Beef has a certain lexicon of flavor attributes and the cooking method may affect the intensity of flavors currently present in beef not alter it to change.

2.1.3.3 Aging

Consumers primarily purchase beef because of its desirable flavor and texture. Morgan et al. (1991) stated that beef flavor was a very important factor in determining overall palatability. Nonetheless, researchers have not extensively evaluated the flavor attributes of meat, and little, if any, research has been conducted to determine the effects of aging on the flavor attributes of steaks. Yancey *et al*., (2005) conducted research to investigate these effects on beef flavor. One-hundred forty carcasses were fabricated to separate the infraspinatus (top-blade steak) from the chuck clod, gluteus medius (top-sirloin steak) from the sirloin, and psoas major (tenderloin steak) from the loin. Small degree of marbling resulted in a more rancid flavor compared with slight marbling, but marbling had no other appreciable effects on the flavor profile. Aging steaks for 35 d increased metallic flavors compared with aging for only 7 or 14 d. Aging meat beyond 21 d decreased beef flavor and its intensity. Flavor is a very complex attribute of meat palatability and must be researched further to improve sensory and consumer acceptability (Calkins & Hodgen, 2007).

2.2 Additives to enhance meat palatability

We have been using additives since prehistoric times beginning with the use of smoke to make meat taste better (USDA-FSIS, 1958). The introduction of the spice trade from Asia, the Middle East, and Europe provided a source of additives to enhance beef palatability. The meat industry has been using additives other than salt to improve palatability for the last two centuries. A food additive is defined by the Food and Drug Administration (FDA) as any substance that directly or indirectly becomes a component or otherwise affects the characteristics of any food (US-FDA, 1960). This definition includes any substance used in the production, processing, treatment, packaging, transportation or storage of food. Additives are used to maintain or improve safety, freshness, nutritional value taste, texture and appearance of meat. Use of food additives has become more prominent in recent years due to the increased production of prepared, processed, and convenience foods. One of the most essential additives of beef has been salt. Salt played an essential role in the Civil War Union. In December, 1864, Union captured Saltville, Virginia, a city with an important salt processing plant that was sustaining the South's armies. Therefore, it is important to discuss the additives we use to enhance beef palatability and the effects on tenderness, juiciness, and flavor. We use different additives to enhance beef; calcium, phosphates, and salts improve tenderness; phosphates and salts increase juiciness and water binders maintain juiciness. When we want to enhance beef flavor we use broths and fats (lipids).

2.2.1 Calcium activated tenderization

Using calcium to improve tenderness is well studied (Koohmariaie *et al.*, 1988, 1989, 1990). The tenderness mechanism is via the activation of the calpain proteolytic system that hydrolyzes key structural myofibrillar proteins during postmortem aging. Calcium injection tenderizes meat by activating m-calpain activity, causing a greater amount of protein degradation than via μ-calpain alone (Koohmaraie *et al*., 1988).

Wheeler *et al.* (1991) conducted two experiments to evaluate the effect of calcium chloride injection on tenderness of round muscles from *Bos indicus* bulls and late-castrated steers. Rounds (n=15) were injected thirty minutes post-exsanguination with 0.3

M $CaCl_2$ at 10% by weight. Calcium chloride injection reduced the shear value at days 1, 8, and 14 compared to the non-injected controls. Biceps femoris muscles injected with the solution required more time to cook and had greater cooking losses.

Whipple & Koohmaraie (1992) obtained steaks five days postmortem and marinated them in 150 *mM* $CaCl_2$ solution for 24 h or 48 h in phase 1 and for 48 h in phases 2 and 3. Steaks were from cows 8-11 years of age and steers fed the β-adrenergic agonist $L_{644.969}$. In phase 1, marination failed to improve shear force values. In phase 2, marination improved meat tenderness. Steaks from β-adrenergic agonist fed steers remained less tender regardless of marination. In phase 3, shear force requirements were decreased with marination.

Milligan *et al*., (1997) collected twenty standard beef rounds to study the effects of $CaCl_2$ injection and degree of doneness on inside round roasts. Inside rounds were halved and each half served either as a control or was injected with $CaCl_2$. Roasts were cooked to 60, 70, or 80 degrees C. Roasts injected with $CaCl_2$ were more tender as exhibited by a higher initial and sustained tenderness scores and decreased Warner-Bratzler shear force values.

Pringle et al., (1999) stated improving tenderness has been identified as a critical need by both the National Beef Quality Audit and the Beef Industry Long Range Task Force. Pringle et al. (1999) reported the effect of calcium chloride (CaCl2) injection on the calpain proteinase system and meat tenderness using three different breeds of steers, (A) Angus (n = 6), (B) Brahman (n = 6), and (F1) Brahman × Angus (n=6) processed at slaughter. Calpastatin activity was increased in muscle from B than in muscle from A and

F1 steers, and $CaCl_2$ injection reduced the activity of the calpains and calpastatin. Striploin and top sirloin steaks from A and F1 steers were more tender than steaks from B steers; however, top round steak tenderness did not differ across breed type. Calcium injection improved strip loin and top sirloin steak tenderness, but it did not affect top round steak tenderness. Pringle (1999) concluded from the data that $CaCl_2$ injection can be used to improve meat tenderness.

2.2.2 Phosphate and Salt

Phosphates are defined as the salt or ester of phosphoric acid, containing PO_4^{3-} (Merriam-Webster, 2015). Phosphates have been used in the meat industry to enhance meat water retention and provide increased meat yields. Phosphate increases the pH of meat and is a definite cause for water retention in meat at a pH isoelectric point of 5.3-5.5 (Shults et al., 1972). Huff-Lonergan & Lonergan (2005) reported that phosphates sequestering calcium and zinc ions of meat will increased water retention of meat. Research has shown that pH effects water retention and swelling of meat. One of the most essential additives to improve tenderness of beef has been salt (NaCl); this crystalline compound is abundant in nature and is commonly used to season and/or preserve food.

Shults et al., (1972) conducted a study to determine the merits of different sodium polyphosphates (sodium tripolyphosphate-TPP, sodium metaphosphate-MP, sodium hexmetaphosphate-HMP, tetrasodium pyrophosphate-PP) with and without sodium chlorides on the swelling of raw beef and the water holding capacity of beef during heating. The primary effect of phosphates on raw meats is to increase pH from approximately 5.2 towards a more alkaline value allowing for increased water-holding capacity and water binding capabilities. The increased pH effect was greatest in the PP mixture.

Sheard and Tali (2004) researched several marinade solutions and tested for their effects on pork tenderness. Loins were injected to a target of 110% of original weight with one of the eight solutions (g/100 g water): un-injected (control), 5% salt, 5% sodium tripolyphosphate ,3% sodium bicarbonate, 5% salt and 5% sodium tripolyphosphate 5%, 5% salt and 3% sodium bicarbonate, 5% sodium tripolyphosphate and 3% sodium bicarbonate, 5% salt, 5% sodium tripolyphosphate and 3% sodium bicarbonate. All marinade solutions significantly reduced the shear force value of treated loins.

