USING HIGH-PRESSURE PROCESSING AS A POSSIBLE TENDERIZATION PROCESS FOR FOODSERVICE TOP SIRLOIN STEAKS

A Dissertation

by

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ABSTRACT

This multi-phase study investigated the use of high pressure processing (HPP) to determine if it resulted in comparable tenderness improvement to that of blade tenderization for beef top sirloin steaks destined for foodservice at varying degrees of doneness, and to determine whether the quality factors such as color, lipid oxidation, shelf-life, and flavor are adversely affected by the use of high pressure processing.

Forty-five top sirloin butts were aged for 35 days, fabricated into three logs each (n=135) (IMPS #184B), and assigned to a treatment group of control, blade tenderization, or high pressure processing (HPP). High pressure processed steaks had higher shear force values when compared to control and blade tenderized steaks. Also, consumer sensory evaluation revealed lower scores for overall like, tenderness like, and tenderness level when compared to the other two treatment groups. For both Warner-Bratzler shear force and consumer sensory evaluations, there were no differences found between the control group and the blade tenderized group. In addition, instrumental cooked color of the cut surface of top sirloin steaks showed higher L^* values and lower a^* values ($P < 0.05$) for high pressure treated steaks when compared to the control and blade tenderized groups. There were no differences ($P > 0.05$) in b^* values between the blade tenderized and high pressure processed groups, but the control group exhibited higher b^* values ($P < 0.05$). Results showed that high pressure processing negatively influenced both tenderness and quality factors. In addition, these results demonstrated that blade tenderization may not be necessary to achieve desirable tenderness in top sirloins that are aged 35 days or more. Additional findings include variation of degrees

of tenderness based on the degree of doneness, and the application of the treatments on the products that have been ‘treated then aged’ versus ‘aged then treated.’

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1. INTRODUCTION

Meat and food companies must consider purchasing and consumption habits of consumers, as well as the perception and trends of consumer demands to keep or reinforce their position in the industry (Patel, Williams-Campbell, Liu, & Solomon, 2005). The demand for high quality and consistent products with natural flavor, taste, and fresh appearance of minimally processed foods is greatly desired by the consumer. Tenderness, flavor, and color are the most important factors affecting beef palatability and marketability (Belew, Brooks, McKenna, & Savell, 2003; Lorenzen et al., 1999). Inconsistent beef tenderness is a major problem in the meat industry. Assurance of acceptable tenderness is especially important to retail and foodservice segments of the industry because of the importance of repeat purchases by their clientele.

The ability of postharvest techniques to enhance tenderness is continually studied to create a more consistent product. Blade tenderization is a postharvest technique that has been shown to effectively tenderize meat by using blades or needle probes to disrupt the myofibrillar apparatus and connective tissue, which leads to lower shear force values and easier mastication (Bowker et al., 2007).

In order to harmonize or blend all of these demands without compromising the safety of the product, it is necessary to implement new preservation technologies in the food industry (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). High pressure processing utilizes water pressure to tenderize meat products by transmitting pressure rapidly and uniformly throughout the product, which causes structural changes to the product. Myofibrillar protein solubility of post-rigor muscle increases when

subject to high pressure processing (Souza et al., 2011). The technology follows Le Chatelier principle, meaning a decrease in volume can be enhanced by pressure, and vice versa. High pressure induces changes in muscle enzyme, meat proteolysis, and myofibrillar proteins, changes the structure and texture of meat change. Further, high pressure influences the tenderization and color of the product (Ma & Ledward, 2004).

Although previous research has shown that blade tenderization and high pressure processing technologies effectively tenderize meat, there is a lack of data characterizing the effects these technologies have on overall quality of foodservice top sirloin butts. Blade or needle tenderization has been used by the foodservice industry for decades as a way to ensure that the tenderness of cuts are improved or made more consistent. Although many beef subprimals are blade tenderized, the top sirloin butts that are most often treated with this technology. The process of blade or needle tenderization has come under attack by some as a possible food safety risk because pathogens can be translocated, at least experimentally, into the interior portion of these subprimals. Finding alternatives to blade or needle tenderization for cuts such as the top sirloin butt would provide options to purveyors who may wish to employ non-penetrating methods to increase tenderness. Furthermore, the implementation of a technology that can improve tenderness without penetrating the exterior surface of the product could be of interest to the meat industry due to the recent FSIS regulations relating to the labeling of mechanically tenderized beef products.

The objectives of Phase 1 of this research were to investigate whether the use of high pressure processing (HPP) will result in comparable tenderness improvement to that

of blade tenderization for top sirloin steaks destined for foodservice. The objectives of Phase 2 of this research were to investigate whether the use of HPP will result in comparable tenderness improvement to that of blade tenderization for top sirloin steaks destined for foodservice when cooked to various endpoint temperatures. The objectives of Phase 3 of this research were to investigate whether the use of HPP will result in comparable tenderness improvement to that of blade tenderization for top sirloin steaks destined for foodservice and to determine whether the quality factors such as color, lipid oxidation, shelf-life, and flavor are adversely affected by the use of high pressure processing when 'treated then aged versus 'aged then treated.'

2. REVIEW OF LITERATURE

Consumer perception and economic conditions have encouraged the meat industry to provide a consistent, tender, highly palatable, and inexpensive product. Factors such as meat color, flavor, aroma, tenderness, and method of cookery play a collective role in meat "taste" (Morgan et al., 1991). The single most important trait affecting consumer satisfaction is tenderness. According to the 2010/2011 National Beef Tenderness Survey, consumers are willing to pay a premium for guaranteed-tender meat products (Guelker, 2013). Meat tenderness is a function of production, processing, value adding and cooking method used to prepare the meat for consumption by the consumer (Thompson, 2002). The texture of meat is of utmost importance to consumer acceptance and therefore much research effort has been put into this issue in order to be able to control and understand it. Industry data from the National Beef Tenderness Survey revealed that in the 1990's there was a 20% increase in tenderness (Guelker, 2013). In addition, from the late 1990's to mid 2000's there was an 18% overall increase in tenderness. Surveys suggested the tenderness increase is in part due to extended aging periods (Guelker, 2013).

In many countries, up to one-third of all meat consumed is prepared by the foodservice industry (Aberle, Forrest, Gerrard, & Mills, 2001). The foodservice industry represents a sizable portion of the total food sales, capturing more than 40% of every consumer dollar spent on food (Riehle, 2015). In 2015, restaurant sales are projected to hit a record of $709.2 billion dollars and continues to grow during a post-recession period (Riehle, 2015). Beef top sirloin steaks are among the most common, cost-

effective steaks served in restaurants around the United States, as well as being in the top 10 most popular steaks purchased at retail by American households (Savell et al., 2005). The top sirloin steak is a low-priced, lean steak desired by consumers (Brooks et al., 2000; Savell et al., 2005). Data from the 2010/2011 National Beef Tenderness Survey showed that consumers rated the top sirloin steaks the lowest for overall like, tenderness like and tenderness level compared to other commonly served beef steaks. Therefore, continued research to improve tenderness consistency is needed.

2.1. Postmortem Tenderization Methods

Aging of fresh beef for foodservice has become essential to meet the high expectations of an exceptional eating experience. Foodservice operators have greater success when marketing premium products to customers who are willing to spend more on the perception of a greater eating experience at a restaurant than at home. Most restaurants offer top sirloin steaks as a lower priced entrée compared to steaks such as the ribeye, filet mignon, New York strip, T-bone, or roasts such as the prime rib. However, the top sirloin continues to pose problems with consistency of tenderness. In a study conducted by Harris, Miller, Savell, Cross, and Ringer (1992), top sirloin steaks showed no significant increase in overall tenderness until 28 days of wet-aged storage. In addition, sensory panelist tended to be more variable with respect to overall tenderness ratings. Connective tissue tended to remain relatively stable and intact during aging. Harris et al. (1992) found highly variable connective tissue ratings on top sirloin steaks. In response to the aging periods, top sirloin steak shear values were higher and did not respond to the aging periods. Harris et al. (1992) concluded that if consistency in

palatability of beef top sirloin steaks is to be optimized, such characteristics must be manipulated, chemically or mechanically, to overcome such inherent tenderness problems. The industry has found aging to be one method that helps limit the amount of inconsistency of tenderness in today's consumer driven beef market. The structure of contractile proteins has a significant effect on the level of tenderness of the muscle. As a muscle enters rigor there is a loss of extensibility and along with that, a change in the texture of the meat. During storage, the product becomes more tender because of proteolytic changes occurring in the structure of the myofibril and the associated proteins. During postmortem aging several key proteins are being modified.

2.1.1. Aging

Aging is shown to be a commonly utilized method for increasing the tenderness of meat products. Aging is the process of holding meat at refrigerated temperatures to allow endogenous proteolytic enzymes in muscle to tenderize the meat. The aging process involves storing carcasses, primals, subprimals, or steaks for sufficient time to maximize palatability characteristics such as tenderness, juiciness, and flavor. The increase in tenderness associated with postmortem aging of meat has been attributed to endogenous enzymes in muscle, a loss of tensile strength of the myofibrillar component of the muscle cell, and shortening of muscle fibers during slow versus rapid phases of rigor mortis (Smith, Culp, & Carpenter, 1978). Protein proteolysis of structural proteins has been determined to be one of the main causes for the increase in tenderness postmortem (Koohmaraie & Geesink, 2006). Products can be aged by two methods: wet aging or dry aging. Wet aging, the most common form, refers to postmortem aging of

meat products in a vacuum package. Due to changes in beef distribution, from the shipment of carcasses as quarters to the shipment of primals or subprimals cuts from areas of production to consumption, aging has become part of the beef industry (Seideman & Durland, 1983). In United States packing plants, beef is routinely vacuum-packaged and distributed. Vacuum packaging provides a method for prolonged shelf-life and palatability of beef during extended periods of shipment and storage. Dry aging is a process whereby beef carcasses, primals, and/or subprimals are stored, without protective packaging, at refrigeration temperatures for one to five weeks to allow the natural enzymatic and biochemical processes that result in improved tenderness and the development of the unique flavor (Savell, 2008). The unpackaged cuts are stored in a uniquely designed area with controlled temperature, relative humidity, and air velocity and allowed to age for a specific period of time. During the dry aging period, the outside surfaces dry out and become moldy due to exposure to air and high humidity and must be trimmed away at the conclusion of the aging period. Dry aging is used less often due to large overhead cost, maintenance of facility, amount of product needed to be stored, and the amount of loss due to moisture loss or trimming.

Another form of aging is high temperature conditioning. High temperature conditioning refers to elevated temperatures during the aging process (Koohmaraie, Seideman, & Crouse, 1988; Whipple, Koohmaraie, Dikeman, & Crouse, 1990). Food safety concerns led to the discontinuation of high temperature conditioning.

During either type of aging, one of the first observable changes in ultrastructure of postmortem muscle occurs in myofibrils where degradation of Z disks begin (Aberle

et al., 2001; Davey & Gilbert, 1969). The longer the storage time the more extensible the muscle will become. Complete loss of the Z disks occurs due to proteolytic degradation of proteins associated with the disk, notably desmin and titin (Aberle et al., 2001; Koohmaraie, 1992, 1994, 1996). Desmin and titin are likely the key substrates that determine meat tenderness. Titin is a mega-protein approximately 3 mega-Daltons in size. In addition to being the largest protein found in mammalian tissues, it is also the third most abundant. In striated muscle, titin spans half the length of the sarcomere with the C-terminal end localizing in the M-line and the N-terminal forming an integral part of the Z-line. Titin degradation during postmortem aging is caused by the weakening of the longitudinal structure of the myofibrillar sarcomere and integrity of the muscle. The weakening of titin can lead to enhanced tenderness. Myosin and actin are two proteins that do not undergo degradation during storage. Myosin is the primary protein in the myofibril, and therefore the contribution of myosin to the structure and tensile strength of meat must not be ignored. Actin is the second most abundant protein in the myofibril and is the primary protein in the thin filament. Traditionally, actin has not been considered to undergo major changes during the postmortem aging period. However, it is suggested that minor degradation of actin occurs in the postmortem muscle. As the resolution of rigor occurs, fragmentation of the myofibrils occurs. Because of the weakening of the myofibers, aged meat yields a higher proportion of smaller fragments upon homogenization than unaged meat. The myofibril fragmentation index, which is based on the fragmentation concept, has been used as an index for meat tenderness, as well as for postmortem tenderization. The myofibril has been shown to be a predictor of

meat tenderness in numerous studies.

