Evaluation Of Exogenous Plant Based Enzymes In A Low-Cost Foodservice Beef Model

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By

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

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and hereby certify that in their opinion it is worthy of acceptance.

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EVALUATION OF EXOGENOUS PLANT BASED ENZYMES IN A LOW-COST FOODSERVICE BEEF MODEL

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ABSTRACT

This study was performed to determine the effects of an exogenous enzyme on the tenderness of beef hanging tenders over a 3-day storage period. The objectives were to evaluate the Slice Shear Force, myofibrillar fragmentation index, level of lipid oxidation, percent cook loss, and sensory panel analysis of treated beef hanging tenders. Thirty-two Choice, whole beef hanging tenders were seperated into 2 individual muscle pillars with the most uniform pillar being randomly assigned to one of four treatments; control, antioxidant, enzyme, and antioxidant+enzyme, with 8 replicates per treatment. The antioxidant treatment consisted of a 10% by weight solution of the Fortium®. The enzyme treatment utilized the Ribeye Tenderizer with Bromelain at 15% pump using a quarter concentration of the manufacturers recommended instructions. The antioxidant+enzyme treatment consisted of a combination of the antioxidant and enzyme treatments. Samples were injected using a single needle injection syringe and placed in a vacuum bag. All samples were stored in a cardboard box and placed in a 4℃ cooler to simulate a storage and transportation environment for a period of 3 days. At the end of the storage period, samples were removed and evaluated for Slice Shear Force, myofibrillar fragmentation index, lipid oxidation, cook loss, and sensory panel evaluation. Results showed a

significant difference among treatments for cook loss percentage (P = 0.0098) with treatments ranking from the highest cook loss percentage to least being antioxidant+enzyme > antioxidant = enzyme > control with means of 33.39, 30.50, 28.18, and 25.69 respectively. Slice Shear Force results showed a significant difference (P = <0.0001) among treatments for Slice Shear Force with antioxidant+enzyme = enzyme < control = antioxidant with mean values of 7.288, 8.417, 18.388, and 19.399 respectively. Anaylsis of data for myofibrillar fragmentation showed a significant difference (P = <0.0001) in the amount of myofibrillar fragmentation between treatment samples with the enzyme and antioxidant+enzyme treatments showing the lowest myofibrillar fragmentation indexes, followed by the control treatment, and then the antioxidant treatment, with mean values of 15.62, 23.21, 47.24, and 115.73 respectively. Results from the sensory panel showed significant differences (P = <0.0001) between treatments for tenderness with panelists results showing a preferred order for tenderness (0 = extremely tough, 15 = extremely tender) being antioxidant+enzyme > enzyme > control > antioxidant, with mean values of 12.8, 11.6, 8.4, and 7.5, respectively. No significant differences (P > 0.05) found between treatments for the attributes of juiciness, flavor, and overall likability. Results for lipid oxidation showed a significant difference (P = 0.0043) between sample treatments with antioxidant = antioxidant+enzyme < enzyme = control and mean values for malonaldehyde recovered of 0.217, 0.259, 0.382, and 0.386, respectively. The data shows that the addition of an exogenous enzyme to beef hanging tenders can increase the perceivable tenderness of beef hanging tenders with little noticeable effect to other sensory eating attributes and can be used in a 3-day storage system.

CHAPTER 1

INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) states that world meat consumption is increasing and it is estimated that by 2030, the consumption of livestock products will be as high as 100 kilograms per person in developed countries (Bruinsma, 2003). During the drought from 2011 to 2012, the cattle industry saw an accelerated decrease in the already shrinking US cattle numbers (Leister et al., 2013), thus presenting a setback to help meet this growing demand for meat products. However, despite high beef prices, consumer demand for beef has remained relatively stable, but there is a large portion of frugal consumers that have found non-ground beef cuts to be priced out of their budget. Thus, there is a need to find alternative ways to provide consumers with whole muscle beef cuts at a more budget friendly price.