The meat industry uses salts, phosphate, alginate and carrageenan to improve juiciness of meat. Lawrence *et al.* (2003) organized an experiment where *semitendinosus* and *longissimus* were injected in stages, calcium lactate followed by phosphate and salt (PS) to evaluate water-binding ability and palatability traits. Both the *semitendinosus* and *longissimus* muscles injected with PS increased pumped yield above those pumped only with calcium lactate only. Pumped yield decreased quadratically as the time between injections increased allowing PS to increase ionic strength and bond to water decreased.

Semimembranosus muscles were injected to 110 percent of the green weight with brines formulated to give 2 percent salt, 1.5 percent glucose, 0.3 percent phosphate, 0.15 percent calcium chloride or 3 percent sodium lactate. The most tender roasts from pre-rigor meat were produced using salt, phosphate or lactate brines and least tender using calcium chloride (Boles & Swan, 1997). The presence of phosphate in the brine or broth resulted in more tender roasts (lower peak shear force values and less energy to break the sample) than calcium chloride, salt or sodium phosphate, or water alone (Boles & Swan, 1996); salt increased the cook yield by altering the charge and solubilizing meat proteins, allowing the tissue to bind more water (Offer & Trinick, 1983; Paterson *et al*., 1988). Smith *et al.,* (1984) concluded that injection of brine containing sodium tripolyphosphate into pork longissimus increased juiciness and reduced Warner-Bratzler shear values, and also improved juiciness when injected into beef semimembranosus.

2.2.3 Carrageenan

Carrageenan is a polysaccharide derived from several varieties of red seaweed and is used in the meat industry as a water binder. It has the ability to retain large amounts of water by forming a solid like elastic gel. Carrageenan forms of kappa and iota are most commonly used in meat production. Form lambda is a non-gelling carrageenan and is more commonly used as a suspension for other ingredients in food products (Glicksman, 1982). It has also been used as a gel binder in ground meat production (Glicksman, 1982). Carrageenan is not to exceed 1.5 percent of product formulation under Directive 7120.1 of FSIS (FSIS, 1999).

Carrageenan is now being used as a fat substitute in processed meats. Levels of 0.2%-0.6% enhance water holding capacity, elasticity, and cohesive binding of meat products. Carrageenan is a major additive in non-dairy products and is under the scrutiny for the induced production of Interleukin-8 (IL-8), a pro-inflammatory chemokine associated with the promotion of neutrophil chemotaxis and degranulation in the digestive tract. Use of carrageenan is prohibited in some food products in the European Union and limited in other food products due to potential health effects it has on humans.

In a study of pale, soft and exudative (PSE) pork meat (Huang & Mikel, 1997), the addition of carrageenan kappa to PSE hams resulted in increased juiciness, tenderness, cohesiveness, and overall acceptability scores than normal hams.

In breakfast sausages, carrageenan was reported to increase the hardness of meat batters and improved the water holding ability (Barbut & Mittal, 1992). DeFreitas *et al*. (1997) reported increased gel strength and water retention when adding carrageenan to salt-soluble meat protein gels. Moreover, Xiong *et al*. (1999) reported that carrageenan increased the cooking yield, hardness and bind strength of low-fat sausages. Ayadi *et al.*, (2009) formulated turkey sausage products with 0.2%, 0.5%, 0.8%, or 1.5% carrageenan powder. They reported no difference in sensory evaluation for tenderness, juiciness or texture; however at 1.5% concentration the flavor score had a marginal decrease.

2.2.4 Alginates

Alginates are derived from brown algae of the family *Phaeophycea* and are used as gelling agents, for synthesis control and improved mouthfeel. The greatest advantage of alginate is it forms heat stable gels at room temperature (Means and Schmidt, 1986). Alginate is a substitution for consumers concerned with salt content (Kolari, 1980).

Means and Schmidt (1986) used alginate in combination with calcium carbonate to bind raw meat pieces together. They reported small quantities of the alginate within the processed meat ingredients are required for successful cold-set binding and successful restructured meat products like chicken fried steak fingers (Means & Schmidt, 1986).

Alginate provides binding properties to meats that are raw and cooked. Raharjo *et al.* (1994) used six trained panelists to evaluate the palatability of restructured steaks.

Steaks restructured with 0.5% Na-alginate/0.5% Ca-lactate had similar juiciness, bind, flavor, texture and color scores as controls. Steaks restructured with Na-alginate/Ca-lactate had less desirable flavor scores (3.6 ± 0.9) than those restructured with salt/phosphate (4.8 ± 0.6). Some panelists reported off-flavors in veal trimmings restructured with Na-alginate/Ca-lactate. Such off-flavors may be related to pockets of Na-alginate and/or Ca-lactate which had not been sufficiently dispersed and hydrated during mixing.

2.2.5 Broths/Brine

Brine is defined in culinary terms as a solution of salt or sugar/salt mixed in water (Krause *et al.,* 2011). In different references, brine may refer to predominately salt solutions ranging from about 2 percent up to about 20 percent. Boles and Swan (1997) studied the effects of brine ingredients on tenderness of pre-rigor processed roast beef. Semimembranosus (SM) muscles were injected to approximately 110% of their original weight with 4°C brine using a four-needle hand injector. The different ingredients were as follows; A. 2% salt (19% in brine), B.1.5% glucose (14.3% in brine), C. 0.3% phosphate (2.85% in brine), D. 0.15% calcium chloride (1.43% in brine), E. l 3% sodium lactate (28.5% in brine), F. water (control). Phosphate (injection C) injected roasts had the lowest peak shear values although these values were not significantly different from those for salt, glucose or calcium chloride injected roasts. This suggests that phosphate can increase product tenderness. Salt, glucose and sodium lactate treated roasts also had significantly lower peak shear force values than the water injected control.

2.2.6 Lipids

Enhancing beef products via lipid injection has been practiced in the meat industry. Lipids act as solvents for volatile compounds that develop during production, handling, and thermal processing (Moody, 1983). Lipids are known to strongly influence flavor, particularly species specific flavors (Moody, 1983). As intramuscular fat increases, the fat flavor which consumers prefer increases (Miller *et al.*, 2000). The use of fat to improve meat palatability can be seen used by the French technique of piqué. The French use of lard in meat using larding needles some with rigid, pointed tip ends or hinged ends. The aiguille has a hollowed handle or tube where you insert the fat and then inject the fat using a plunger. The other technique lardoire has a clip on the back where you attach the fat to and force the fat in, as though you were sewing the fat into the meat (The Culinary Institute of America, 1988).

Following French larding, the technique for injecting liquid edible beef fat into beef carcasses was developed in the early sixties (Durham *et al.*, 1961). Edible tallow was heated to 60˚C, and pumped directly into the muscles of the carcass at a pressure of 9-13 Pa. This technique was successfully used in hot and cold carcasses as well as individual cuts. The technique of pumping tallow into low quality carcasses could be used to improve palatability and visual marbling. The research of Durham *et al.*, (1961) paved the way for innovation by Seaboard Foods Inc., which offers a pork loin that has been enhanced with a mixture of salt, water, phosphate and a 7% mixture of fine particles of pork fat. Beef proprietors such as Cargill also use salt and water mixtures with phosphate to improve beef loins (steaks) to be prepared by low end restaurants.