The following is a summary provided by Aberle et al. (2001) of changes that occur in skeletal muscle during postmortem aging:

1) Z disk degradation, which leads to weakening and fragmentation of myofibrils.
2) Degradation of desmin, which causes disruption of transverse cross-linking between myofibrils and leads to fragmentation of myofibrils.
3) Degradation and disappearance of troponin-T.
4) Degradation of titin and nebulin. Because of their ability to maintain longitudinal stability of myofibrils, disruption of these structures would lead to fragmentation of myofibrils.
5) Degradation of these myofibrillar proteins results in appearance of new polypeptides seen by gel electrophoresis.
6) The most significant observation is that the major contractile proteins, myosin and actin, are not affected even after 56 days of postmortem aging (Figure 1.)

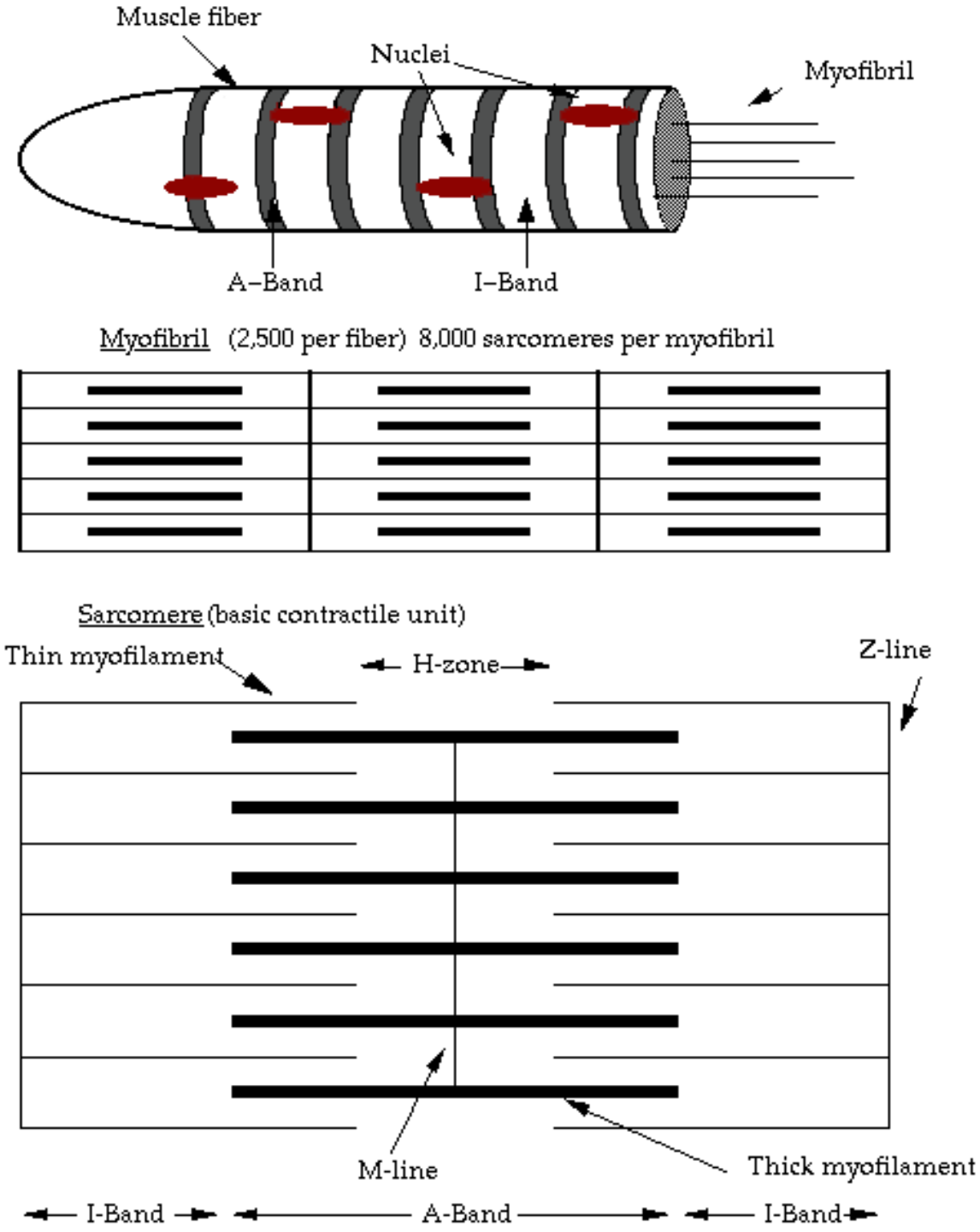
Improvement in tenderness during storage is due almost entirely to proteolytic degradation of myofibrillar proteins. Meat tenderness could be dramatically improved if collagen and intermolecular cross-linkages were degraded (Aberle et al., 2001).

Several hypotheses have been researched to determine the causes of the breakdown of myofibrillar proteins during postmortem aging. Researchers have thoroughly investigated the roles of lysosomal enzymes (cathepsins), calcium-dependent proteases (calpains) and caspases in postmortem tenderization of meat. Goll et al. (1983) and Koohmaraie, Babiker, Merkel, and Dutson (1988) investigated ways to quantify and determine differences in postmortem proteolysis of proteins. Goll et al. (1983); Koohmaraie, Babiker, et al. (1988) studies indicated that the proteinases had to be present in the muscle tissue, and have access to the substrate needed to activate the proteinase to cause degradation in aged meat. Stored in the lysosome, cathepsins are

acidic enzymes purposed to aid in postmortem tenderization (Calkins & Seideman, 1988). However, according to Koohmaraie (1992), cathepsins have not been shown to be released from the lysosome postmortem, therefore not affecting postmortem proteolysis. Cathepsins primary targets are actin and myosin, as suggested by Aberle et al. (2001) however, no degradation was reported in actin or myosin after 56 days of aging. These results suggest that cathepsins are not involved in postmortem tenderization.

Calcium-dependent proteases known as calpains were found to be the primary cause for an increase in postmortem tenderization caused by structural protein degradation (Olson, Parrish, Dayton, & Goll, 1977). Calpains cause the breakdown in Z-disk structural proteins. According to the amount of calcium required for activation, calpains are categorized into m-calpain and μ-calpain. In order to control calpain degradation, calpastatin an endogenous inhibitor is released.

Figure 1. Sarcomere. (Savell, 1995a).



Postmortem tenderization can be affected by type of aging, postmortem proteolysis, and several unknown factors.

2.1.2. Blade Tenderization

Connective tissue is one of the components that can have a significant impact on the tenderness of meat. There are two types of connective tissue that we generally refer to: supportive connective tissue (cartilage and bone) and connective tissue proper (ground substance and fibrous connective tissue that is associated with muscles). Of the two types, connective tissue proper is the primary concern as it pertains to meat tenderness. The ground substance portion of connective tissue proper has minimal impact on meat tenderness, as it is mostly structureless and made up of soluble glycoproteins. The fibrous connective tissue is composed of different combinations of collagen, elastin, and reticulin. The most abundant of these three is collagen, and it is made up of amino acids like glycine, hydroxyproline, and proline. Type I and Type III collagen fibers compose the primary portion of the three connective tissue layers in muscle. The three main layers of connective tissue associated with muscles are epimysium, perimysium, and endomysium. Endomysium is the layer that surrounds the individual muscle fibers. Perimysium is the connective tissue layer that surrounds the muscle bundle. Epimysium is the layer that surrounds groups of muscle bundles and provides support for the structure of the muscle. The epimysium is not as large of a concern pertaining to tenderness because it is often trimmed during foodservice preparation, while the perimysium and endomysium (intramuscular connective tissues) play a larger role in cooked meat tenderness due to the inability to remove them

manually.

The effects of intramuscular connective tissue on tenderness have been extensively examined (Light, Champion, Voyle, & Bailey, 1985; Nishimura, Liu, Hattori, & Takahashi, 1998; Ramsbottom, Strandine, & Koonz, 1945). Many different factors such as muscle, animal age, breed, feeding regime, and aging can greatly affect the contribution of connective tissue to the toughness of meat. Muscles that are responsible for repetitive motions, such as locomotion, tend to have a higher amount of intramuscular connective tissue. Also, as the age of the animal increases, the amount of insoluble collagen can increase, which can result in a decrease in muscle tenderness. The combination of these factors can cause an increase in the variation in meat tenderness. To help provide an eating experience for a consumer that is consistently desirable, measures must be taken to help reduce that variation.

Consumers can identify differences in beef tenderness and are willing to pay more for more tender beef (M. F. Miller, Carr, Ramsey, Crockett, & Hoover, 2001). The industry challenge is to decrease variation and improve tenderness through multiple ante- and postmortem technologies. Of beef steaks regularly offered on restaurant menus, top sirloin steaks are the toughest and most variable in tenderness, but are typically lower in price than most other menu offerings. One of the major types of mechanical tenderization utilized in the beef industry is needle/blade tenderization. Blade tenderization is a commonly used technology shown to improve tenderness through physical disruption of muscle fibers and connective tissue. Blade tenderization utilizes a set of blades, which pierce the meat, cutting through muscle fibers and

connective tissue and improving tenderness. Blade tenderization is utilized for the improvement of tenderness of relatively tough muscles to make them more consistent and comparable to muscles of more favorable tenderness and consistency (Seideman, Smith, Carpenter, & Marshall, 1977; Tatum, Smith, & Carpenter, 1978). This process can be performed on wholesale cuts, or individual steaks and roasts. Blade tenderization can be effectively utilized to reduce variability and inconsistency in tenderness of beef cuts as well as improve the overall palatability. Mechanical tenderization will improve tenderness, but inherently tough cuts cannot be made as desirable as tender cuts (Tatum et al., 1978). Essentially all beef cuts can be blade tenderized, but tougher cuts will have the greatest improvement in the level of tenderness. The tenderization process is used on raw products, generally after rigor. When beef is mechanically tenderized, desirability ratings for flavor and tenderness of most cuts are greatly improved.

In a survey of North American Meat Processors, 61.8 % of processors used blade tenderization on top sirloins. The specific number of passes through the tenderizer and the speed of the conveyor does not significantly affect the overall tenderness. One pass at medium to high conveyor speeds is adequate to improve tenderness of most cuts. Of the processors that used blade tenderization, an average of 1.6 passes were used to achieve desired tenderness levels (George-Evins, Unruh, Waylan, & Marsden, 2004). Other forms of mechanical tenderization have been investigated experimentally, but have not been implemented due to cost or lack of effectiveness (Maddock, 2008). Processors will continue to search for ways to improve consistency in tenderness by disrupting connective tissue and muscle structure while maintaining the safety of the

product.

2.1.3. High Pressure Processing

The modern consumer requires foods that are safe and nutritious, free from additives, taste good, and for certain products, have a longer shelf-life. High pressure processing is one method that allows the industry to meet the consumer requirements. High pressure processing on food systems was first reported by Hite, in 1899 (Simonin, Duranton, & de Lamballerie, 2012). Due to technological difficulties and cost, high pressure processing was not readily utilized in the food industry. High pressure processing is gradually being adopted by the food industry for processing and preservation of meat and meat products (Sun & Holley, 2010). The effect of high pressure processing is dependent upon protein susceptibility, applied pressure and temperature, and the duration of the pressure treatment (Sun & Holley, 2010). High pressure processing is most frequently carried out in a liquid pressure-transmitting medium such as water, the sample being protected for direct contact by using sealed flexible packaging.

High pressure processing is the technology by which a product is treated at or above 100 MPa. Megapascal (MPa) is the unit utilized to measure the amount of pressure being applied to a commodity. The pressure is transmitted uniformly and instantaneously throughout the food, which allows very homogenous products to be obtained. Pressure affects the conformation of macromolecules, the transition temperature of water and lipids, and some chemical reactions. Biochemical systems exposed to pressure follows the principle of Le Chatelier, which indicates that any

phenomena accompanied by a decrease in volume are enhanced by an increase in pressure, and vice versa. The other scientific principle used in food applications of high pressure is the isostatic transmission, which is the uniform transmission of pressure throughout the food. Thus, the product does not become deformed almost instantaneously. This uniform and instantaneous process, independent of product size and shape, allows very homogenous foods to be obtained.

In the processing of a food system, temperature and pressure may work synergistically to bring about a change in product confirmation. This is because temperature exerts its effects through the enthalpy and entropy changes involved in a given chemical reaction, while any effect of pressure is related to the volume changes involved (Ma & Ledward, 2013). If a given reaction involves a decrease in volume then it will be favored by an increase in pressure while one involving a volume increase will be inhibited. Pressure treatment causes the driving forces for the unfolding or denaturation of the breaking of ionic linkages and some hydrophobic interactions. Therefore, moderate pressures may stabilize a protein against heat denaturation and conversely a moderate temperature increase may stabilize a protein against pressure denaturation.