Tenderness has been referred to by most customers as the trait that most affects the palatability of a meat product (Huffman et al., 1996; Savell et al., 1989). Tenderness can be described as a trait made up of three primary components: actomysoin effect of myofibrillar proteins, the background effect of connective tissue, and the bulk density of intramuscular fat (Savell & Cross, 1988). Considerable interest exists in the evaluation and development of methods to produce meat with consistent tenderness and to improve the tenderness of tougher red meat cuts, while still maintaining meat quality (Koohmaraire, 1996). Different methods exist that allow producers and packers to manipulate these attributes of tenderness, with the end goal of producing a consistently tender product.

The beef hanging tender has the potential to be a low cost, whole muscle cut for both consumers and the foodservice market. The hanging tender is a two-pillared muscle that weighs approximately 2 to 3-pounds. Due to its constant use as an anchor for the diaphragm to the backbone, the hanging tender has a higher connective tissue content, thus perceivably lacking in tenderness. Exogenous plant based enzymes offer the ability to improve the perceivable tenderness through the proteolytic effect these enzymes have on both myofibrillar proteins and connective tissue (Miller et al., 1995; Sullivan & Calkins, 2010). Currently, when they are traded, the price per pound of beef hanging tenders is on the low end of the spectrum compared to other popular whole muscle beef cuts. This creates room for additional processing to be added through enzyme application to improve tenderness, while remaining at a competitive price point. The objective of this study was to determine the effects of an exogenous enzyme on the tenderness of beef hanging tenders over a 3-day storage period.

CHAPTER 2

LITERATURE REVIEW

NATIONAL BEEF TENDERNESS SURVEY

The National Beef Tenderness Survey has been conducted four times over the last couple decades to provide the beef industry with a benchmark as to where beef products stand in terms of tenderness at a given time point (Morgan et al., 1991; Brooks et al., 2000; Voges et al., 2007; Guelker et al., 2012). Furthermore, these surveys work to provide an image of how beef tenderness has changed over time due to potential industry or environment factors. The original National Beef Tenderness Survey conducted in 1990, came about as results from the National Consumer Retail Beef Study (Savell et al., 1987, 1989) and other studies revealed the importance of how taste, and more specifically tenderness, impacts how consumers make their purchasing decisions for beef products. At this time many meat retailers indicated that they seldom received any complaints or returns of meat for being tough, thus it caused some to question where the tenderness issue fell in the chain of supply (Morgan et al., 1991). Thus, the National Beef Tenderness Survey was first performed to measure the magnitude of beef tenderness and its consumer related issues at the retail level. All of the National Beef Tenderness Surveys pulled retail cuts from retailers in major cities across the United States, with the later surveys attempting to follow the city or region precedents set by the first National Beef Tenderness Survey. All of these studies utilized Warner-Bratzler Shear Force and sensory panels to evaluate the mechanical tenderness of retail cuts, as well as the consumer perceived tenderness and eating experience. Results from the first National Beef Tenderness Survey reported that retail cuts from the chuck

and round had shear force values indicating that these cuts would receive overall tenderness ratings of less than "slightly tender" (Morgan et al., 1991). Furthermore, the first tenderness survey showed that continued work was needed to improve the tenderness of retail cuts from the chuck and round. Results from the second National Beef Tenderness Survey conducted in 1998, showed that the tenderness of retail cuts from the chuck had improved; however, retail cuts from the round still required more attention during processing and preparation to improve tenderness (Brooks et al., 2000). The third National Beef Tenderness Audit in 2006, showed that all retail cuts showed improvement in shear force values compared to previous surveys, which could be a result of increased aging times, longer, slower chill rates, and more programs focused on beef tenderness (Voges et al., 2007). Regardless of this across the board improvement, retail cuts from the round still would require more attention postmortem to ensure acceptable tenderness and the study recommended that decreasing the number of retail cuts that are not sufficiently aged before consumption may help improve tenderness (Voges et al., 2007). The most recent National Beef Tenderness Survey found no major changes in shear force values for beef tenderness when compared to the results from the survey performed by Voges et al. (2007), thus concluding that a plateau may have been reached in beef tenderness due to the numerous programs within the beef industry that focus on tenderness (Guelker et al., 2012).