Post-mortem injection of beef fat into beef sub-primals has been recently shown to improve tenderness and sensory attributes (Holmes *et al*., 2013). Holmes *et al.*, (2013) injected edible (71°C) beef tallow into low quality beef striploins. The research evaluated un-injected and injected steak samples on proximate analyses, Warner-Bratzler shear force test, fatty acid lipid profiling, and in-home consumer sensory evaluation. It was concluded that fat is important in cooking, as it melts and keeps the meat from drying out it also adds flavor.

The Japanese Meltique process uses lean beef produced in Australia and is injected with beef fat. The Hastings Food Processing plant (Establishment Number: 429) located in Wauchope, New South Wales, Australia produces the Meltique Beef products for Hokubee Australia PTY LTD. Their method injecting soluble oil perfects the French 'larding' process and improves primal frozen beef products. The 'Marbling' effect enhances the product's performance and ensures moisture, tenderness, and flavor is retained in cooking due to the internal basting process. The meltique product is trimmed and shaped to specifications set by the consumer such as restaurants, chefs, and private cooks (Hokubee Australia PTY LTD, 2014).

2.3 Methods to Enhance Meat

2.3.1 Immersion/marination

Marination is a method of reducing aging time for meat tenderization (Goodwin & Maness, 1984). Marinade ingredients such as salt, phosphates, acids, tenderizers, sugar, seasonings, and flavorings have various functions when added to chicken, pork, and beef. These include increasing water-holding capacity and moisture retention, decreasing cooking loss, improving tenderness and retarding warmed-over flavor development (Landes, 1972; Shults & Wierbicki, 1973; Chen, 1982; Young *et al*., 1992).

Marinades are primarily a surface treatment and may only penetrate 1/8" into the meat. After immersion of a cut of meat in a marinade for 24 hours, typically 1 to 2 % of the marinade gets into the meat depending on the fat content (Shrestha *et al.*, 2010). The main ingredient to penetrate deep into a retail cut is salt. Marinades must be saturated with salt and then they are considered brines and unless injected, the brine only penetrates a 1/8" at the minimal immersion time of 30 to 60 minutes.

Dhanda *et al*., (2002) used *semimembranosus* whole meat cuts divided longitudinally into two, almost equal, sections. One of these two sections was injected with brine containing sodium chloride and sodium tripolyphosphate to achieve 10% addition by weight, whereas the other side was kept as a non-injected control. The treatments were rotated so that both were represented equally in the muscle sections of all 20 *semimembranosus*. Marination by injection reduced shear force values of SM; that is, injected steaks/roasts had less shear force values (63.9 N) compared to control samples (102.3 N). A panel of 80 consumer's preferred injected steaks cooked to 77°C endpoint over other combinations, followed by non-injected steaks cooked to 71°C; whereas, injected steaks cooked to 71°C and non-injected steaks cooked to 77°C were equally least preferred.

Hashim *et al.*, (1999) experimented on immersion and injection of chicken breast with seasonings and immersed them in marinades hydrated with either water or a water/liquid honey mixture. Chicken breasts were either immersed overnight using lemon-pepper marinade seasoning (5.27% marinade powder and 94.73% tap water) or injected with lemon-pepper at 14.37% over green weight (14.37% marinade powder and 85.63% tap water). Three levels of honey were substituted for water in the marinade (10, 20, and 30%) to understand the functionality of honey as a marinade ingredient. Honey flavor of immersed chicken was not affected by honey level. For injected chicken, the influence of honey level on honey flavor was most apparent at the 20 and 30% honey levels where the honey flavor was the most noticeable. Juicy texture of injected chicken was not affected by honey level. For the immersion method, chicken marinated without honey was rated as the juiciest whereas that immersed in 20% honey marinade was rated least juicy. Injected chickens retained more (9.24%) marinade than immersed (1.75%) chickens.

2.3.2 Massaging/ Tumbling

Whole meat cuts are placed into a mechanical tumbler along with a cold, seasoned liquid such as a brine or marinade. The liquid needs to be cold to keep the meat at a safe temperature (< 4°C). The tumbler is usually a stainless steel drum that rotates slowly, about 15 to 20 rpm. The drum is placed under a vacuum to facilitate absorption and to eliminate the ambient environment for proliferation of aerobic bacteria. As the meat cuts rub each other inside the rotating tumbler (with or without paddles), abrasion loosens the protein network of the meat, allowing proteins in the muscle fibers to absorb liquid. The goal of tumbling meat commercially is to extract salt-soluble proteins such as myosin and add water back into meat. In most countries, regulations dictate how much water meat processors are allowed to add to a piece of meat, otherwise the meat must be sold as "water added". With a tumbler, up to 12% or more of the marinade may be absorbed into the meat. (Froning & Sackett, 1985).

Turkey breast muscle was injected with salt and various types of phosphate (sodium tripolyphosphate; 90% sodium tripolyphosphate and 10% sodium hexametaphosphate; 75% sodium tripolyphosphate and 25% sodium hexametaphosphate; 90% sodium tripolyphosphate and 10% tetrasodium pyrophosphate) solutions to 103% of green weight (Froning and Sackett, 1985). After injection, breasts were tumbled in a cold room in a laboratory-sized tumbler for 2 hr at 20 rpm with alternate intervals of tumbling for 10 min and 10 min of rest for a total of 1 hr of tumbling. Sensory properties (binding ability, juiciness, flavor) of rolls made from tumbled meat were significantly improved when the breast muscle was tumbled in the presence of salt and phosphates.

2.3.3 Injection

The use of needles and syringes first appeared in the 17th century for experiments with remedies and drugs to heal the sick. Until the late 19th century, the use of needles and syringes were exclusively for medical purposes. As time progressed, the use of needles and syringes integrated into the culinary and meat industry as devices to facilitate additive administration to improve meat palatability. The meat industry faced the need for faster and more effective methods of flavoring addition and improving the uses of brines and marinades. Currently, consumer use of injecting spices, herbs, and juices to enhance their meat cuts is increasing at home. Injection has long improved meat palatability at the commercial level and more recently has moved into household use to improve meat tenderness, juiciness, and flavor.

Rodas-González *et al.*, (2015) used 19 loins from control steers and 20 loins from zilpaterol hydrochloride fed steers, injected them with 200m*M* food grade $CaCl_2$ and aged them 7, 14, 21, and 28 d. Previous work had shown injection at 24 or 48 h with 200 mM $CaCl_2$ at 5% could be applied without detrimental effects on palatability (Wheeler *et al.*, 1993; Diles *et al*., 1994; Kerth *et al*., 1995, Lansdell *et al.*, 1995). Injection with $CaCl_2$ resulted in more desirable scores for flavor intensity and beef flavor compared with steaks from non-injected strip loins aged 14 d. In addition, mean scores for off flavor were greater for steaks from strip loins injected with $CaCl_2$. Panelists did not detect differences in any other palatability traits due to $CaCl_2$ injection in steaks aged 14 d. They concluded that zilpaterol hydrochloride reduced tenderness of USDA Select strip loins.