The application of pressure on proteins leads to different degrees of protein structure modification. The pressure induces an unfolding of the protein structure and subsequent folding after pressure release. Complete denaturation of proteins can occur under high pressure. Covalent bonds have a low compressibility and are much less sensitive to changes in pressure (Cheftel & Culioli, 1997). High pressure processing

induces the breakdown of salt bonds and also parts of hydrophobic interactions (Cheftel & Culioli, 1997). Hydrophobic interactions are very sensitive to pressure and primarily make up the quaternary structure. Major changes in the tertiary structure are observed beyond 200 MPa and changes in the secondary structure require very high pressure above 700 MPa (Rastogi et al., 2007). Muscle proteins including myofibrillar proteins are unfolded up to a pressure of 300 MPa. Pressures beyond 300 MPa result in increased denaturation, gel formation and agglomeration of proteins (Bajovic, Bolumar, & Heinz, 2012). High pressure processing has a remarkable effect on the actin-myosin complex. In addition, z-line in myofibrils is not apparent in pressurized muscle. Unfortunately, Beilken, Macfarlane, and Jones (1990) found limited effect on visible connective tissue at ambient and high temperatures.

High pressure processing systems consist of a pressure vessel and a pressure-generating device. The vessel is loaded with the food commodity and closed. From the bottom of the unit the pressure medium, usually water, is pumped into the vessel. Once the desired pressure is reached, the pumping is stopped, valves are closed, and pressure can be maintained. High pressure processing is typically conducted in a batch process and pressure vessels used for commercial food production have capacities of 35-350 L (Patterson, 2005).

High-pressure processing has been used by the food industry primarily as a method to improve product shelf-life and food safety (Simonin et al., 2012). The application of high pressure processing to meat and meat products results in the modification of quality parameters such as color, texture, and water holding capacity

(Bajovic et al., 2012). High pressure processing affects quality parameters of fresh meat, particularly depending on the pressure level applied, and thus typical characteristic associated with fresh meat like texture and especially color can be remarkably modified. The meat becomes more gel-structures and paler losing the typical appearance of fresh meat. Therefore, most of the previous research on using pressure to tenderize beef has been conducted on pre-rigor meat rather than post-rigor meat so there are limited studies available to determine the impact of this process on the possible tenderization of top sirloin steaks (Bajovic et al., 2012; Ma & Ledward, 2004; Suzuki, Watanabe, Iwamura, Ikeuchi, & Saito, 1990). In addition, the use of high-pressure processing may result in some color and flavor problems in fresh meats. Therefore, if this process is used for increasing tenderness, it cannot negatively impact other important quality factors, ultimately affecting consumer appeal.

High pressure processing induces color modification on meat color criteria such as lightness (L^*), redness (a^*), and yellowness (b^*). Thus in some conditions, the lightness of meat could be heightened by high-pressure treatment and the redness increased or decreased. The increase in L* values begins from 200 MPa and becomes stabilized for pressures around 300-400 MPa. The lighter appearance of meat could be due to globin denaturation and heme displacement or release, an increase in drip losses leading to changes of water content of meat.

This suggested that high pressure treatment could induce the same kind of myoglobin modification than cooking, such as the denaturation of metmyoglobin and displacement towards the ferric state of the heme iron. Meat discoloration could be a

problem for marketing pressurized raw meat, as meat color is one of the most important criterions for consumers.

2.2. Meat Quality Evaluation Methods

Consumer satisfaction is important, because it is generally assumed to be a significant determinant of repeat sales, positive word-of-mouth, and consumer loyalty. Satisfaction is important to the individual consumer because it reflects a positive outcome from the outlay of scarce resources and or the fulfillment of unmet needs (Resurreccion, 2003).

Meat products are similar to all other food products in that they are developed, produced, and marketed to appeal to the consumer. Ultimately, the success of a food product depends on its acceptance to the consumer, who is the user or potential user of the product and thus the one who purchases the product (Resurreccion, 2003). Research and developers of meat products have to be involved in consumer studies to collect and understand consumer response to the food products and variables or factors that are being studied in order to ensure meat products will have high consumer acceptance (Resurreccion, 2003). Consumer affective tests are necessary for better understanding of the consumer, especially tests that ask for preference and acceptance (Resurreccion, 2003). Central location consumer panels are commonly used in the industry. Central location consumer panels are usually conducted where large numbers of consumers can be intercepted to evaluate samples (Resurreccion, 2003).

Objective evaluations allow for the comparison of different treatments as well as ascertaining their effect on a particular characteristic, but do not provide information

concerning product acceptability or preference for one kind of meat over another (Destefanis, Brugiapaglia, Barge, & Dal Molin, 2008). Therefore, consumer opinion is a key factor to establish meat value and justify purchase decision.

Palatability is defined as the interaction between several factors including tenderness, juiciness, and flavor. Boleman et al. (1997) and Savell and Shackelford (1992) found that tenderness was the primary economic factor for beef palatability.

2.2.1. Tenderness & Warner-Bratzler Shear Force

The most important qualitative characteristic of meat is tenderness (Destefanis et al., 2008). Unfortunately, tenderness is also a highly variable characteristic. Therefore, tenderness inconsistency is a priority issue for the meat industry. Muscle tenderness can be affected in a small way by many different factors. The contractile state of the sarcomere, the smallest contractile unit of the muscle, is known to have a considerable effect on the tenderness of meat (Locker, 1960). The effects of aging on beef tenderness have also been well documented (Calkins & Seideman, 1988; Goll et al., 1983; Koohmaraie, 1992; Koohmaraie, Babiker, et al., 1988). Another highly researched tenderness component is intramuscular fat. Conflicting reports have been generated about the impact of quality grade on overall beef tenderness (Berry, Smith, & Carpenter, 1974; Carpenter, Smith, & Butler, 1972; Cover, Hostetler, & Ritchey, 1962; Parrish, Olson, Miner, Young, & Snell, 1973; Tatum et al., 1980). Research about tenderness will likely continue in the future, as it remains a critical factor in creating repeat customers at both the retail and foodservice level.

Due to the expense and availability of consumer panels, the Warner-Bratzler

shear force machine is utilized to predict tenderness ratings obtained by a taste panel to replicate shearing, penetrating, biting, mincing, compressing, and stretching the meat. The origins of Warner-Bratzler shear force were recounted at some of the first Reciprocal Meat Conferences. In the late 1920's, K. F. Warner and his associates had the idea of shearing a sample of cooked meat as an indication of its tenderness. Years later, L. J. Bratzler refined the shearing methods to include the blade shape, thickness, dullness of cutting edge, shearing speed, etc. (Bratzler, 1932). Today, Warner-Bratzler shear force is the most widely used method to determine tenderness.

Pressure induces texture modifications by affecting the myofibrillar protein structure and their gel forming properties. In general, low pressures (<200 MPa) can tenderize pre-rigor meat, whereas tenderization post-rigor with high pressure processing can only be achieved by higher temperatures (Sun & Holley, 2010). The influence of high pressure processing on the meat tenderness is dependent on the rigor stage, pressure and temperature level applied, and their combination (Sun & Holley, 2010).

2.2.2. Color

When selecting beef, bright cherry-red color is one of the most important quality attributes in a consumer's mind when they purchasing from the retail case (Lynch, Kastner, & Kropf, 1986). Consumers routinely use product color and appearance to select or reject products, and suppliers of muscle food products must also create and maintain the desired color attributes (Hunt & King, 2012). Perception of muscle color, either raw or cooked, influences the human perception of product acceptability (R. K.

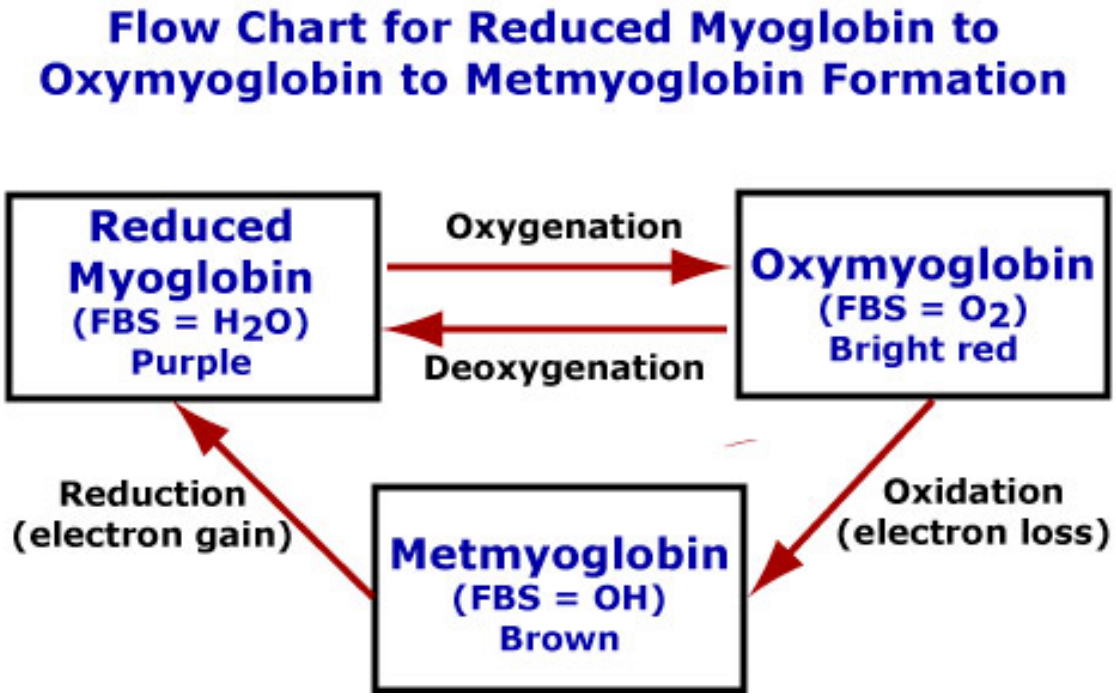
Miller, 1994). Unfortunately, several factors can attribute to a desirable or undesirable color.

Meat color is a complicated system and the prominent contributor to meat color is myoglobin. In the living cell, it serves as both an oxygen storage and an oxygen delivery function (Faustman & Cassens, 1990). Product's color is determined by the interaction of myoglobin pigment chemistry with the physics of light absorbance and reflectance (Hunt & King, 2012). The color of meat depends on the optical properties of the meat surface as well as on the myoglobin content of the muscle (Bajovic et al., 2012). Myoglobin is a water-soluble protein responsible for meat color. An iron atom has six bonds. The ligand present at the sixth bond and the valence state of iron determines meat color (Hunt & King, 2012). Deoxymyoglobin, metmyoglobin, and oxymyoglobin are the primary chemical states of meat color.

As myoglobin content increases, the muscle food increases in color intensity from white or pink to very dark red; therefore myoglobin content is directly related to final muscle color. Higher myoglobin content in beef muscle is the major factor that differentiates the bright cherry red color of beef when compared to the lighter color of pork or poultry meat. Myoglobin is the major pigment in meat, accounting for 50-80% of the total pigment. Meat color, although strongly influenced by myoglobin concentration, is also affected by handling and storage prior to presentation to or consumption by the consumer. Additionally, the length of time at which meat is held in storage and temperature during storage ultimately influences meat color. After meat is exposed to air, beef slowly turns to a bright cherry-red color, and this process is typically

referred to as blooming (Lee, Apple, Yancey, Sawyer, & Johnson, 2008). Blooming is the result of oxygen binding to the iron atom and, in this state; the myoglobin molecule is called oxymyoglobin. Blooming is defined as the amount of time it takes to oxygenate the cut surface transitioning from deoxymyoglobin to oxymyoglobin (Lee et al., 2008). The lack of oxygen in vacuum packaged meat converts beef exposed to the atmosphere from a bright, cherry-red color to a purple-red color in the vacuum package, the conversion of oxymyoglobin to deoxymyoglobin. Deoxymyoglobin is a dark purplish-red color typical of the interior color of fresh meat (Hunt & King, 2012). Deoxymyoglobin is the result of ferrous iron with a vacant sixth binding site (Hunt & King, 2012). Maintaining a dark purplish-red color requires low oxygen exposure. The formation of oxymyoglobin is the process of oxygenating deoxymyoglobin meat. The attachment of oxygen at the sixth ligand will form a bright-red color. Metmyoglobin is a tan to brown color form of myoglobin and most often equated with spoilage in fresh meat by consumers. Jeremiah, Carpenter, and Smith (1972) suggested that consumers do not prefer steaks that are extremely dark or extremely pale in muscle color. Exposure to low oxygen concentrations and hydroxide attaching at the sixth position causes metmyoglobin color (Bendall & Taylor, 1972; O'Keeffe & Hood, 1982; Renerre & Labas, 1987). By focusing on the biochemical aspect of muscle and an increase in the understanding of muscle color can help determine how to treat a muscle (Figure 2).

Figure 2. Color Conversion. (Savell, 1995b).