BEEF MUSCLE PROFILING PROJECT

In the late 1990s, the beef packing industry was in need of a revitalization. During this time, the Cattle Fax organization found that the value of cuts from the chuck and round, unquestionably the largest portions of the beef carcass, had decreased in value 20 to 30% over a five-year period (Redson, 2011). The beef industry soon took notice to this decrease in value

and came together to develop a plan to increase carcass value by finding the best way to utilize the attributes of each muscle. This project performed physical and chemical analyses on more than 5,000 muscles across 142 beef carcasses and compiled the results to create a database of muscle characteristics on muscles from the chuck and round (Von Seggern et al., 2005). The 39 muscles found in the chuck and round were analyzed for objective color, expressible moisture, proximate composition, emulsion capacity, pH, total collagen content, total heme-iron concentration, and Warner-Bratzler Shear Force (Von Seggern et al., 2005). Results from this study, provided insight to packers on which cuts offered the potential for added value (Von Seggern et al., 2005). Packers developed new processing methods to isolate individual muscles from the complex multi-muscled chuck and round into value added cuts that would appeal to consumers (Redson, 2011). The price of these value-added cuts would fall between premium steaks and budget friendly ground beef. The program, with the help of company merchandising and marketing groups, added 13 "Value Cuts" to the market, which include: from the shoulder clod, the Flat Iron, Petite Tender, Petite Tender Medallions and Ranch Steak, from the chuck roll, America's Beef Roast, Boneless Country-Style Beef Chuck Ribs, Delmonico Steak, Denver Cut and Sierra Cut, and from the round, Sirloin Tip Side Steak, Sirloin Tip Center Steak, Western Griller Steak and Western Tip (Redson, 2011). Von Seggern et al. (2005) estimated that new value cuts introduced by the muscle profiling project would increase beef carcass values by $50 to $70 per head. This research initiated a new path of research and product development with the goal to reinvent how consumers see traditional cuts that have been offered to them, while at the same time providing the potential to increase carcass profits.

TENDERNESS

Actomyosin Effect of Myofibrillar Proteins

The actomyosin effect deals with the interactions of muscle proteins, the contractile state of actin and myosin, and the structural integrity of the Z-disk (Aberle et al., 2012). It is reported that disruption of these components in meat structure contributes to overall tenderness (Koohmaraire, 1994). This interaction of myofibrillar proteins encompasses sarcomere length, muscle fiber diameter, and muscle fiber proteolysis. A longer sarcomere or stretching of a sarcomere results in less overlapping of the actin and myosin protein chains, thus causing less resistance to the tooth when biting and is more tender (Wheeler et al., 2002). On the other hand, shorter sarcomeres have a greater amount of overlap between the actin and myosin chains, which causes an increased amount of resistance to the tooth, resulting in decreased tenderness. Furthermore, there is discussion that sarcomere length can also influence tenderness through its ability to affect the interaction of proteases and their substrates (Weaver et al., 2008). The diameter of a muscle fiber can also be utilized to determine tenderness as a larger diameter muscle fiber offers greater bite resistance versus a smaller diameter muscle fiber, thus larger diameter fibers are tougher and less tender compared to smaller diameter muscle fibers. It appears that the main determinant of ultimate tenderness is the extent of proteolysis of key target muscle proteins within muscle fibers (Koohmaraie & Geesink, 2006; Hopkins & Taylor, 2002). During post-mortem aging proteolysis is responsible for ultrastructural changes in skeletal muscle, which leads to the improvement of meat tenderness (Koohmaraie, 1992a, 1992b). The system responsible for proteolysis during post-mortem aging the calpain-calpastatin enzyme system (Koohmaraie, 1996). Dayton et al. (1976) reported that calpain degraded troponin and tropomyosin, but had almost no effect on myosin, actin, and α-actin. Taylor et al. (1995) also

confirmed that the calpain system played a major role in post mortem tenderization by removing Z-disks, but did not degrade α-actin and myosin.