McGee *et al.*, (2002) used 30 pairs of inside rounds from USDA Select graded carcasses. Paired muscle samples were cut in half (20.32 x 15.24 x 15.24 cm) and assigned to one of four injection treatment groups (0, 5, 7, and 9% injection levels), using 0.25% sodium tripolyphosphate, 0.35% sodium chloride, and 2% sodium lactate. The injection resulted in a difference between control and injected rounds at 9%. Stites et al., (1989) concluded that injecting beef roasts to 10% of their original weight with a solution containing sodium tripolyphosphate and sodium chloride decreased WBS force when compared to controls. The 5% treatment group at 35 days was significantly different from both the 7 and 9% treatments for all sensory days. This data is in agreement with the findings of Papadopoulos *et al.* (1991) who reported that the addition of sodium lactate, 0.5% sodium chloride, and 0.3% sodium tripolyphosphate increased sensory panel tenderness. Consumer sensory evaluation, detected significant differences in palatability attributes between the control and injected beef inside rounds and between injection treatment levels of 5, 7, and 9% (McGee *et al.*, 2002).

Holmes *et al.*, (2014) used an injection system to administer edible cooked beef tallow into beef strip at 13.4 % more (by weight) than the control steak samples. They reported an increase in cooking loss (8.6% greater) from the injected samples; however shear force values were 6.0 N less. In the consumer evaluation, in-home consumers preferred fat-injected steaks 2 to 1 over the non-injected controls. This product research exhibited that the use of injection systems can improve tenderness, juiciness, flavor, and distribution of the solution being injected into the meat product.

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CHAPTER III

POST-MORTEM MECHANICAL INJECTION OF LOW QUALITY BEEF LOINS WITH PORK BACK FAT IMPROVES PALATABILITY ATTRIBUTES

3.1 Abstract

Palatability attributes of beef striploin steaks mechanically enhanced with pork fat were evaluated. Beef striploins were collected from USDA Standard steer carcasses. Loins were longitudinally cut into halves (lateral or medial) and assigned randomly to pork fat injection (PFI) or non-injected control (CON). Loin halves assigned to PFI were enhanced with cooked (>71°C) pork fat using a multi-needle injector. Steaks were analyzed via Warner-Bratzler shear force, trained and consumer sensory panels, and proximate analysis (cooked and uncooked). Shear force values for PFI steaks were lower ($P<0.01$) than CON steaks (2.50 vs. 4.44 kg, respectively). Trained panelists detected ($P=0.02$) an off-flavor for PFI steaks but were unable to discern other attribute differences. Consumer panelists denoted ($P=0.05$) improved tenderness and overall preference ($P=0.01$) for the PFI treatment. Cooked PFI steaks had less ($P<0.01$) moisture (-1.0%) and more ($P<0.01$) fat (+1.3%) than CON steaks; protein did not differ ($P=0.14$). This processing method deserves further investigation for new product development.

3.2 Introduction

Meat palatability can primarily be attributed to the three essential tasting qualities tenderness, flavor, and juiciness. Approximately 48% of 1,090 consumers surveyed ranked tenderness as the most important trait when purchasing meat, 36% ranked flavor the second most important trait, and juiciness ranked 19% (Chichester, L. M., Consumers' Perception and Preferences of Meat and the Meat Industry, Unpublished doctoral dissertation; West Texas A&M University, 2009). For some consumers, it can be rather expensive to purchase premium cuts of meat that are considered more tender, juicier, or flavorful (e.g. tenderloin, ribeye, etc.). For the past 30 years, innovations in improving the tenderness of meats and using inexpensive additives have been on the forefront of research. French chefs have used larding needles to insert lard into meat using a hollowed handle or tube. The method of lardoire has a clip on the back where you attach the fat to and force the fat in, as though you were sewing the fat into the meat (The Culinary Institute of America, 1988).

A technique to improve tenderness using the principle of French larding by injecting liquid edible beef fat into beef carcasses was reported by (Durham et al. 1961). Post-mortem injection of beef fat into beef subprimals has been recently shown to improve tenderness and sensory attributes (Holmes et al. 2013). Based on current market prices, Select boxed beef is priced at $229.32/cwt. Market prices for IMPS 180 prime is priced at $316.54/cwt, premium choice is priced at $297.93/cwt, choice is priced at $264.59/cwt, select is priced at $229.32/cwt and no roll is priced at $193.70/cwt.

Pork is known to add a desirable flavor to other meats, such as beef, which may improve palatability and increase the euphoria that is associated with the combination of beef and pork.

The objective of the experiment was to examine the palatability attributes of low quality beef striploin steaks mechanically enhanced with pork subcutaneous fat.

3.3 Materials and Methods

3.3.1 Muscles

Beef striploin subprimals (IMPS 180; n = 40) from USDA Standard quality grade steer carcasses (one loin per carcass) were collected from the fabrication line of a commercial beef processor (Tyson Fresh Meats; USDA Est. 245E), vacuumed packaged, transported to the West Texas A&M University meat laboratory and stored at 2° C until 14 d post-mortem. Carcass data including 12th rib subcutaneous fat, ribeye area, estimated percentage kidney, pelvic, and heart fat, hot carcass weight, estimated yield grade, and marbling score were collected by an E + V Vision Grading camera.

3.3.2 Fabrication

At 14 d post-mortem, the forty loins were assigned randomly to pork fat injection (PFI) treatment or to non-injected control (CON). Subcutaneous fat was trimmed to the epimysial connective tissue of each loin using a mechanical knife (Whizard Knife Series II, 1000M2, Bettcher Industries, Inc. Vermilion, OH, USA). After fat removal, the vein portion (containing the *gluteus medius* and *longissimus lumborum* muscles) of each loin was removed and loins were longitudinally cut into halves (denoted lateral or medial). Beef loins assigned to the control treatment were vacuum packaged and stored in a freezer at -28.9°C. A green weight (kg) was recorded for each control and treated half.

3.3.3 Fat Processing

One-hundred and thirty six kg of edible pork subcutaneous fat was purchased from a pork processing plant (Seaboard Foods, Inc.; USDA Est. 13597). Fat was coarse ground (1.27 cm plate hub) via a grinder (BIRO® MODELS 548SS, The Biro MFG. Co., Marblehead, Ohio, USA) and stored at 2°C for 1 d. A propane fired oil heater was used to melt and fully cook (>71°C) the fat to facilitate separation of collagen from fat. Melted fat was poured through 25.4 cm shortening filter cones (10" Filter Cones, FC-10-3, Disco Manufacturing Company, McDonough, GA, USA) to strain the pure fat from the solids; solids filtered from the liquid edible pork fat were discarded. Filtered fat was allowed to cool and held at 60°C using a drum belt heater (710-55-230 Heater, 55GAL STL, 230V, 1500W, Morse Manufacturing Co., Inc., East Syracuse, NY, USA) stored in a 57 L pot for 24 h.