The physical appearance of a retail cut in the display case is the most important factor determining consumer selection or purchase of meat products. In a consumer's mind the most important quality attribute during purchasing is the bright, cherry-red color (Lee et al., 2008). Therefore, visual determinations are the gold standard for assessing treatment effects and estimating consumer perception (Mancini & Hunt, 2005). Beef top sirloin steaks are among the top 10 most popular steaks purchased at retail by the American households (Savell et al., 2005). However, McKenna et al. (2005) classified the *gluteus medius* as an "intermediate" color stability muscle, indicating great discoloration rates during display. Color can be evaluated by a trained panel using a predetermined scale and can be a viable determinate of meat quality. If not provided

references, panelist descriptions of color may depend on individual cognition (Mancini & Hunt, 2005). Color photographs closely illustrate or anchor the panelists to the descriptors used to define the reference points for color descriptor scales. Panelists should be trained, screened, and selected based on their abilities to consistently evaluate desired color traits. Trained panelists produce more repeatable data with a normal distribution compared to untrained panelists.

Additionally, instrumental color measurements have been used to measure muscle color. Instrumental color will provide an objective assessment to muscle color. Several types of instruments are available to conduct instrumental color analysis. Each instrument offers a variety of options that allow researchers to choose from several (1) color systems (Hunter, CIE, and tristimulus); (2) Illuminants (A, C, D65, and Ultralume); (3) observers (2° and 10°); and (4) aperture sizes (0.64-3.2 cm) (Mancini & Hunt, 2005). Determining which light source and aperture is project specific and dependent on the objectives of the research.

Cooked meat color is determined by myoglobin's response to heat (King & Whyte, 2006). Heating causes the denaturation of the globin, which then precipitates with other meat proteins. Denaturation of myoglobin and other proteins begins between 55 °C and 65 °C in meat, and most denaturation has occurred by 75 °C or 80 °C (King & Whyte, 2006). As meat temperature increases there is likely to be an increase in pH, therefore slowing down the rate of myoglobin denaturation. Oxymyoglobin, deoxymyoglobin, and metmyoglobin differ in their sensitivity to heat. Deoxymyoglobin

is the least sensitive to heat; oxymyoglobin and metmyoglobin have very similar heat sensitivities.

Adequate cooking of meat produces a color change to off-white, grey, or brown depending on the protein source (King & Whyte, 2006). The final cooked color depends on the extent of the ferrihemochrome formation and the final concentration of undenatured oxymyoglobin and deoxymyoglobin (Varnam & Sutherland, 1995).

The color intensity of meat is determined by antemortem factors that include species, stress, sex and age of the animal, postmortem pH rate of decline and ultimate pH of the meat (Seideman, Cross, Smith, & Durland, 1984).

The modification induced by high pressure treatment on meat color is related to the color criteria such as lightness (L^*), redness (a^*) or yellowness (b^*) (Jung, Ghoul, & de Lamballerie-Anton, 2003). High pressure processing could heighten the lightness of meat and the redness increased or decreased (Jung et al., 2003). The increase in the L^* value begins from 200 MPa and becomes stabilized for pressures around 300-400 MPa (Carlez, Veciana-Nogues, & Cheftel, 1995).

2.2.3. Flavor

Meat flavor is the result of compounds stimulating the olfactory and taste receptors in the oral and nasal cavity of humans. These chemical compounds can vary in concentrations due to the influence of heat on the chemical structure, the degree of oxidation, the initial level of compounds, and the interactions between the compounds. The muscle system can be divided into the lean portion and the lipid portion. Meat flavor is composed of (1.) meatlike flavor derived from water-soluble reducing sugars

and amino acids, (2.) species-specific flavors which are due to differences in fatty acid composition and aromatic water-soluble compounds stored in lipid depots of the animal; and (3.) off-flavor development as the result of oxidation of lipid double bonds, defined as lipid oxidation or autooxidation, and other degradation processes.

2.2.4. Juiciness

Juiciness conveys the overall impression of palatability to consumers (Aberle et al., 2001). Juiciness contains many important flavor components and assists in fragmenting and softening meat during chewing. Regardless of other meat attributes, the absence of juiciness severely limits its acceptability and destroys its unique palatability characteristics (Aberle et al., 2001). The sources of juiciness in meat are intramuscular lipids and water. The greater the amount of intramuscular lipids the less the product will shrink during cooking, therefore the more juicy. The major contributor to juiciness is water remaining in a cooked product. Because fat-free water content of meat is relatively uniform, differences in juiciness relate primarily to the ability of muscles to retain water during cooking (Aberle et al., 2001). Cook loss is a common way to predict the potential juiciness of a meat product.

2.2.5. Thiobarbituric Acid Reactive Substances (TBARS)

The problem of oxidative deterioration is of greatest economic importance in the production of lipid containing foods (Frankel, 1980). Oxidation of unsaturated lipids not only produces offensive odors and flavors but can also decrease the nutritional quality and safety by the formation of secondary reaction products in foods after cooking and processing (Frankel, 1980). There have been many instances consumers report an off-

odor or a rancid or warmed-over flavor in fresh meats. The term warmed over explains the rapid development of an oxidized flavor in refrigerated cooked meats. Raw meats or fatty tissues that have been stored weeks or months prior to preparation can have a rancid taste, derived from the same processes as the warmed over flavor. Warmed over flavor and rancid taste are both attributes associated with lipid oxidation.

Lipid oxidation or oxidative rancidity results from autooxidation, chemical changes that occur upon exposure to atmospheric oxygen. The change in expected flavor can be due to oxidative rancidity. The susceptibility of fatty acids present in meat lipids to undergo lipid oxidation is dependent up on the type of unsaturated fatty acids in addition to their degree of unsaturation. Autooxidation is predominantly associated with the attack of double bonds by oxygen and consequently involves phospholipids which characteristically contain polyunsaturated fatty acids having three or more double bonds (Faustman & Cassens, 1990). Phosphatidyl ethanolamine is the phospholipid of utmost concern in the development of oxidative rancidity. Initiation is responsible for the formation of free radicals, propagation instigates the chain reaction of the free radicals and termination encompasses the formation of nonradical products. When unsaturated lipid molecules react with oxygen in the presence of a catalyst such as heat, light or metallic ions, and free radicals are produced which can evenly yield lipid peroxy radicals. These lipid peroxy can react with unoxidized lipid molecules to form unstable hydroperoxides which, upon decomposition, can form compounds such as hexanal, pentanal, and malonaldehyde.

Because malonadehyde, an end product of lipid oxidation, is correlated with the

development of rancid flavors, this compound is frequently utilized as a measure of oxidative rancidity. Malonaldehyde produces a chromogen with it reacts with 2-thiobarbituric acid. This color reaction is thought to result from the condensation of one molecule of malonaldehyde with two molecules 2-thiobarbituric acid and can be spectrophotometrically measured at 530-570 nm. The spectrophotometric determination of this red pigment has been used to determine rancidity in a wide variety of food products. The 2-thiobarbituric acid test is often used to determine the extent of antioxidation occurring in fat-containing food systems such as meat. Instead of reporting arbitrary absorbance units, which in view of the diversity and empirical nature of the methods employed cannot be compared from laboratory to laboratory, the formulation of the TBA number was developed. The TBA number is defined as mg of malonaldehyde per 1,000 g of sample (Tarladgis, Watts, Younathan, & Dugan, 1960).

Lipid oxidation is a concern in the meat industry as it causes deterioration in the quality of the meat and meat products. The propensity of meat and meat products to undergo oxidation depends on several factors to include pre-slaughter stress and post-slaughter conditions, postmortem pH, carcass temperature, cold shortening, and processing techniques. Understanding the effects of lipid oxidation and the factors that affect this process will help find techniques to decrease the occurrences and the extent of lipid oxidation in products.

According to Yagiz et al. (2009), high pressure processing can increase lipid oxidation and induce color changes in red meat, which make it have a cooked appearance. Although, high pressure processing has preservative effects, pressure

makes meat more susceptible to lipid oxidation. Also, the application of heat increases the oxidative susceptibility of muscle foods. The combination of heat and pressure damage the cell membrane and is thought to be at least partially responsible for the negative quality effects (Ma & Ledward, 2004). The mechanisms by which high pressure processing induces lipid oxidation are not fully understood. High pressure processing triggers lipid oxidation by two mechanisms: increased accessibility for iron from hemoproteins and membrane disruption (Bajovic et al., 2012). The release of iron from hemoproteins can promote lipid oxidation (Bajovic et al., 2012). Due to the extended storage period of top sirloins, TBARS will be conducted on the product to determine levels of lipid oxidation of the final product post aging and treatments.

To measure lipid oxidation in meat products the 2-thiobarbituric acid (TBA) test has extensively been used. The 2-thiobarbituric acid test is a colorimetric technique, which measures the absorbance of the pink compound formed between TBA and TBARS. Two methods for sample preparation commonly used are the distillation technique and aqueous extraction. Both methods have drawbacks; therefore improvements have been made on each of these techniques, including the addition of antioxidants and metal chelators (Rhee, Anderson, & Sams, 1996; Tarladgis et al., 1960). Performing sensory analysis in conjunction with a chemical means such as TBARS will provide the most accurate assessment on warmed over flavor and rancidity.

2.2.6. Shelf-life

Fresh meat is naturally a highly perishable product due to its biological composition. The average composition of meat is approximately 73% water, 21%

protein, 6% lipid, and less than one percent soluble, non-protein substance. Post mortem muscle will also have a certain amount of glucose (the preferred substrate of aerobic spoilage microorganisms) still present in the cells. Normal aerobic packaging conditions can limit the number of days that a meat product can be held due to the growth and biochemical activities of pyschotrophic aerobic microorganisms. High pressure processing is commercially used mainly as a non-thermal decontamination technology for processed and ready-to-eat meat products with high consumer acceptance, in comparison to other non-thermal decontamination technologies such as ionizing radiation (Bajovic et al., 2012). In 1899, B. H. Hite, a researcher at West Virginia University Experiment Station, became the first to successfully demonstrate the use of pressure to as treatment to kill microorganisms. His original work was in milk, but his findings lead to further investigations involving high pressure processing of foods. High pressure processing at low or moderate temperature causes inactivation of certain enzymes and the destruction of microbial vegetative cells without changing, in general, the sensorial attributes of the product. The mode of action of high pressure processing involves destabilization in the functional and structural integrity of the cytoplasmic membrane of the microbial cells. However, the resistance of the microorganisms is variable depending on the strain and the meat matrix to be treated. The efficacy of the treatment also depends on the achieved pressure and on the exposure time. The effectiveness and consumer acceptance alone, is leading to an increased number of commercial installations. The most interesting commercial applications for food industry have been achieved by combining pressures from 400 to 600 MPa with temperatures

from 5 to 90 °C for 10 to 30 minutes. Such applications result in products with low microbial counts, greater safety, and longer shelf-life at refrigeration temperature storage (Yuste, Capellas, Pla, Fung, & Mor-Mur, 2001). Pressure levels applied for the pasteurization of meats and meat products, range in an area of 400 to 600 MPa with short processing times of 3 to 7 min and at room temperature (Bajovic et al., 2012). These treatments lead in most cases to an inactivation of more than four log units for the most common vegetative pathogenic and spoilage microorganisms resulting in an increased shelf-life and improved safety (Bajovic et al., 2012). High pressure processing of meats is generally regarded as an alternative method of extending shelf-life without using preservatives or antimicrobial ingredients.

3. PHASES OF RESEARCH

3.1. Preliminary Research

Before conducting the studies, Choice, Beef Loin (*n = 4*), Top Sirloin Butt, Center-Cut, Boneless, Cap Off (IMPS #184B) (*Gluteus medius*), aged 35 days were obtained from a local purveyor. Top sirloin butts were separated medial to lateral into 3 pieces (logs). Each log was systematically assigned to a treatment. The three treatments included: control, blade tenderized, and high pressure processed. Control logs were portioned into 2.54 cm thick steaks and vacuum packaged. Blade tenderized products were subjected to a Model 700WI blade tenderizer, (Ross Industries, Inc., Midland, VA.), cut into 2.54 cm thick steaks and vacuum packaged. High pressure processed logs were taken to Universal Pasteurization, Coppell, Texas. Each log was assigned a different pressure to determine which pressure achieved satisfactory tenderness and quality levels. The logs were pressurized to 40,000, 50,000, 60,000, and 70,000 psi for 120 sec (Avure Technologies, Quintas Food Press 350L – 600L, Middletown, OH), and portioned into 2.54 cm thick steaks and vacuum packaged. Shear force data from preliminary trials was utilized to determine the appropriate treatment.

3.2. Phase 1

3.2.1. Product Collection

Choice, Beef Loin (*n = 45*), Top Sirloin Butt, Center-Cut, Boneless, Cap Off (IMPS #184B) (*Gluteus medius*) aged 35 days were obtained from a local purveyor for each of the three replications. Top sirloin butts were separated medial to lateral into 3 pieces (logs) to generate 135 logs.