Background Effect of Connective Tissue

The background effect involves the amount and solubility of connective tissue with a muscle. Connective tissue exists in the form of collagen and elastin, but the most abundant form is collagen and is a contributing factor in meat tenderness (Cross et al., 1973). Collagen molecules are bound together through intermolecular crosslinks that help provide structure and strength to muscles. These collagen crosslinks have a higher turnover rate during an animal's youth, but this turnover rate slows as an animal matures. Once this happens, the more soluble crosslinks are replaced with thermally stable and less soluble crosslinks (Aberle et al., 2012). It is the proportion of these mature, permanent crosslinks, rather than the amount of collagen, that influences the collagen related toughness in meat. The proportion of permanent to soluble crosslinks increases with age, thus resulting in tougher, less tender meat being found in older animals versus their younger counterparts. Collagen crosslink degradation and formation is also influenced by animal growth rate, species, breed, and nutrition (McKeith et al., 1998; Purslow, 2005). Post-mortem degradation of collagen and the use of collagenases appear to play a major role in providing the desired tenderness and texture by altering the connective tissue structure (Weston et al., 2002).

Bulk Density Effect of Intramuscular Fat

The bulk density effect of intramuscular fat, also known as the lubricating effect, takes into account the amount and distribution of intramuscular fat, or marbling, within a muscle. The

amount of intramuscular fat is used by many countries, including the United States, as a leading indicator of quality and is the driving force behind their quality grading system for beef carcasses. The function behind the bulk density effect can be viewed by a couple different theories. The bite theory is simply explained by the fact that it is much easier for a consumer to bite, or chew, through fat than muscle fibers, therefore, more fat equals a more tender product. The strain theory looks at the standpoint that fat will stretch and strain the connective tissue that provides strength and structure. This in-turn will weaken the supporting connective tissue making it more tender. The final theory is known as the insurance theory. Its reasoning is that the added intramuscular fat with a cut of meat will allow it to be cooked to a higher degree of doneness and still "insures" a good eating experience as the increased amount of fat provides added juiciness and flavor. Results from a study done by Starkey et al. (2016) show that the main factors which influence Shear Force, a mechanical measurement of tenderness, of Longissimus lumborum muscles were intramuscular fat, sarcomere length, and desmin degradation, but for sensory tenderness, intramuscular fat, ultimate pH, and gender were the main factors. The findings from this study are supported by those from Corbin et al. (2015), where investigators found that in general tenderness, as indicated by both consumer and trained sensory panels, increased with higher fat levels. However, the effect of intramuscular fat on tenderness is somewhat disputed, as according to a study performed by Koohmaraie et al. (2002) sarcomere length, connective tissue content, and proteolysis of myofibrils and associated proteins account for most, if not all, of the explainable variation in tenderness or meat after post-mortem storage. Furthermore, Wheeler et al. (1994) concluded that marbling explained at most only 5% of the variation in beef tenderness.

Valuing Tenderness

Studies performed by Boleman et al. (1997), Miller et al. (2001), Platter et al. (2005), and Wheeler and Koohmaraie (1994) have all looked into determining how consumers perceive tenderness and their willingness to pay for guaranteed tender products. These studies all indicate that consumers do have the ability to detect differences in tenderness between samples. At the same time, they determined that consumers are willing to pay premiums for guaranteed tender products. Lusk et al. (2001) determined that relying on a taste test alone, consumers in a grocery store setting were willing to pay an average premium of $1.84 per pound for a tender versus tough steak. Results from these studies lend support to the decision made by Boleman et al. (1997), that economic incentives could be used by retailers and packers to promote the production, identification, and marketing of tender beef cuts.