3.3.4 Fat Injection

Strip loins were injected with the melted and fully cooked edible pork fat using a Günther Pickle Injector (Injectomatic 280/282 PI 9-21 Brine Injector, Koch Equipment, Kansas City, MO, USA) with a series of perforated needles. Loin halves were put through the machine three at a time side by side. Treated halves were allowed to cool for thirty minutes so the liquid fat would solidify, weighed to obtain an injected weight for calculation of percentage of lipid enhancement, vacuum packaged and stored in a freezer at -28.9°C. Fat that accumulated on the external surface of the strip loin halves was removed once it solidified.

3.3.5 Processing

Once the beef loins were frozen the control and treated halves were matched according to their identification. Beginning at the anterior end, loins were cut into 2.54cm steaks (Figure 3.1) and were assigned respectively: 1st and 2nd pair-Warner Bratzler Shear Force analysis, 3rd and 4th pair-Proximate analysis, 5th pair-Trained sensory panel analysis, 6^{th} to 8th pair - Consumer analysis (Figure 3.2).

3.3.6 Warner-Bratzler Shear Force Determinations

Steaks were defrosted at 2°C for 24 h then cooked in a forced-air convection oven (Blodgett, model CTB/R, G.S. Blodgett Co., Burlington, VT) set at 177°C. Internal temperature of each steak was monitored using copper-constatan thermocouples (Omega Engineering, Stamford, VT) positioned in the geometric center of each steak and connected to a temperature monitoring device (Omega Engineering Stamford, VT); steaks were removed from the oven at 69.5°C in order to reach a target endpoint temperature of 71°C. Steaks were cooled on a rack for approximately 10 minutes, wrapped in cellophane and chilled for 24 h at 2°C. After chilling, six cores (1.27 cm diameter) were removed from each steak parallel to the muscle fiber orientation using a mechanical coring device. Cores were immediately sheared once through the center using a V-shaped blade on a Warner-Bratzler shear force machine (G-R Manufacturing, Manhattan, KS). Peak shear force was displayed in newtons on a Mecmesin BGN-500 Shear Force Gauge (Newton House, United Kingdom) and recorded.

3.3.7 Trained Sensory Evaluation

One hundred and sixty pairs of sample steaks (80 CON/80 PFI) were evaluated at Kansas State University by trained panelists for sensory attributes; using a 1 to 8 scale panelists scored (8 Extremely juicy/tender/none/intense, 7 Very juicy/tender/practically none/intense, 6 Moderately juicy/tender/traces/intense , 5 Slightly juicy/tender/slight/intense , 4 Slightly dry/tough/moderate/ bland , 3 Moderately dry/tough/slightly abundant/bland , 2 Very dry/tough/moderately/bland , 1 Extremely dry/tough/abundant/none. Off-flavor: asparagus, apricot, barnyard, beet, buttery burnt, chemical, chocolate/cocoa, cooked milk, cumin, dairy, floral, green-haylike, heated oil, refrigerator, stale, rancid, warmed-over), steaks for myofibrillar tenderness, juiciness, beef flavor intensity, overall tenderness, and off-flavor intensity. Each panelist received two samples from treated and control steaks that were adjacent from the same loin. Steaks were defrosted at 2°C for 24 h then cooked in a forced-air convection oven (Blodgett, model CTB/R) set at 177°C. Internal temperature of each steak was monitored using copper-constantan thermocouples (Omega Engineering) positioned in the geometric center of each steak and connected to a temperature monitoring device (Omega Engineering); steaks were removed from the oven at 69.5°C to target an endpoint temperature of 71°C. Each steak was labeled to their perspective loin and position. Samples were then cut into 1.27 x 1.27 cm x steak thickness cubes.

3.3.8 Consumer Sensory Evaluation

One hundred and forty pairs of sample steaks (70 CON / 70 PFI) were evaluated at West Texas A&M University by untrained consumers for sensory attributes; using a 1 to 9 scale panelists scored (1-dislike extremely, 9-like extremely) samples for tenderness, flavor, juiciness, and texture. Investigators prepared and served 56 steaks per-day at a temperature of 71°C and each participant completed a consent form, demographics information, and palatability attributes survey (Figure 3.3). In addition, panelists were asked to provide their overall preference. Each consumer received one treated and one control steak that were adjacent (medial/lateral) from the same loin. Each participant was informed upon completion of the demographics and survey they would receive a ten dollar gift certificate redeemable at the WTAMU meat lab. Steaks were defrosted at 2°C for 24 h then cooked in a forced-air convection oven (Blodgett, model CTB/R) set at 177°C. Internal temperature of each steak was monitored using copper-constantan thermocouples (Omega Engineering, Stamford, VT) positioned in the geometric center of each steak and connected to a temperature monitoring device (Omega Engineering Stamford, VT); steaks were removed from the oven at 69.5°C to target an endpoint temperature of 71°C. Each steak was labeled to their respective loin and position and wrapped in foil to be transported to a warming oven. Samples were kept at temperature (71°C) until a consumer was ready to consume the samples. Samples were then cut into 1.27 x 1.27 cm x steak thickness cubes to mimic the trained sensory samples.

3.3.9 Proximate Analysis

Beef striploin samples were trimmed of any excess fat, cut into 2.54 cm cubes, frozen in liquid nitrogen, and pulverized in a food processor (Cuisinart, East Windsor, NJ). Upon removal from the processor, 13g ± 5g samples from each steak (2 uncooked and 2 cooked samples) were placed in a labeled sample bag and frozen (-18°C). Quantification of moisture, fat, and protein were performed in duplicate per procedures reported by Servi-Tech laboratories in Amarillo, Texas (Moisture, AOAC 934.01; Crude protein, AOAC 990.03; Crude fat, AOAC 2003.06). At Servi-Tech laboratories the samples were re-thawed and weighed to obtain a wet weight and placed in an oven to dry for 3 h at 130°C. After removal from the oven, samples were placed into a desiccator to allow samples to cool without producing moisture. Upon removal from the desiccator, samples were weighed again to obtain a dry weight. Moisture concentration (percent) was calculated using the following formula 100 * [sample weight- (last re-weigh – tare weight)] / sample weight. Crude protein was calculated using the formula, crude protein, % (w/w) = % N * 6.25. A plug of defatted cotton was placed on top of the sample to keep it in the thimble during extraction; 70–90 mL of solvent was used. The sample was raised and suspended over the boiling solvent. During rinsing, residual traces of the extractable material were flushed out of the sample and retained in the extraction cup. The control and pork-fat injected samples lipid concentration were weighed into the extraction thimbles and calculated: % Fat = (W2 – W1)/W3*100, where W1 = weight of the extraction cup; W2 = weight of the extraction cup + extract; W3 = weight of the sample.

3.3.10 Statistical Analysis

A randomized complete block experimental design was used for the experiment; the forty beef strip loin subprimals were the blocks and individual steaks were sampling units. A one-way treatment structure of was used; and the experimental unit was ½ of striploin (medial or lateral) with treatments assigned randomly using a random number generator. An LSMEANS statement generated means and PDIFF statement was used to determine where the differences (α=0.05) occurred between treatments. The analysis was conducted using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) for interval scale values of WBSF and proximate analysis. The fixed effect was treatment and random effects were animal and location (lateral or medial half). Ordinal data were analyzed using the Wilcoxon rank-sum test (NPAR1WAY) test procedure of SAS (SAS Institute, Inc. Cary, NC). The sensory analysis responses were separated by palatability attributes: tenderness, juiciness, flavor, texture, and overall acceptability. The UNIVARIATE procedure was used to obtain quartile deviations for the analysis of the trained sensory traits: myofibrillar tenderness, juiciness, beef flavor intensity, overall tenderness, and off-flavor intensity. Overall preference data were analyzed using a chi-square test via PROC FREQ in SAS.