3.2.2 Treatment Design

Each log was assigned systematically to a treatment. The three treatments included: control, blade tenderized, and high pressure processed. Control logs were portioned anterior to posterior into 2.54 cm thick steaks (Portioner, Model X600, Marel USA, Lenexa KS) and vacuum packaged. Blade tenderized product were subjected to a Model 700WI blade tenderizer, (Ross Industries, Inc., Midland, VA.), cut into 2.54 cm thick steaks, and vacuum packaged. High pressure processed logs were taken to Universal Pasteurization, Coppell, TX. The logs were pressurized to 60,000 psi (413.68 MPa) for 120 sec (Avure Technologies, Quintas Food Press 350L – 600L, Middletown, OH). Post treatment, the logs were portioned into 2.54 cm thick steaks and assigned to Warner-Bratzler shear force (WBSF) or consumer panel. All products were transported in insulated containers with dry ice, to the Rosenthal Meat Science and Technology Center (College Station, TX) for further analysis.

At the Rosenthal Meat Science and Technology Center, the products were held at 2 °C. Steaks were transported to the sensory kitchen before cooking on a preheated (177 °C) electric griddle (National Presto Industries, Inc., Eau Claire, WI). Before cooking, steaks were weighed and initial internal temperatures were recorded. Steaks were cooked to an internal temperature of 35 °C then turned over and cooked to a final internal temperature of 70 °C. Internal cooking temperatures were monitored by a 0.02 cm diameter copper constantan Type-T thermocouple wire, inserted in the geometric center of the steak and connected to a Type t-thermometer (Omega^{TM} HH506A Engineering, Inc, Stamford, CT). Electric griddle temperatures were monitored to

maintain a surface temperature range of 173 °C to 180 °C. Final internal temperatures and steak weights were recorded. Initial and final steak weights were recorded to determine cook loss.

3.2.3. Instrumental Color Analysis

A portable HunterLab miniscan EZ spectrophotometer (Hunter Associates Laboratory, Reston, VA) was used to evaluate three-color scale indices: *L** (lightness), *a** (redness), and *b** (yellowness) values. The spectrophotometer was standardized before starting and after finishing by using a black and white glass tile. For the control, initial color (D65/10°) was assessed on the surface of the log. Logs were portioned into steaks and post treatment color was collected on the surface of the steak prior to vacuum packaging. For blade tenderized and high pressure processed, initial color before treatment was assessed to provide a baseline color assessment on the surface of the log. Initial and final color assessment measurements were taken using D65/10°. Blade tenderized and high pressure processed logs were portioned into steaks, then post treatment color was collected on the steak surface to determine color changes caused by the treatments. Post cooking, instrumental color was taken on the cut surface of the cooked product using D65/10°. Additionally, a trained panel of three individuals determined subjective degrees of doneness and color of the cut surface using the Beef Steak Color Guide, degrees of doneness.

3.2.4. Warner-Bratzler Shear Force Determination

After cooking, steaks designated for shear force were placed on trays and wrapped in plastic film and stored in a cooler (2 to 4 °C) for 16-18 h before coring. At

least six 1.27 cm diameter cores from each steak were removed parallel to the longitudinal orientation of the muscle fibers. The cores were sheared perpendicular to the muscle fibers orientation using a United Testing machine (United Model SSTM-500, Huntington Beach, CA) with a Warner-Bratzler shear device and cross-head speed set at 500mm/min using a 226.8 kg load cell, and a 1.02 cm thick V-shape blade with a 60° angle and a half-round peak. Peak shear force (N) measurements were recorded and averaged to obtain a mean WBSF value for each steak.

3.2.5. Sensory Evaluation

For sensory evaluation, the product was held between 2 to 4 °C, then cooked on a preheated (177 °C) electric griddle (National Presto Industries, Inc., Eau Claire, WI). Before cooking, steaks were weighed and initial internal temperatures were recorded. Steaks were cooked to an internal temperature of 35 °C, turned over, and cooked to a final internal temperature of 70 °C. Internal cooking temperature was monitored by a 0.02 cm diameter copper constantan Type-T thermocouple wire, inserted in the geometric center of the steak and connected to a Type t-thermometer (Omega^{TM} HH506A Engineering, Inc, Stamford, CT). Electric griddle surface temperature was monitored to maintain a surface temperature range of 173 °C to 180 °C. Each steak was cut into 1.27 cm cubes and served warm to the consumer panelists in individual booths equipped with red theater gel lights. On average, 4 panelists evaluated each sample. Steak identification numbers, as well as order of service to consumer panelists, were assigned randomly to each of the 20 panelists per session. Subsequent samples were

evaluated at intervals of about four minutes. Each panelist was involved in only one session.

Consumer panelists were recruited from the Bryan/College Station, TX area and contacted by telephone and email to ensure that they were at least 18 years of age and consumers of beef products. Demographic data was collected on each panelist. Each session consisted of 20 panelists and included a total of 180 consumers in the entire panel. Sensory evaluation was performed under controlled conditions by a consumer panel differing in sex, age, and ethnic background. Before evaluation, instructions regarding the structure of the ballot and sampling procedures for the steak samples were provided verbally to the consumers before each session. Panelists were provided with double-distilled, deionized water and unsalted crackers and were instructed to take a bite of cracker and a drink of water before evaluating each sample to cleanse their palates and to minimize sensory fatigue between samples. In each session, the consumer panelist evaluated 3 samples, selected considering overall like/dislike, tenderness, level of tenderness, flavor, level of flavor, juiciness, and level of juiciness using a ten-point, end-anchored hedonic scale. The sensory ballots included the following attributes: overall like (OLIKE)(1 = dislike extremely; 10 = like extremely), flavor like (FLAV)(1 = dislike extremely; 10 = like extremely); level of beef flavor (FLVBF)(1 = extremely bland or no flavor; 10 = extremely flavorful or intense), tenderness like (TEND)(1 = dislike extremely; 10 = like extremely), level of tenderness (LEVTEND)(1 = extremely tough; 10 = extremely tender), juiciness like (JUIC)(1 = dislike extremely; 10 = like

extremely), and level of juiciness (LEVJUIC)(1 = extremely dry; 10 = extremely juicy). Consumers were given a monetary award of $25 for their participation in this study.

3.2.6. Statistical Analysis

Data were analyzed using SAS (SAS Institute Inc., Cary, NC). Data were analyzed using PROC GLM to evaluate treatment differences between control, blade tenderized, and high pressure processed. Least squares means were calculated; where ANOVA testing indicated significance, means were separated using the PDIFF procedure and an $\alpha < 0.05$.

3.2.7. Results and Discussion

3.2.7.1. Post Treatment Color

Least squares means of instrumental color of the top sirloins after treatment are presented in Table 1. High pressure processed top sirloins exhibited higher L^* values and lower a^* and b^* values ($P < 0.05$) when compared to the control and blade tenderized groups. Carlez et al. (1995) suggested utilizing high pressures (150 – 300 MPa) significantly increase L^* values and lowers a^* values. High pressure treatment of 325 MPa or higher have been shown to have a negative effect on a^* values and metmyoglobin levels of beef when compared to control products (Jung et al., 2003). There were no differences ($P > 0.05$) for L^*, a^*, or b^* between control and blade tenderized top sirloins. High pressure denatures proteins depending on the protein type, processing conditions, and the applied pressure (Jung, de Lamballerie-Anton, & Ghoul, 2000). Pressure ranges from 100-300 MPa can result in reversible protein denaturation. The application of 300 MPa or greater will result in irreversible protein denaturation.

Above 150 MPa, there are color changes similar to cooked meat products (Hugas, Garriga, & Monfort, 2002). Based on results from this study, combining temperature and pressure directly affected overall quality of the product.

3.2.7.2. Warner-Bratzler and Sensory Tenderness

High pressure processed steaks had higher WBS values ($P < 0.0001$) than control and blade tenderized (Table 2). Consumer panel overall tenderness data (Table 3) indicated the high pressure processed samples were less tender than the control and blade tenderized steaks. According to Buckow, Sikes, and Tume (2013), pressure of several hundred megapascals (MPa) favors the van der Waals forces, as they tend to maximize the packing density of the proteins. In addition, Buckow et al. (2013) suggested pressure levels of 200 – 400 MPa at 20 to 50 °C for 10 min is required to denature bovine protein to reduce tenderness levels. However, Ma and Ledward (2004) found that the higher the temperature and pressure, the more likely protein hardness occurs. Also, a high temperature, low pressure treatment will significantly decrease tenderness levels (Ma & Ledward, 2004). Studies conducted by Jung et al. (2000) concluded high pressure treatment influences the area of myofibrils. Pressurization of meat leads to significantly larger myofibrillar size. There is a direct relationship between tenderness and myofibrillar size and sarcomere length; the highest shear force is correlated with the largest fiber size and shortest sarcomere (Lewis, Brown, & Heck, 1977). Results from Jung et al. (2000) found that product treated at 300 MPa observed no improvement in tenderness. Although high pressure treatment caused ultrastructural modifications, the changes would not cause tenderness.

Research suggests much higher pressure is required to achieve comparable tenderness levels to blade tenderized (Patel et al., 2005). Tenderness is considered the most important qualitative trait characteristic of meat. Blade tenderization (BT) is commercially utilized to increase tenderness by partial severance of both connective tissue and muscle fibers, which leads to lower shear force and easier mastication supporting the research findings (Patel et al., 2005). George-Evins et al. (2004) concluded tenderness of sirloin steaks can be improved with extended postmortem aging or blade tenderization, regardless of degree of doneness. Additional enhanced tenderness can be obtained when using extended postmortem aging followed by blade tenderization (George-Evins et al., 2004).

The rupture of non-covalent interactions within protein molecules and subsequent reformation of intra- and inter- molecular bonds within or between the molecules is caused by high pressure. Further research using high pressure on long-aged beef is needed to determine if the high pressure treatment may be causing an effect on the crosslinking of muscle proteins, and therefore contributing to toughness.

3.2.7.3. Consumer Sensory Panel

Consumer sensory data are presented in Table 3. High pressure processed top sirloin steaks received less favorable scores ($P < 0.05$) for overall like than those from the control group, but there were no differences ($P > 0.05$) than those from the blade tenderized group. There was no difference between the control and the blade tenderized groups for overall like ($P > 0.05$). Tenderness heavily contributed to the consumers "overall like." As previously mentioned, values for tenderness like and level of

tenderness also were lower ($P < 0.05$) for the high pressure group when compared to the other treatments. There were no differences ($P > 0.05$) among the three groups for level of beef flavor, juiciness like, or level of juiciness. There also were no differences ($P > 0.05$) among the treatment groups for cooking yield during the sensory panel.

3.2.7.4. Cooked Color and Subjective Degree of Doneness

Data for cooked color and degree of doneness are shown in Table 4. Instrumental cooked color of the cut surface of top sirloin steaks showed higher L^* values and lower a^* values ($P < 0.05$) for high pressure treated steaks when compared to the control and blade tenderized groups. There were no differences ($P > 0.05$) in b^* values between the blade tenderized and high pressure processed groups, but the control group exhibited higher b^* values ($P < 0.05$). Subjective evaluation of degree of doneness was the same for all three treatment groups ($P > 0.05$). As previously stated, the denaturation of proteins during high pressure processing could contribute to the decreased redness of the cooked product; however, it does not appear that it had an effect on the visual degree of doneness that would be perceived by the end consumer.

3.3. Phase 2. Cooking Endpoint Temperatures

3.3.1. Product Collection

Choice, Beef Loin ($n = 45$), Top Sirloin Butt, Center-Cut, Boneless, Cap Off (IMPS #184B) (*Gluteus medius*) aged 35 days were collected from a local purveyor for each of the three replications. Top sirloin butts were separated medial to lateral into 3 pieces (logs) to generate 135 logs.

3.3.2. Treatment Design

Each log was assigned to a treatment. The three treatments included: control, blade tenderized, and high pressure processed. Control logs were portioned anterior to posterior into 2.54 cm thick steaks and vacuum packaged. Blade tenderized product were subjected to a Model 700WI blade tenderizer, Ross Industries, Inc., Midland, VA., cut into 2.54 cm thick steaks, and vacuum packaged. High pressure processed logs were taken to Universal Pasteurization, Coppell, TX. The logs were pressurized to 60,000 psi (413.68 MPa) for 120 sec (Avure Technologies, Quintas Food Press 350L – 600L, Middletown, OH). Post treatment, the logs were portioned into 2.54 cm thick steaks (Portioner, Model X600, Marel USA, Lenexa KS) and assigned randomly to Warner-Bratzler shear force (WBSF). All products were transported in insulated containers with dry ice, to the Rosenthal Meat Science and Technology Center (College Station, TX) for further analysis.