METHODS OF EVALUATING TENDERNESS

As tenderness is one of the most important attributes to meat products and due to its potential variability between similar products, researchers need multiple ways to evaluate tenderness to be able to provide the desired parties with an accurate tenderness analysis for a product. Two categories exist for the types of analyses that can be performed on a sample to gain insight on its level of tenderness: direct measures of tenderness and indirect measures of tenderness. Direct measures of tenderness are used to provide feedback on a sample stating whether a sample is tender or not, while results from indirect measures of tenderness are used to predict the potential tenderness of a sample. Indirect measures of tenderness are used to give support to the results of direct measures of tenderness, thus strengthening the final tenderness verdict of a sample.

Direct measures of tenderness include Warner-Bratzler Shear Force (WBSF), Slice Shear Force (SSF), and use of "trained" and "untrained" sensory panels. Warner-Bratzler Shear Force and Slice Shear Force and both mechanical methods for measuring tenderness. Both methods of shear force load bearing blade to forcefully shear through samples and sensors provide a reading for the force it takes to shear the sample. Readings are recorded in either Newtons or kilograms and translate into the amount of force it takes to "bite" through the sample. Both methods only account for the myofibrillar protein interactions in a sample. Samples used for this analysis must be free of fat and connective tissue as these types of tissues can cause inaccurate measurements due to compositional differences compared to myofibrillar proteins. These methods differ in their form of sample, number of replicates needed per sample, and type of blade. WBSF utilizes 6 cores that are a ½ inch thick, taken parallel to the muscle fibers, while SSF uses one 1-centimeter-thick and 5-centimeter-long slice that is cut parallel to the muscle fibers (Shackelford et al., 1999). Cores to be analyzed for WBSF are sheared using a blade with a triangle portion cut out of the middle, whereas SSF samples are sheared using a straight edge blade. Sensory panels can be utilized to gain insight to how consumers rank quality attributes, such as tenderness, of meat products. The two forms of panels used are "trained" and "untrained". The American Meat Science Association (AMSA) sets forth guidelines for conducting sensory panels in the Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat (AMSA, 2015). Due to the benefit of saving time and money and the difficulty in maintaining a well-trained sensory panel, tenderness of cooked meat samples can be assessed much more easily via Warner-Bratzler shear force than trained sensory panel analysis (Shackelford et al., 1995).

Common indirect measures of tenderness include sarcomere length, myofibrillar fragmentation index, hydroxyproline concentration, calpastatin assay, ionic strength assay, fat extraction, microscopic imaging, blots, and muscle fiber typing. Sarcomere length, myofibrillar fragmentation index, calpastatin assay, ionic strength assay, blots, and muscle fiber typing are methods that are used to measure variations in myofibrillar protein interactions. Sarcomere length has been shown by numerous studies to be a factor in tenderness, as a positive correlation exists between improved tenderness and a longer sarcomere length (Wheeler et al., 2002). Measuring the activity or quantity of calpastatin, the inhibitor of calpains, can provide insight to the tenderness of a product. A greater activity or quantity of calpastatin will yield a greater inhibition of the endogenous calpains, thus limiting the proteolysis of myofibrillar proteins, resulting in a less tender product (Dargelos et al., 2007). Hydroxproline concentrations within a sample can be used to calculate the amount of collagen found within a sample. Microscopic imaging offers researchers the ability to visualize the physical disruption of both myofibrillar proteins and connective tissue matrixes. These methods of analysis are best suited to lend supporting data to direct methods of measuring tenderness.