3.4 Results and Discussion

3.4.1 Carcass Measurements

Loins were collected from carcasses that had marbling scores with minimum of 230 (Traces ^{30}) – and a maximum of 380 (Slight ^{80}) and the average camera marbling score equaled Traces^{90} (Table 3.1). Hot carcass weight data indicated the sample population weighed 368 ± 44 kg, with an LM area of 109 ± 10 cm^2, 12^{th} rib subcutaneous fat depth of 0.50 ± 0.25 cm. and average USDA calculated yield grade of 1.08 ± 0.26. For comparison, the 2011 National Beef Quality Audit calculated yield grade average was 2.6 and the average marbling score Small^{40} (Moore et al. 2012). Estimated KPH percentage had a mean value of 1.59%, minimum of 1.26% maximum of 1.99% estimated values based on an algorithm of other data according to the E+ V camera data.

3.4.2 Injection and Proximate Analysis

Fat injection was calculated at 12.61 %, which is similar to previous research studies which reported fat injection ≥13%. Loin halves were injected three simultaneously to allow equilibrium of the injector, and no one loin percentage pump was significantly increased or decreased. Proximate analyses were performed to obtain an overview of the steak samples protein, moisture, and fat content prior to and after cooking. Proximate analyses of uncooked striploins injected with pork fat revealed they had less (P < 0.01) moisture (-5.2%) and protein (-1.9%) concurrent with greater (P < 0.01) fat (+7.3%) than control striploins (Table 3.2). Proximate analysis of cooked striploins injected with pork fat revealed less (P < 0.01) moisture (-1.03%) and more fat (+1.28%) with no difference (P=0.14) in protein content. Protein and fat increased based on proportion of the samples initial percentage. There was not an increase of weight in protein and fat, only the amount of moisture and fat cooked loss of those components(Table 3.2). As hypothesized, the fat-injected steaks had more fat than the control and less moisture based on proportions due to the displaced water by the fat in the muscle. The greater the fat content of whole meat muscle, the less total water holding capacity of the muscle (Romans *et al*., 2001). Holmes *et al*., (2013) reported fat-injected steaks had the greatest intramuscular fat concomitant with less moisture content due to fat displacement of water. Because fatty tissues contain little moisture, the greater the fat content of muscle, the less total water content (Romans *et al*., 2001).

3.4.3 Warner-Bratzler shear force

Warner-Bratzler shear force data (Table 3.2) demonstrated lower ($P < 0.01$) peak force values for PFI steaks as compared to the CON treatment (24.51 vs. 43.54 N, respectively). These data are in agreement with Holmes et al. (2013) which indicated that steaks injected with fat had an average shear force value of 25.4 N compared to non-injected control steaks average shear force value of 31.4 N. These data are also in agreement with Durham *et al.* (1961), whom noted that the steaks injected with fat were using less pounds of cutting pressure using the Warner-Bratzler shear force. USDA quality grades for beef carcass beef have been evaluated using visual assessments by graders for degree of marbling in the *longissimus* muscle in relation to maturity (USDA, 1997). Standard beef carcasses historically have produced shear force values greater than Prime beef carcasses (Emerson et al., 2014). Timm et al. (2003) report Standard steaks had greater ($P < 0.05$) values for Warner-Bratzler shear force test than Prime steaks, indicating that LM steaks from carcasses graded Standard were less tender.

During the cooking process, the drip and evaporative loss for both control and pork-fat injected steaks were monitored and recorded. Pork-fat injected steaks averaged 28.40% cooking loss compared to controls that lost an average of 19.6% with difference of 8.77% ($P < 0.01$). As in relation to previous study of Holmes *et al*. (2013) reported 31.06% average loss by the fat-injected and an average loss of 22.45% by the control with a difference of 8.62% ($P < 0.01$).

3.4.4 Trained sensory

Trained panelists detected (P=0.02) an off-flavor for pork fat injected steaks (Table 3.3) and rated the PFI steaks (6.46±0.80) more off-flavor (e.g. peanut, burnt, porky, cow-like) than the CON steaks (6.14±0.75). However, scores for tenderness, juiciness, myofibrillar tenderness, and connective tissue were not different ($P \geq 0.05$). However, we hypothesized a "Halo effect" on the trained sensory panelist, where they were likely overwhelmed by the off-flavor of pork in beef and were unable to discern the other palatability attributes (Lawless, 1995). Panelists that already have an impression about the product when asked about a second trait – will form a logical association (e.g. dry= tough) (Clark & Lawless, 1994; Stone *et al*., 2012)

3.4.5 Consumer sensory evaluation

During the week of February 16-20, 2015, 140 pairs of steaks from beef striploins were cooked (71°C) at the WTAMU meat lab. The steaks once cooked were transported to the commons building on campus to be kept warmed and served to panelist. The population of the sensory were university faculty, staff, and students each participant completed a consent form, demographics sheet, and palatability attributes survey.

Demographic data indicated that the participants were 56% male and 44% female consumers (Table 3.4). Of the one-hundred and forty consumers, they ranged in ages from 18 years to 73 years old, with a median age of 27. Highest levels of education completed were reported as: High School- 44%; Trade School-1%; Associates Degree - 21%; Bachelor's Degree -13%; Master's Degree- 11%; Professionals Degree (Ph.D., Ed.D., M.D., J.D., or D.V.M.) -10%. Consumers were represented by 13% African-American/Black, 0% Asian Pacific/Islander, 19% Hispanic/Latino, 7% Interracial, 0% Native American/American Indian, 61% Caucasian/ White. Annual household income was indicated as: less than 10,000- 23%; 10,001-30,000 -26%; 30,001-50,000 - 14%; 50,001-70,000-11%; 70,001-90,000- 10%; 90,001-100,000 - 3%; and above 100,001- 13%.

Frequency of beef consumption (Table 3.5) was indicated as: daily- 22%; weekly- 66%; monthly- 9%; yearly- 2%; Never/NA- 1%. Grades of beef preferred when purchasing a steak were indicated as: Prime- 39%; Choice- 32%; Select- 9%; Standard- 1%; No Preference -19%. Consumers were asked to score on the 1 to 9 point scale the research steaks visually and their preference of them the means were indicated as: pork-fat injected- 7.14, non-injected control- 6.20. The visual appraisal of the research steaks indicated the preference as: 15 (11%) visually preferred both steaks, 38 (27%) visually preferred the control steak, 88 (62%) visually preferred the injected steak. Based on previous research beef consumption decreased from daily intake of 26% to 22%, increased the weekly consumption of 64% to 66%, and consumption on monthly basis or never consumed beef showed no difference (Holmes *et al*., 2013). There was also differences in age range and ethnicity of consumers when compared to recent injection study, where an increase of minority beef consumers were evaluated and younger age group participation increased.