At the Rosenthal Meat Science and Technology Center (College Station, TX), the products were held at 2 to 4 °C. The steaks were transported to the sensory kitchen to cook on a preheated (177 °C) electric griddle (National Presto Industries, Inc., Eau Claire, WI). Before cooking, steaks were weighed and initial internal temperatures were recorded. Steaks were cooked to an internal temperature of 35 °C then turned over and cooked to three final internal temperatures of 63 °C, 71 °C, and 77 °C depending on assignment. Internal cooking temperature was monitored by a 0.02 cm diameter copper constantan Type-T thermocouple wire, inserted in the geometric center of the steak and connected to a Type t-thermometer (Omega^{TM} HH506A Engineering, Inc, Stamford,

CT). Electric griddle surface temperatures were monitored to maintain a surface temperature range of 173 °C to 180 °C.

3.3.3. Warner-Bratzler Shear Force Determination

After cooking, steaks were placed on trays and wrapped in plastic film and stored in a cooler at (2 to 4 °C) for 16-18 h before coring. At least six 1.27 cm diameter cores from each steak were removed parallel to the longitudinal orientation of the muscle fibers. The cores were sheared perpendicular to the muscle fibers orientation using a United Testing machine (United Model SSTM-500, Huntington Beach, CA) with a Warner-Bratzler shear device and cross-head speed set at 500mm/min using a 226.8 kg load cell, and a 1.02 cm thick V-shape blade with a 60° angle and a half-round peak. Peak shear force (N) measurements were recorded and averaged to obtain a mean WBSF value for each steak.

3.3.4. Statistical Analysis

Data were analyzed using JMP®, Pro 11 (SAS Institute Inc., Cary, NC). Data were analyzed using ANOVA: Fit Model to evaluate treatment differences between control, blade tenderized, and high pressure processed. Least squares means were calculated; where ANOVA testing indicated significance, means were separated using the students' t procedure and an $\alpha < 0.05$.

3.3.5. Results and Discussion

3.3.5.1. Warner-Bratzler Shear Force Tenderness

Previous research has shown that cooking methods and end point temperature affect beef palatability (Belk, Luchak, & Miller, 1993; Berry & Bigner, 1995; Berry &

Leddy, 1990; Savell et al., 1987; Savell et al., 1989). The ability to serve a product that maximizes customer satisfaction, maintains customer loyalty, and increases patronage is a very complex issue facing the foodservice industry (Cox, Thompson, Cunial, Winter, & Gordon, 1997). The cooking processes for steak requires a balance between, enhancing or maintaining tenderness, ensuing product safety and delivering a steak which is in accordance with customers preference for degree of doneness. Changes in meat tenderness with cooking results from alterations in connective tissue and myofibrillar proteins. It is widely accepted that heat solubilizes collagen that results in tenderization, whereas heat denatures myofibrillar proteins that results in toughening (Obuz, Dikeman, & Loughin, 2003). Least squares means for WBS force data from control, blade tenderized, and high pressure processed are reported in Table 5. The difference in WBS force across treatment groups and endpoint temperatures varied. As endpoint temperature increased the WBS value tended to increase. For the 63° C and the 77° C endpoints, steaks from high pressure processed sirloins required higher shear force than steaks from the control and blade tenderized sirloins ($P < 0.05$). For the 71° C endpoint, sirloins that were blade tenderized had the lowest shear force values ($P < 0.05$) when compared to high pressure and control groups for the same endpoint, which did not differ from one another. Similar findings were reported by Lorenzen et al. (2003) as they studied increasing endpoint temperature and found an increase in WBS values. Furthermore, Lorenzen et al. (1999) reported that consumers detected no tenderness differences ($P > 0.05$) among cooking methods when steaks were cooked to medium rare or less, medium, or medium well degrees of doneness. However, steaks that were

cooked to well done or greater degrees of doneness by indoor grilling were more tender ($P < 0.05$) than steaks cooked to the same degree of doneness by other cookery methods. Changes in shear force values are time and temperature dependent, and the net effect of this toughening or tenderization depends on cooking conditions. Steaks cooked to 63 °C tended to be more tender than those cooked to 71 and 77 °C within each of the treatment groups. Luchak et al. (1998) reported decreased trained panel ratings with increased degree of doneness for overall tenderness. High pressure processed steaks had higher WBS values than blade tenderized (Table 5).

Blade tenderized and control product when cooked to 63 °C were not statistically different for WBS ($P < 0.05$). When cooked to 71 °C, blade tenderized product was the most tender. At 77 °C, high pressure processed recorded the highest WBS values. Overall, steaks from the high pressure processed group that were cooked to 77° C exhibited the highest shear force values, while steaks from the blade tenderized group that were cooked to 63° C required the lowest amount of shear force ($P < 0.05$). Kim, Homma, Ikeuchi, and Suzuki (1993) observed that the conversion of connectin, as well as the degradation of nebulin, were limited at higher pressures (400 MPa). Kim et al. (1993) suggested calpain activity decreased with increasing pressure above 100 MPa, therefore the conversion of connectin increased. As reported by Obuz et al. (2003), the endpoint temperature is a significant factor affecting tenderness. Parrish et al. (1973) stated that endpoint temperature was a more important modifier of tenderness than marbling or maturity. In a study of consumers in a restaurant setting, Cox et al. (1997) found when consumers received beef steaks cooked to their ordered degree of doneness,

customer satisfaction was the highest, but when steaks were served over or under cooked compared with their ordered degree of doneness, customer satisfaction was significantly lower. Higher WBS values are reported with higher endpoint temperatures.

Direct evidence of the tenderization of meat pressurized post-rigor has not been clearly reported. Combining pressure with heat does tenderize meat, but the final products have a cooked appearance, and therefore cannot be sold as fresh meat. High pressure processing would also have to compete with other tenderizing processes used for fresh and cooked meats. The change in meat color caused by the application of pressure above 300 MPa, even at low temperature, means products are not consumer appealing and could not be sold in a fresh retail market. Due to innate challenges with top sirloin steaks, the variation in degree of doneness desired by consumers should be assessed before deterring tenderness quality factors.

3.4. Phase 3. Age Treat vs. Treat Age

3.4.1. Product Collection

A total of 30 Select, Beef Loin, Top Sirloin Butt, Center-Cut, Boneless, Cap Off (IMPS #184B) (*Gluteus medius*) were selected from electrically stimulated intact carcasses from a local packer. Top sirloin butts were separated medial to lateral into 3 pieces (logs) to generate 90 logs over three replications.

3.4.2. Treatment Design

Each log was assigned randomly to a treatment. The three treatments included: control, blade tenderized, and high pressure processed. Once assigned to a treatment, the

product was further divided into “treat then age” or “age then treat” groups. All products were aged in the form of log.

Following selection, ‘treat then age’ products were transported in insulated containers with ice, to the Rosenthal Meat Science and Technology Center (College Station, TX) for further analysis. Product was fabricated into logs then treated. Blade tenderized product was subjected to a Model 700WI blade tenderizer, Ross Industries, Inc., Midland, VA., and then vacuum packaged. High pressure processed logs were taken to Universal Pasteurization, Coppell, TX. The logs were pressurized to 60,000 psi (413.68 MPa) for 210 sec (Avure Technologies, Quintas Food Press 350L – 600L, Middletown, OH). Post treatment the product was aged in the form of a log for 35 days. After treatment the product was assigned randomly to Warner-Bratzler shear force (WBSF) or consumer panel. After the treatments and aging periods the product was portioned into 2.54 cm thick steaks for consumer sensory and Warner-Bratzler shear force analysis.

Following selection, ‘age then treat’ products were transported in insulated containers with ice, to the Rosenthal Meat Science and Technology Center (College Station, TX) for further analysis. Product was fabricated into logs then aged for 35 days prior to treating. Following aging, blade tenderized product was subjected to a Model 700WI blade tenderizer, Ross Industries, Inc., Midland, VA., and then vacuum packaged. High pressure processed logs were taken to Universal Pasteurization, Coppell, TX. The logs were pressurized to 60,000 psi (413.68 MPa) for 210 sec (Avure Technologies, Quintas Food Press 350L – 600L, Middletown, OH). Post treatment, the

product was assigned randomly to Warner-Bratzler shear force (WBSF) or consumer panel. After the treatments and aging periods the product was portioned into 2.54 cm thick steaks for consumer sensory and Warner-Bratzler shear force analysis.

At the Rosenthal Meat Science and Technology Center (College Station, TX), the products were held at 2 to 4 °C. The steaks were transported to the sensory kitchen to cook on a preheated (177 °C) electric griddle (National Presto Industries, Inc., Eau Claire, WI). Before cooking, steaks were weighed and initial internal temperatures were recorded. Steaks were cooked to an internal temperature of 35 °C then turned over and cooked to a final internal temperature of 70 °C. Internal cooking temperature was monitored by a 0.02 cm diameter copper constantan Type-T thermocouple wire, inserted in the geometric center of the steak and connected to a Type t-thermometer (Omega™ HH506A Engineering, Inc, Stamford, CT). Electric griddle surface temperatures were monitored to maintain a temperature range of 173 °C to 180 °C.

3.4.3. Shelf-life Analysis

Swab samples were collected from product surfaces on day 1 and day 35 prior to and post treatments. Before sample collection, all sponges (3M, St. Paul, MN) were hydrated with 25 ml of buffered peptone water (BD Diagnostics, Spark, MD). Sponges were then wrung-out in the bag to remove excess buffered peptone water, removed from the bag, and were used to swab a 100-cm^2 area of each sample surface. Samples were taken by making five horizontal passes with a sponge, flipping the sponge over, and utilizing the opposite side of the sponge to make an additional five vertical passes over the sample surface. Non-sterile nitrile gloves were worn at all times. Samples were

stored at refrigerated conditions (approximately 4 °C) until arrival to the Food Microbiology Laboratory at Texas A&M University (College Station).

Upon arrival, samples were hand-pummeled for 1 min. To accommodate aerobic plate count analysis, pummeled samples obtained on day 1 and 35 were plated in duplicate onto aerobic plate count Petrifilm plates (3M, St. Paul, MN) by using appropriate serial dilutions and pipetting 1 ml of sample onto the center of the bottom film. When necessary, a spreader was used over the top film of the Petrifilm plates to distribute the sample over the circular area before gel formed. Aerobic plate count plates were incubated aerobically for 48 h at 35 °C. Following incubation, plates were counted.

3.4.4. Instrumental Color Analysis

A portable HunterLab miniscan EZ spectrophotometer (Hunter Associates Laboratory, Reston, VA) was used to evaluate three color scale indices: L^* (lightness), a^* (redness), and b^* (yellowness) values. The spectrophotometer was standardized before starting and after finishing by using a black and white glass tile. For the control, initial color (D65/10°) was assessed on the surface of the log and the cut surface. Logs were portioned into steaks and post treatment color was collected on the surface of the steak prior to vacuum packaging. For blade tenderized and high pressure processed, initial color before treatment was assessed to provide a baseline color score on the surface of the log. Measurements were taken using D65/10°. Blade tenderized and high pressure processed logs were portioned into steaks, then post treatment color was collected on the steak surface to determine color changes caused by the treatments. Post

cooking, instrumental color was taken on the cut surface of the cooked product using D65/10°. Additionally, a trained panel of three individuals determined subjective degrees of doneness and color of the cut surface using the Beef Steak Color Guide, degrees of doneness.

3.4.5. Warner-Bratzler Shear Force Determination

After cooking, steaks designated for shear force were placed on trays and wrapped in plastic film and stored in a cooler at 2 to 4 °C for 16-18 h before coring. At least six 1.27 cm diameter cores from each steak were removed parallel to the longitudinal orientation of the muscle fibers. The cores were sheared perpendicular to the muscle fibers orientation using a United Testing machine (United Model SSTM-500, Huntington Beach, CA) with a Warner-Bratzler shear device and cross-head speed set at 500mm/min using a 226.8 kg load cell, and a 1.02 cm thick V-shape blade with a 60° angle and a half-round peak. Peak shear force (N) measurements were recorded and averaged to obtain a mean WBSF value for each steak.