DETERMINATION OF TENDERNESS BY SENSORY PANEL

Sensory panels offer researchers the ability to analyze how consumers respond to certain traits that are important for meat products to be deemed desirable for initial and repeat purchase by consumers. The American Meat Science Association (AMSA) sets forth guidelines for conducting sensory panels in the Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat (AMSA, 2015). Two types of sensory panel exist, “trained” or “untrained”. A “trained” sensory panel is usually educated on what should be

considered desirable and undesirable for a trait of study. Panelists will be trained using standards that allow them to have a basis to compare the tested samples to from memory. An "untrained" sensory panel uses everyday with common knowledge, or user experience, of a type of product, but they do not receive any training prior to participation in a study. "Untrained" panels are also known as consumer panels as they use the opinions of everyday consumers to provide a glimpse of how a product or production method of a product may be received in the market place. Panelists are asked to rank samples on a given scale for desired attributes that ranges from one extreme to the opposite extreme with more moderate scores in between. Tenderness attributes that may be considered when evaluating samples include tenderness, texture, mouthfeel, and overall like. Panelists can also be surveyed to inquire how they value tenderness versus price by asking them to choose a change in dollar value they would be willing to pay for a guaranteed tender product.

EXOGENOUS ENZYMES

Enzymes that are taken from sources outside the body are known as exogenous enzymes. Enzymes such as these originate from plant, bacterial, and fungal sources. Exogenous enzymes have been utilized for centuries to improve tenderness through proteolytic activity (Sullivan & Calkins, 2010). The United States Food and Drug Administration (FDA) recognizes five exogenous enzymes as Generally Recognized as safe (GRAS) to be used in meat products (Sullivan & Calkins, 2010). These exogenous enzymes are papain, bromelain, ficin, *Aspergillus oryzae* protease, and *Bacillus subtilis* protease.

Papain, bromelain, and ficin all originate from plant sources, while Aspergillus oryzae protease and Bacillus subtilis protease are bacterial sources. Papain is produced by collecting

latex from the fruit of the papaya tree (Feiner, 2006). This latex is then dried and purified to create a concentrated source in the form of a powder of liquid, which can then be added to a meat product. Bromelain is found in the in the stump or root of the pineapple plant (Feiner, 2006). The stump or root is peeled and crushed to extract the juice, which is then turned into a powder form (Feiner, 2006). Ficin is extracted from the milky juice of the fig tree (Feiner, 2006). These enzymes have a large spectrum of action in the degradation process of myofibrillar muscles, affecting both myofibrillar proteins and collagen (Miller et al., 1995).

Methods for the application of these exogenous plant based enzymes include dipping, marinating, and injecting. Furthermore, tumbling treated products or vacuum packing can help ensure that the enzyme is uniformly distributed. A antemortem treatment method has also been investigated to use the circulatory system of the animal to help distribute the enzyme throughout the muscles (Buek et al., 1959). An injectable form of papain was patented by Swift & Company to be used in live animals, however it was found to over tenderize the final product and animals treated had an increase in the rate of condemned livers and kidneys due to the filtration function of these organs (Buek et al., 1959). Thus, antemortem injection was not found to be a successful method for improving tenderness through enzyme application. A study performed by Bradley et al. (1987) did review the effect of a commercial antemortem papain injectable called Pro Ten and found that the injectable solution did not cause any detectable liver and kidney damage in treated cattle verses untreated controls, thus concluding that the decision to ban Pro Ten could not be based on the idea that it affected an animal's welfare.

The main risk associated with the use of exogenous enzymes is due to the indiscriminate protein degradation action of these enzymes, a product may become over tenderized if the enzymes are allowed to remain in an active state within the product for too long or if they are

introduced in too great of a concentration (Kang and Rice, 1970; Cronlund and Woychik, 1986). Meat that has been over tenderized is characterized by a mushy texture with no bite resistance (Feiner, 2006). A study performed by Takagi et al. (1992) showed that papain highly degraded myosin, which makes up 60% of myofibrillar protein. Bernholdt (1975) and Rolan et al. (1988) say that bromelain and ficin both have properties similar to papain and may also excessively degrade myofibrillar protein.