The objective of the consumer palatability analysis was to evaluate each steak and rank tenderness, juiciness, flavor, and texture using a 9-point hedonic scale: 9= extremely like, 8= like very much, 7= like moderately, 6= like slightly, 5= Neutral, 4= dislike slightly, 3= dislike moderately, 2= dislike very much, 1= dislike extremely) and to specify overall preference between the PFI steak and the CON steak (Table 3.6). Consumers tended (P = 0.08) to rate the PFI steaks (6.72±0.6) more tender than the CON steaks (6.30±0.7). For juiciness, consumers rated the PFI steaks (6.30±0.75) as juicier (P = 0.05) than the CON steaks (5.70±0.70). However, scores for flavor and texture were not different ($P \geq 0.13$). Consumers were asked to choose which sample they preferred based on tenderness, juiciness, flavor, and texture ratings. Of the 140 consumers, 83 (59%) of consumers preferred the PFI steaks (P<0.01) whereas 57 (41%) of the consumers preferred the CON steaks. Holmes *et al.*, (2013) reported consumers rated enhanced steaks (8±0.3) more tender and juicy (P<0.01) than the non-enhanced control (7±0.05). Of the 127 consumers, 87 (69%) preferred the injected steak striploins (P<0.01) whereas 40 (31%) preferred the control steaks.

3.4.6 Discussion

Because the trained sensory data are in contrast to the consumer panel and WBSF data, a plausible theory is the Halo effect – caused by evaluating too many factors at one time (Lawless, 1995). Panelists already have an impression about the product when asked about one particular attribute and will often form an association between traits. We hypothesized that the plausibility of a halo effect was exhibited based on the data received and comments made by the sensory panelist organizers. Trained panelists were so overwhelmed by the pork flavor in a beef steak that they were not able to discern between the other palatability attributes. It is possible that the halo effect can be avoided by asking the panelist to rate each individual attribute rather than a one bite evaluation of all attributes.

Review of these data suggests that mechanically injecting low quality beef striploins with pork subcutaneous fat altered proximate analysis and improved palatability. This processing method deserves further investigation and may offer an opportunity for new product development where the use of pork fat would improve palatability and consumer euphoria of a beef/pork integrated product. Consumers during the controlled evaluation and survey requested seasoning to increase the flavor of the meat sample. Comments at the end of the survey suggest consumers associate flavor as an essential attribute correlated with tenderness (i.e. no salt flavor = less tender meat). Consumers' preferred PFI steaks over CON steaks but an undesirable flavor of pork that appeared to mask the desired beef flavor made it difficult for the trained sensory panelists to discern the other palatability attributes. Further investigations should include use of anti-oxidants such as rosemary or thyme to retard lipid oxidation and increase consumer acceptability. Future research should investigate methods to reduce the needle streaks that appeared (Figure 3.1) in the steak in an effort to more closely mimic naturally deposited marbling. Possibly increasing the injector pump pressure to accommodate pork fat will improve equal dispersion. This project has investigated the possibility of using pork-fat injection to improve the current market where approximately 5% of beef carcasses do not meet the minimal quality standards to grade USDA Select.

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Table 3.1 Descriptive statistics of carcass traits for sample population.

Item	Mean	Median	Std. dev.	*Quartile dev.*	Min.	Max
12th rib subcutaneous fat, cm	0.50		±0.25		0.05	1.04
LM area, cm^2	109.01		±10.15		96.77	133.81
Estimated KPH,%	1.59		±0.17		1.26	1.99
HCW, kg	368.09		±43.85		285.00	489.60
USDA Yield Grade[a]	1.08		±0.26		1.0	2.0
Marbling Score[b]		290		±20	230	380

[a]USDA yield grade= 2.5 + 2.5 (Fat thickness, in.)+ 0.0038 (hot carcass weight, lbs)+ 0.2 (Kidney, Pelvic, Heart fat %) – 0.32 (loin muscle area, in^2)

[b]Marbling Score: (*Prime*) abundant=900, moderately abundant=800, slightly abundant=700, (*Choice)* moderate=600, modest=500, small=400, (*Select*) slight=300, (*Standard*) traces= 200, practically devoid= 200, e.g.(290= traces 90)

Table 3.2 Injection, proximate analysis and objective tenderness attributes of control and pork-fat injected beef striploin steaks

Items	Control (CON)	Pork-fat injected (PFI)	SEM	P-value
Green weight, kg	1.80	1.87	0.06	0.14
Injected weight, kg	1.80	2.12	0.11	<0.01
Injection,%		12.61		
Moisture, % (uncooked)	72.79	67.58	0.21	<0.01
Moisture, % (cooked)	62.84	61.81	0.23	<0.01
Protein, % (uncooked)	24.36	22.47	0.14	<0.01
Protein, % (cooked)	32.63	32.12	0.24	0.14
Fat, % (uncooked)	2.26	9.55	0.25	<0.01
Fat, % (cooked)	4.17	5.45	0.22	<0.01
Cooking time, minutes	24.80	21.60	0.13	<0.01
Cooking loss,%	19.67	28.44	0.21	<0.01
WBSF, N	43.54	24.51	0.17	<0.01

Table 3.3 Trained sensory panel evaluation of control and pork-fat injected beef striploin steaks cooked to 71°C

Item	Control	Pork-Fat Injected	P-value
Tenderness[a]	5.8±0.45	6.0±0.43	0.62
Juiciness[b]	4.6±0.35	4.6±0.23	0.15
Myofibrillar tenderness[a]	6.0±0.88	6.5±1.00	0.50
Connective tissue[c]	6.5±0.63	6.7±0.88	0.71
Off-flavor[d]	8.0±1.00	6.0±1.50	0.02

[a] 5=slightly tender, 6=moderately tender

[b] 4=slightly dry, 5=slightly juicy

[c] 6=traces, 7=practically none

[d] 6=traces, 7=intense, 8= extremely intense

Table 3.4 Demographics of consumer sensory survey participants

Variables	Frequency
Gender	
Male	56%
Female	44%
Age	
< 21	30%
22-29	44%
30-39	9%
40-49	9%
50-59	5%
> 59	3%
Education	
Less than high school	0%
High school	44%
Trade school	1%
Associates degree	21%
Bachelor's degree	13%
Master's degree	11%
Professionals degree	10%
Other	0%
Ethnicity	
African-American/Black	13%
Asian Pacific/Islander	0%
Hispanic/Latino	19%
Interracial	7%
Native American/American Indian	0%
Caucasian/White	61%
Other	0%
Income, $	
Less than 10,000	23%
10,001-30,000	26%
30,001-50,000	14%
50,001-70,000	11%
70,001-90,000	10%
90,001-100,000	3%
100,001 or more	13%

Table 3.5 Frequency of beef consumption and beef quality grade preference

Variables	Frequency
Beef Consumption	
Daily	22%
Weekly	66%
Monthly	9%
Yearly	2%
Never/NA	1%
Grade or Beef Preferred	
Prime	39%
Choice	32%
Select	9%
Standard	1%
No Preference	19%

Table 3.6 Consumer sensory evaluation of control and pork-fat injected beef striploin steaks cooked to 71°C

Table 3.6 Consumer sensory evaluation of control and pork-fat injected beef striploin steaks cooked to 71°C

Item	Control	Pork-Fat Injected	P-value
Tenderness[a]	6.3±0.70	6.7±0.60	0.08
Juiciness[b]	5.7±0.70	6.3±0.75	0.05
Flavor[c]	5.7±0.45	5.9±0.50	0.23
Texture[d]	6.1±0.75	6.4±0.80	0.13
Overall preference[e]	57	83	0.02

[a] 5= neutral, 6=like,7= like slightly

[b] 5=neutral, 6=like, 7= like slightly

[c] 4=dislike,5=neutral, 6= like

[d] 4=dislike, 5=neutral, 6=like.