3.4.6. Thiobarbituric Acid Reactive Substances (TBARS) Evaluation

Thiobarbituric acid reactive substances for fresh meat procedure were determined by distillation according to Tarladgis et al. (1960) as modified by Rhee (1978) with the following modifications. Steaks were cooked as previously described and allowed to chill at 4°C for 16 hrs. Steaks were diced and duplicate ten gram samples were added to a 125 ml poly bottles containing 50 ml of distilled deionized water. Five ml of Propyl Gallate and 5 ml of EDTA were added to each sample and then the sample was homogenized at 15,000 RPM for 1 minute, using an Ultra Turrax T-25 (IKA Works,

INC. Wilmington, NC) with an 18 mm rotor/stator (IKA Works, INC.). The homogenized 66 ml meat sample and an additional 31.5 ml of distilled deionized water were added to a 500 ml kjeldahl distillation flask. Five to six glass boiling beads and 2.5 ml of 4N HCl (BDH) was added to the kjeldahl flask. Before connecting the kjeldahl flask to the distillation unit, Slipicone silicone release spray (DC Products PTY Ltd. New Waverly, Australia) was sprayed into the neck of the flask. Then the flask was connected to the distillation unit and allowed to distill until 50 ml of distillate was collected. The 50 ml of distillate was transferred into 50 ml centrifuge tubes and stored covered from light at 4°C for no longer than 18 hrs. In triplicate, 125 µl of distillate and 125 µl of 0.02M TBA solution was pipetted into each well of a 96-well microplate. For each 96 well microplate, 125 µl of distilled deionized water and 125 µl of 0.02M TBA solution was pipetted into 3 wells to be used as blanks. The 96 well microplates were incubated for 130 min at 40 °C. The plates were removed from the incubator and read at 532 nm on a Bio-Tek microplate reader (EPOCH) within 1 hour.

For each replication (3), a standard curve was calculated by pipetting 125 µl of $1x10^{-3}$M 1,1,3,3-tetraethoxypropane (TEP) ranging from $1x10^{-8}$ to $7x\ 10^{-8}$ M MDA/5 ml and 125 µl of 0.02M TBA solution in triplicate into wells on a 96 well microplate. The 96 well microplates were incubated for 130 min at 40 °C. The plates were removed from the incubator and read at 532 nm on a Bio-Tek microplate reader (EPOCH) within 1 hour. Results were plotted as absorbency versus concentration (M MDA/5 ml). The absorbency values were transformed into TBA numbers by multiplying them by a distillation factor (K), which was calculated as described in Tarladgis et al. (1960).

3.4.7. Consumer Sensory Evaluation

For sensory evaluation, the product was held at 2 °C, then cooked on a preheated (177 °C) electric griddle (National Presto Industries, Inc., Eau Claire, WI). Before cooking, steaks were weighed and initial internal temperatures were recorded. Steaks were cooked to an internal temperature of 35 °C then turned over and cooked to a final internal temperature of 70 °C. Internal cooking temperature were monitored by a 0.02 cm diameter copper constantan Type-T thermocouple wire, inserted in the geometric center of the steak and connected to a Type t-thermometer (Omega^{TM} HH506A Engineering, Inc, Stamford, CT). Electric griddle temperatures were monitored to maintain a surface temperature range of 173 °C to 180 °C. Each steak was cut into 1.27 cm cubes and served warm to the consumer panelists in individual booths equipped with red theater gel lights. On average, 4 panelists evaluated each sample. Steak identification numbers, as well as order of service to consumer panelists, were assigned randomly to each of the 20 panelists per session. Subsequent samples were tested at intervals of about four minutes. Each panelist was involved in only one session.

Consumer panelists were recruited in the Bryan/College Station, TX area and contacted by telephone and email to ensure that they were at least 18 years of age and consumers of beef products. Each session consisted of 20 panelists and included a total of 121 consumers. Sensory evaluation was performed under controlled conditions by a consumer panel differing in sex, age, and ethnic background. Before evaluation, instructions regarding the structure of the ballot and sampling procedures for the steak samples were provided verbally to the consumers in each session. Panelists were

provided with double-distilled, deionized water and unsalted crackers and were instructed to take a bite of cracker and a drink of water before evaluating each sample to cleanse their palates and to minimize sensory fatigue between samples. In each session, the consumer panelist evaluated 3 samples, selected considering overall like/dislike, tenderness, level of tenderness, flavor, level of flavor, juiciness, and level of juiciness using a nine-point, end-anchored hedonic scale. Panelists were asked to evaluate the steak samples using 10-point scale. The sensory ballots included the following attributes: overall like (OLIKE)(1 = dislike extremely; 10 = like extremely), flavor like (FLAV)(1 = dislike extremely; 10 = like extremely); level of beef flavor (FLVBF)(1 = extremely bland or no flavor; 10 = extremely flavorful or intense), tenderness like (TEND)(1 = dislike extremely; 10 = like extremely), level of tenderness (LEVTEND)(1 = extremely tough; 10 = extremely tender), juiciness like (JUIC)(1 = dislike extremely; 10 = like extremely), and level of juiciness (LEVJUIC)(1 = extremely dry; 10 = extremely juicy). Consumers were given a monetary award of $25 for their participation in this study.

3.4.8. Statistical Analysis

Data were analyzed using JMP®, Pro 11 (SAS Institute Inc., Cary, NC). Data were analyzed using ANOVA: Fit Model to evaluate treatment differences between control, blade tenderized, and high pressure processed. Least squares means were calculated; where ANOVA testing indicated significance, means were separated using the students' t procedure and an $\alpha < 0.05$.

3.4.9. Results and Discussion

3.4.9.1. Shelf-life

High pressure processing of meat has been investigated for years due to its potential to inactivate microorganisms and extend shelf-life. Under specific conditions high pressure processing can inactivate microorganisms in meat products. Consequently pressure treatment may be a suitable method to extend the shelf-life of fresh meat without any additives. Least squares means are reported in Table 6. Initial aerobic plate count samples across all treatments and groups showed no statistical difference ($P < 0.0001$). Initial samples provided a baseline for potential microbial growth throughout the treatments and aging. Final aerobic plate counts recorded between 'treat then age' versus 'age then treat', treat then age samples showed a higher log count (3.81 $\log_{10}$/CFU) than age then treat (2.10 $\log_{10}$/CFU) ($P < 0.0001$). According to Jung et al. (2003), samples pressurized with 130 MPa total microbial load of the samples remained similar to the control. In contrast, a treatment of 520 MPa led to a decrease of 2.5 log. This shows that moderate pressures do not improve microbiological quality of meat (Jung et al., 2003). Additional research concluded that the higher the intensity of pressure led to the greater reduction in microorganisms. The effect of high pressure on microorganisms is dependent on the type of microorganisms present, and the composition of food.

High pressure processing is currently being used to eliminate pathogenic microorganisms, extend shelf-life, maintain sensory quality, and improve the safety of commercial processed meat products.

3.4.9.2. Post Treatment Color

The color of fresh meat is one of the most important evaluation parameters consumers use when purchasing. The modification induced by high pressure treatment on meat color is related to the color criteria such as lightness (L^*), redness (a^*) or yellowness (b^*). Thus in some conditions, the lightness of meat could be heightened by high-pressure treatment and the redness increased or decreased. Jung et al. (2003) determined that high pressure has an effect on metmyoglobin production. At pressures up to 300 MPa, with a pressurization liquid of 10 °C, the production of metmyoglobin was decreased leading to an increase in a^* value, but both concur that the discoloration of meat at higher pressure >200 MPa (Carlez et al., 1995) and > 325 MPa (Jung et al., 2003) are the result of denaturation to myofibrillar and sarcoplasmic proteins, particularly myoglobin.

Post-rigor minced beef samples were reported to develop a gray color after being pressurized at 150 MPa for 20 min with a pressurization liquid at 50 °C (Carlez et al., 1995). However, when pressure was greater than 150 MPa L^* values increased and a^* decreased, appearing lighter and less red (Carlez et al., 1995). Additionally, Carlez et al. (1995) concluded that pressure at or above 200 MPa causes a 'whitening' effect on meat. The same research also determined total myoglobin content was less for samples pressurized in a range of 200 – 300 MPa.

In this study, cut surface color was assessed instrumentally post treatment (Table 7). The cut surface of high pressure processed top sirloins displayed higher lightness (L^*) and yellowness (b^*) values when compared to all other groups of top sirloins ($P < 0.0001$ and $P = 0.0472$, respectively). High pressure processed sirloins also exhibited lower overall values for redness (a^*) when compared to the other treatments ($P < 0.0001$).

3.4.9.3. Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric acid reactive substances are expressed in TBAR numbers, which is expressed as milligrams of malonaldehyde per kilogram of sample, using a conversion factor of 21.64. In all cases duplicate was completed to average the absorbance value. When evaluating the 'treat then age' versus 'age then treat' across all treatments there was no statistical difference between the two groups ($P = 0.9744$). As shown in Table 8, high pressure processed expressed a significantly ($P < 0.0001$) higher TBA number when compared to blade tenderized and control. Ma, Ledward, Zamri, Frazier, and Zhou (2007) reported that the pressure required to initiate a decrease in the oxidative stability for beef can be as low as 200 MPa. It has been suggested that this phenomenon is due to the release of 'free' iron from the iron complexes present in meat, as the concentration of 'free' iron increased after pressure treatment and chelating agents, such as EDTA effectively prevented the increased rates of oxidation seen in pressure treated proteins (Defaye & Ledward, 1999). The use of high pressure in meat has shown to accelerate lipid oxidation particularly with pressures at or above 300 MPa (Cheftel & Culioli, 1997; Ma et al., 2007). Research using beef and poultry found that lipid

oxidation rates were five times higher at pressures above 400 MPa; samples tempered to higher temperatures (50 °, 60 °, and 70 °C) and then pressurized, had higher TBARS in beef only. Additionally, when comparing the treatments across both groups blade tenderized and control tended ($P = 0.0673$) to show a difference in TBA numbers. This is theorized to be due to the disruption of the muscle tissue, which can allow a greater opportunity for oxidative effects to occur. In Table 8, treatments across groups are reported. High pressure processing tends to have higher TBA values compared to blade tenderized and control. The oxidative stability of fresh meat is important to ensure that consumers get a product of the highest sensory quality.

3.4.9.4. Cooked Color and Subjective Degree of Doneness

For instrumental cooked color, the blade tenderized sirloins from the 'treat then age' group displayed the lowest L^* values (Table 9). From the 'age then treat' group, high pressure processed sirloins exhibited the highest values for lightness (L^*) and the lowest values for redness (a^*) when compared to the other two treatments. There were no differences between treatments for b^* values. When compared to the blade tenderized and control groups, cooked steaks from high pressure processed sirloins displayed a higher degree of doneness based on trained visual assessment ($P < 0.05$). There was no difference in visual degree of doneness between the group that was high pressure processed prior to aging and the group that was high pressure processed at the end of the aging period ($P > 0.05$). High pressure processed sirloins from the 'age then treat' group produced cooked steaks with a higher visual degree of doneness than steaks from the blade tenderized and control groups ($P < 0.05$). The increase in L^* values

begins from 200 MPa and becomes stabilized for pressures around 300 to 400 MPa (Jung et al., 2003). The lighter the appearance could be due to globin denaturation and heme displacement or release, an increase in drip losses leading to changes of water content of the meat or a damage of porphyric ring and protein coagulation (Carlez et al., 1995). The decrease in redness could be related to the increase in metmyoglobin therefore resulting in a brown coloration of meat which is undesirable to consumers (Jung et al., 2003).

3.4.9.5. Warner-Bratzler and Sensory Tenderness

Gruber et al. (2006) showed Warner-Bratzler Shear Force values of select *Gluteus Medius* to continue to improve up to 28 days postmortem. In general, Select muscles required 20 days or longer postmortem aging to achieve a majority of their respective aging response (Gruber et al., 2006). All top sirloins in this study were wet aged 35 days postmortem.

Steaks from high pressure processed top sirloins exhibited higher Warner-Bratzler shear force values overall, but there was no difference in between control and blade tenderized within each aging group ($P < 0.05$) (Table 10). In previous experiments, pressure of 400 to 600 MPa caused significant increases in hardness at all temperatures (Ma & Ledward, 2004). Ma and Ledward (2004) found increasing pressures causes increases in springiness and cohesiveness. Ma and Ledward (2004) states at relatively low pressures (200 – 400 MPa) myosin initially aggregates by the two heads fusing together to form a one headed structure, these fused heads then subsequently aggregate to give a clump of heads with tails extending outwards. The

three major endothermic transitions seen have been attributed to myosin, collagen, and actin. The peak maxima for myosin is 54.6 °C, collagen 67.1 °C, and actin 77.3 °C. Pressure of 200 MPa decreased the myosin peaks, 400 MPa or higher the actin peaks were not visible. The collagen peak is not affected by pressure (Ma & Ledward, 2004). At ambient pressure about 43 % of the myosin is denatured, but there is no obvious effect on collagen and actin. Myosin is the first structure to denature due to increased pressure (Ma & Ledward, 2004).

3.4.9.6. Consumer Sensory Panel

Consumer sensory data are presented in Table 11. High pressure processed top sirloin steaks from the 'Treat then Age' group received less favorable scores ($P < 0.05$) for overall like than blade tenderized sirloins from the same aging group. There also was no difference between the control (treat then age) and the both blade tenderized groups for overall like ($P > 0.05$). For overall like, blade tenderized product in the 'treat then age' versus the 'age then treat' group received equal evaluation from the consumer panelists. There was no difference in overall flavor between the three treatment groups within 'age then treat' and 'treat then age'. Consumer panelist perceived the beefy flavor to be higher in products that were 'aged then treated' compared to those 'treated then aged'. High pressured steaks from 'age then treat' group possessed the highest overall level of beef flavor ($P < 0.05$). Tenderness heavily contributed to the consumers "overall like." As previously mentioned, values for tenderness like and level of tenderness also were less favorable ($P < 0.05$) for the high pressure group when compared to the other treatments. Blade tenderized product was perceived to be more

tender compared to high pressure processed treated product ($P < 0.05$). Juiciness level and juiciness like varied across groups and treatment types. There also were no differences ($P > 0.05$) among the treatment groups for cooking yield during the sensory panel.

Foods produced or processed by many of these technologies pose challenging problems for researchers interested in the factors responsible for consumer choice, acceptance and purchase behavior. Like most food products, optimizing the sensory quality of these foods is critical to their success in the marketplace (Cardello, Schutz, & Lesher, 2007). However, optimal sensory quality, on its own, will not guarantee success. Consumer perceptions about safety, cost, and risk/benefits associated with novel technologies can negatively influence consumer choice and purchase decisions. A small number of studies have used sensory panel to evaluate the parameter of tenderness and juiciness. Taste panel assessment of high pressure processed beef determined that tenderness scores for treated samples were significantly different while juiciness values were not (Riffero & Holmes, 1983)

4. CONCLUSIONS

The challenge to decrease variation and improve tenderness through multiple ante- and postmortem technologies is one that has been of interest to the beef industry for many years. Postmortem aging and blade tenderization of *Gluteus medius* steaks can improve tenderness, as measured by Warner-Bratzler and sensory panel, without decreasing flavor or juiciness. All of these factors are important to provide consumers with a consistent product. In recent years, enhancements in equipment design have ensured worldwide recognition of high pressure processing as a new food processing technology.

Moderate pressures may stabilize a protein against heat denaturation and conversely a moderate temperature increase may stabilize a protein against pressure denaturation (Ma & Ledward, 2013). The texture of meat is, to a large extent dependent on the connective tissue and contractile systems. The triple helix of collagen is predominantly stabilized by hydrogen bonds and accordingly is relatively inert to pressure, under normal circumstances. In fact pressure treatment at 150 MPa increased the thermal stability of tendon collagen by 6 °C, from 58 to 64 °C, as would be expected from the volume increase associated with rupture of these bonds (Ma & Ledward, 2013). Thus, it is unlikely that this background toughness will be amenable to pressure treatment (Ma & Ledward, 2013). When pressure treated at room temperature (20 °C) meat toughens due to denaturation of the myofibrillar proteins (Ma & Ledward, 2013), the hardness increases up to about 400 MPa but at higher pressures a small decrease appears to occur. Subsequent cooking of such pressure treated meat at 70 °C increases

the hardness still further up to the values seen in meat cooked directly to 70 °C with no pressure treatment. However if meat cooked directly to 70 °C is then subjected to pressures of 200 to 600 MPa little or no increase in hardness is observed (Ma & Ledward, 2013).

The results of this study suggest that more research may be needed to determine the full effects of high pressure processing on muscle protein structure. Furthermore, findings of this research show that blade tenderization of subprimals is not necessary to achieve acceptable product quality in top sirloins that are aged 35 days or more.

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APPENDIX A

Table 1.
Initial mean (± SEM) lightness (*L**), redness (*a**), and yellowness (*b**) values of top sirloin steaks stratified by treatment.

Treatment	*n*	*L**	*a**	*b**
Control	45	47.77[b]	20.84[a]	17.41[b]
Blade Tenderized	45	48.09[b]	20.83[a]	17.14[b]
High Pressure Processed	45	54.53[a]	19.88[b]	18.98[a]
SEM[A]		0.50	0.25	0.24

Means within the same column lacking a common letter (a-b) differ ($P < 0.05$).
[A]Standard error of the mean.

Table 2.
Least squares means (± SEM) of Warner–Bratzler shear (WBS) values in Newtons (N).

Treatment	WBS (N)
Control	23.35[b]
Blade Tenderized	22.42[b]
High Pressure Processed	27.65[a]
SEM[A]	0.08

Means within the same column lacking a common letter (a-b) differ ($P < 0.05$).
[A]Standard error of the mean.

Table 3.
Least squares means of palatability characteristics of beef steaks from top sirloin for consumer (n=186 consumers) evaluation stratified by treatment.

Treatment	*n*	Overall Like[A]	Flavor Like[A]	Level of beef flavor[B]	Tenderness like[A]	Level of tenderness[C]	Juiciness like[A]	Level of juiciness[D]	Cook yield (%)
Control	186	7.40[a]	7.00	7.32	7.48[a]	7.31[a]	7.10	7.00	76.42
Blade Tenderized	186	7.32[ab]	6.98	7.33	7.52[a]	7.39[a]	6.99	7.00	75.11
High Pressure Processed	186	7.02[b]	7.00	7.32	6.85[b]	6.62[b]	6.89	6.97	74.43
SEM[E]		0.13	0.14	0.13	0.15	0.14	0.15	0.14	0.98

Means within the same column lacking a common letter (a-b) differ ($P < 0.05$).
[A] 10 = Like extremely; 1 = dislike extremely.
[B] 10 = Extremely flavorful or intense; 1 = extremely bland or no flavor.
[C] 10 = Extremely tender; 1 = extremely tough.
[D] 10 = Extremely juicy; 1 = extremely dry.
[E]Standard error of the mean.

Table 4.
Least squares means of cooked color (± SEM) lightness (*L**), redness (*a**), and yellowness (*b**) values of top sirloin steaks stratified by treatment.

Treatment	*n*	*L**	*a**	*b**	Subjective Degree of Doneness[A]
Control	45	53.40[b]	10.99[a]	20.05[a]	4.62
Blade Tenderized	45	53.56[b]	10.79[a]	18.84[b]	4.61
High Pressure Processed	45	55.33[a]	9.77[b]	18.74[b]	4.78
SEM[B]		0.59	0.36	0.31	0.06

Means within the same column lacking a common letter (a-b) differ ($P < 0.05$).
[A] (1=Very Rare, 2=Rare, 3=Medium Rare, 4=Medium, 5=Well Done, 6=Very Well Done) Evaluated by trained personnel according to the Beef Steak Color Guide for Degrees of Doneness, National Cattlemen's Beef Association.
[B]Standard error of the mean.

Table 5.
Least squares means (± SEM) of Warner–Bratzler shear (WBS) values in Newtons (N) when cooked to different degrees of doneness.

Treatment	WBS (N)					
	63 °C	SEM[A]	**71 °C**	SEM[A]	**77 °C**	SEM[A]
Control	19.22[e]	0.06	22.46[abc]	0.06	22.36[bc]	0.06
Blade Tenderized	18.04[e]	0.06	19.61[de]	0.06	21.28[cd]	0.06
High Pressure Processed	21.97[bc]	0.06	23.14[ab]	0.06	24.22[a]	0.06
SEM[A]						

Means within the same column lacking a common letter (a-c) differ ($P < 0.05$).
[A]Standard error of the mean.

Table 6.
Least squares means of post treatment aerobic plate counts (APC) by treatment (d_log 10 CFU)

Treatment	APC	
	TA[B]	AT[C]
Control	1.90[c]	1.63[c]
Blade Tenderized	5.19[a]	3.44[b]
High Pressure Processed	4.35[ab]	1.24[c]
SEM[A]	0.27	0.27

Means within the same column lacking a common letter (a-c) differ ($P < 0.05$).
[A]Standard error of the mean.
[B]TA: 'treat then age'
[C]AT: 'age then treat'

Table 7.
Least squares means of cut surface color (± SEM) lightness (*L**), redness (*a**), and yellowness (*b**) values of top sirloin steaks (n = 30) stratified by treatment.

Treatment	*L**		*a**		*b**	
	TA[B]	AT[C]	TA[B]	AT[C]	TA[B]	AT[C]
Control	47.37[b]	44.62[c]	19.31[a]	18.61[ab]	17.31[ab]	16.63[b]
Blade Tenderized	46.19[bc]	45.72[bc]	19.25[ab]	17.71[b]	17.02[b]	16.84[b]
High Pressure Processed	50.32[a]	51.34[a]	15.51[c]	15.17[c]	18.92[a]	17.50[ab]
SEM[A]	0.89		0.55		0.59	

Means within the same column lacking a common letter (a-c) differ ($P < 0.05$).
[A]Standard error of the mean.
[B]TA: 'treat then age'
[C]AT: 'age then treat'

Table 8.
Least squares means (± SEM) of TBARS (TBA value = 21.64).

Treatment	TBA Number
Control	1.58[b]
Blade Tenderized	1.72[b]
High Pressure Processed	1.90[a]
SEM[A]	0.05

Means within the same column lacking a common letter (a-b) differ ($P < 0.05$).
[A]Standard error of the mean.

Table 9.
Least squares means of cooked color (± SEM) lightness (*L**), redness (*a**), and yellowness (*b**) values of top sirloin steaks (n = 30) stratified by treatment.

Treatment	*L**		*a**		*b**		Subjective Degree of Doneness[A]	
	TA[C]	AT[D]	TA[C]	AT[D]	TA[C]	AT[D]	TA[C]	AT[D]
Control	55.11^{ab}	52.63^{c}	10.55^{bc}	11.97^{a}	17.32^{ab}	17.51^{ab}	4.75^{cd}	4.67^{d}
Blade Tenderized	53.00^{c}	53.44^{bc}	10.69^{abc}	11.26^{ab}	17.20^{ab}	18.04^{a}	4.85^{bc}	4.73^{cd}
High Pressure Processed	56.64^{a}	55.24^{a}	9.67^{cd}	9.22^{d}	16.56^{b}	16.89^{ab}	4.96^{ab}	5.06^{a}
SEM[B]	0.62		0.48		0.44		SEM[B] 0.05	

Means within the same column lacking a common letter (a-d) differ ($P < 0.05$).
[A] (1=Very Rare, 2=Rare, 3=Medium Rare, 4=Medium, 5=Well Done, 6=Very Well Done) Evaluated by trained personnel according to the Beef Steak Color Guide for Degrees of Doneness, National Cattlemen's Beef Association.
[B]Standard error of the mean.
[C]TA: 'treat then age'
[D]AT: 'age then treat'

Table 10.
Least squares means (± SEM) of Warner–Bratzler shear (WBS) values in Newtons (N) of 'treat then age' versus 'age then treat'.

Treatment	WBS (N)	
	TA[B]	AT[C]
Control	22.16[c]	24.71[c]
Blade Tenderized	25.00[bc]	21.87[c]
High Pressure Processed	31.97[a]	29.32[ab]
SEM[A]	0.16	0.16

Means within the same column lacking a common letter (a-c) differ ($P < 0.05$).
[A]Standard error of the mean.
[B]TA: 'treat then age'
[C]AT: 'age then treat'

Table 11.
Least squares means of palatability characteristics of beef steaks from top sirloin for consumer (n=122 consumers) evaluation stratified by treatment.

Treatment	Overall Like[A]		Flavor Like[A]		Level of beef flavor[B]		Tenderness like[A]		Level of tenderness[C]		Juiciness like[A]		Level of juiciness[D]	
	TA[F]	AT[G]	TA[F]	AT[G]	TA[F]	AT[G]	TA[F]	AT[G]	TA[F]	AT[G]	TA[F]	AT[G]	TA[F]	AT[G]
Control	4.34^{ab}	3.93^{b}	4.57^{ab}	4.08^{c}	4.56^{a}	4.03^{bc}	4.35^{b}	3.99^{bc}	4.66^{a}	4.12^{cd}	4.66^{abc}	4.24^{bc}	4.94^{a}	4.36^{bc}
Blade Tenderized	4.27^{b}	4.12^{b}	4.94^{a}	4.27^{bc}	4.65^{a}	4.40^{ab}	3.46^{d}	3.63^{cd}	3.65^{d}	3.71^{d}	4.15c	4.55^{abc}	4.34^{c}	4.35^{ab}
High Pressure Processed	4.82^{a}	4.27^{b}	4.60^{ab}	3.98^{c}	4.55^{a}	3.94^{c}	4.99^{a}	4.40^{b}	5.13^{a}	4.48^{b}	5.02a	4.74^{a}	5.04^{a}	4.97^{a}
SEM[E]														

Means within the same column lacking a common letter (a-b) differ ($P < 0.05$).
[A] 10 = Like extremely; 1 = dislike extremely.
[B] 10 = Extremely flavorful or intense; 1 = extremely bland or no flavor.
[C] 10 = Extremely tender; 1 = extremely tough.
[D] 10 = Extremely juicy; 1 = extremely dry.
[E]Standard error of the mean.
[F]TA: 'treat then age'
[G]AT: 'age then treat'