Figure 3.1 Control beef striploin steaks (top) and pork-fat injected beef striploin steaks (bottom)

MEDIAL	LATERAL
WBSF (2.54 cm thick)	WBSF (2.54 cm thick)
WBSF (2.54 cm thick)	WBSF (2.54 cm thick)
Proximate Fat Analysis (2.54 cm thick)	Proximate Fat Analysis (2.54 cm thick)
Trained Sensory (2.54 cm thick)	Trained Sensory (2.54 cm thick)
Proximate Fat Analysis (cooked sample/2.54 cm)	Proximate Fat Analysis (cooked sample/2.54 cm)
Consumer Analysis (2.54 cm)	Consumer Analysis (2.54 cm)
Consumer Analysis	Consumer Analysis
Removal of Vein Portion	Removal of Vein Portion

Figure 3.2 Allocation of steaks to analyses

PLANT CARCASS ID: 012210
RIBEYE AREA: 92.07 cm^2
MARBLING SCORE: 300 – Slight 0
YG: 1
WT ID: 118

Figure 3.3 Breakout of steaks allocated for analyses

Beef Striploins

Ballot Instructions and Demographic Information

Please complete the demographic information below. After you have received your cooked steak sample we ask that you consume it as you would normally do and not share your thoughts with those around you, as you fill out this ballot. Please, remember to insert your Participant ID number in the appropriate blank. ATT: YOU MUST BE 18 YEARS OF AGE TO COMPLETE THIS SURVEY. Thank you again for participating in this consumer survey. REMINDER: ALL SURVEYS WILL REMAIN CONFIDENTIAL. Once you have completed the survey please place in the basket provided. This survey should only take 10-15 minutes of your time.

❶ YOUR PARTICIPANT NUMBER __________

❷ GENDER

◯ MALE

◯ FEMALE

❸ AGE

____

❹ HIGHEST LEVEL OF EDUCATION COMPLETED.

◯ Less than High School

◯ High School

◯ Trade School

◯ Associates Degree

◯ Bachelor’s Degree

◯ Master’s Degree

◯ PhD, EdD, MD, JD, DVM, or other professional degree

◯ Other (Please Specify)___________________________________

❺ **ETHNICITY**

◯ AFRICAN-AMERICAN ◯ASIAN PACIFIC/ISLANDER ◯HISPANIC/ LATINO

◯ INTERRACIAL ◯NATIVE AMERICAN/ AMERICAN INDIAN ◯WHITE/

CAUCASION ◯ OTHER (PLEASE

SPECIFY)________________________________

❻ ANNUAL HOUSEHOLD INCOME. PLEASE ONLY CHOOSE ONE.

◯ LESS THAN $10,000

◯ $10,001-$30,000

◯ $30,001-$50,000

◯$50,001-$70,000

◯$70,001-$90,000

◯$90,001-$100,000

◯$100,001 OR MORE

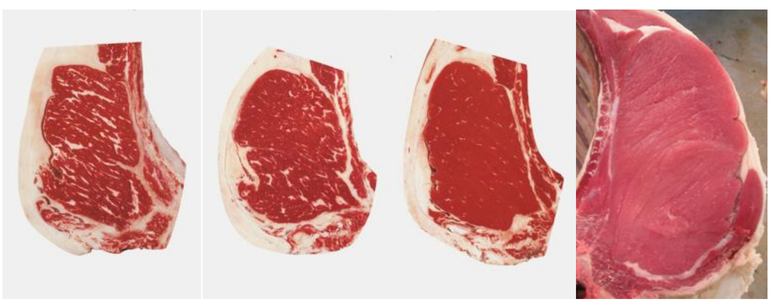
❼ **HOW OFTEN DO YOU CONSUME BEEF?**

CONSUMPTION OF BEE F: ◯DAILY◯WEEKLY◯

MONTHLY ◯YEARLY ◯NEVER

❽ **WHICH GRADE OF BEEF DO YOU PREFER?**

◯ PRIM E ◯ CHOICE ◯ SELECT ◯STANDARD ◯ NO PREFERENCE



PRIME **CHOICE** **SELECT** **STANDARD**

SAMPLES AND RATINGS

On this section of the ballot enter the three digit code for each of your steak samples in the boxes as the code is displayed on the label. Next, rate each sample based on tenderness, juiciness and flavor. After you have rated each sample please enter the code of the sample you prefer overall.

❾ REVIEW THE VISUAL APPRAISAL OF THE UN-COOKED STEAKS & PLEASE RATE EACH SAMPLE

Like extremely, like very much, like slightly, like, Neutral, Dislike, dislike slightly, dislike very much, dislike extremely

STEAK A ◯◯◯◯◯◯◯◯◯

STEAK B ◯◯◯◯◯◯◯◯◯

PORK-FAT INJECTED SAMPLE

VISUAL APPRAISAL

A.

CONTROL SAMPLE

VISUAL APPRAISAL

B.

❿PLEASE ENTER THE CODE FOR STEAK #1 HERE._______

⓫RATE THE FIRST STEAK BASED UPON TENDERNESS, JUICINESS, TEXTURE, AND FLAVOR.

Like extremely, like very much, like slightly, like, Neutral, dislike, dislike slightly, dislike very much, dislike extremely

Tenderness ◯◯◯◯◯◯◯◯◯

Juiciness ◯◯◯◯◯◯◯◯◯

Flavor ◯◯◯◯◯◯◯◯◯

Texture ◯◯◯◯◯◯◯◯◯

⓬PLEASE ENTER THE CODE FOR STEAK #2 HERE._______

⓭RATE THE SECOND SAMPLE BASED UPON TENDERNESS, JUICINESS, TEXTURE AND FLAVOR.

Like extremely like very much, like slightly, like, Neutral, dislike, dislike slightly, dislike very much, dislike extremely

Tenderness ◯◯◯◯◯◯◯◯◯

Juiciness ◯◯◯◯◯◯◯◯◯

Flavor ◯◯◯◯◯◯◯◯◯

Texture ◯◯◯◯◯◯◯◯◯

⓮PLEASE ENTER THE CODE FOR THE STEAK YOU PREFER OVERALL BASED ON YOUR RATINGS ABOVE.______________________

⓯COMMENTS:

Flavor-

Tenderness-

Juiciness-

Texture-