Recent developments in the availability and function of highly specific and active food grade enzymes has allowed the application of enzyme technology to be a viable option to assist beef processors in meeting consumer expectations for high quality and consistent product (Pietrasik and Shand, 2011). Shin et al. (2008) found that enzyme treated beef samples had significant increases in tenderness compared to non-treated control samples, with the enzyme treated samples having lower WBSF values. Similar results of the ability of exogenous plant based enzymes on their ability to increase tenderness in beef samples have shown by Qihe et al. (2006), Sullivan & Calkins (2010), Ha et al. (2012) and Zhao et al. (2012).

MYOFIBRILLAR FRAGMENTATION INDEX

Myofibrillar fragmentation index measures the amount of myofibrillar protein degradation within a sample by utilizing the amount of residual myofibrillar protein capture in a screen, which is then inserted into a formula to yield an index value. This value can be used to correlate a sample being more or less tender, with a lower myofibrillar fragmentation index concluding that a greater amount of myofibrillar protein degradation occurred, which could lead to a sample having greater tenderness. A positive correlation between the rate of myofibrillar fragmentation and the tenderness of the meat is well known (Dosler et al., 2007). Results from a

study performed by Culler et al. (2006) showed that myofibril fragmentation index accounted for more than 50% of the variation in loin steak tenderness. Furthermore, it was found that myofibril fragmentation was a more important effector of tenderness in loin steaks than collagen solubility or sarcomere length (Culler et al., 2006). The steaks used in Culler et al. (2006) varied widely in marbling degree and maturity, thus indicating that myofibrillar fragmentation index should be an ideal predictor of loin steak tenderness.

LIPID OXIDATION

Lipids are primarily responsible for both desirable and undesirable flavors and aromas in meat (Cheng, 2016). Oxidative damage to lipids occurs in the living animal because of an imbalance between the production of reactive oxygen species and the animal's cellular defense mechanisms (Morrissey et al., 1998). This imbalance may be caused by a high intake of oxidized lipids or poly-unsaturated fatty acids, or due to a low intake of nutrients that act as antioxidants. Pro-oxidants are introduced into cascade known as autoxidation, which occurs in a three-step process consisting of initiation, propagation, and termination (Aberle et al., 2012). Initiation occurs when oxygen is exposed to the product and cleaves the fatty acid double bonds to form free radicals (Aberle et al., 2012). As initiation occurs, oxygen is produced or introduced from the surrounding atmosphere and will continue to attack unsaturated fatty acids, producing more free radicals (Aberle et al., 2012). This process is known as the propagation step, which continues as long as oxygen is available to cleave the double bonds (Aberle et al., 2012). Termination will occur naturally as non-reactive products are formed and the propagation can no longer occur (Aberle et al., 2012). An increase in lipid oxidation can cause a decrease in flavor, color, texture, and nutritional value in meat (Buckley et al., 1989). Other detrimental effects

caused by lipid oxidation that have been found in meat include decrease in shelf life, increase in off-flavor, changes in functional, and sensory characteristics. Lipid oxidation can be accelerated or slowed during the handling, processing, storage, and cooking of meat products.

Effect of Antioxidants in meat products

In recent years, pressure to reduce artificial additive use in foods has led to attempts to increase meat stability by dietary strategies (Morrissey et al., 1998). Supplementation of animal diets with vitamin E, ascorbic acid, carotenoids, and withdrawal of trace mineral supplements have been used to try to reduce the oxidation potential of meat products. Mielnik et al. (2003) found that direct addition of mechanically deboned turkey meat with antioxidants could be an alternative method to prevent oxidative degradation of the meat during frozen storage when product cannot be placed in an anaerobic environment, such as vacuum package. A study performed by Ahn et al. (2007) found that grape seed extract and pine bark extract are promising additives for maintaining the quality and safety of cooked beef.

DETERMINATION OF LIPID OXIDATION IN MEAT BY TBARS ANALYSIS

The most common analysis to measure lipid oxidation in meat is the Thiobarbituric Acid Reactive Substances (TBARS) analysis. This analysis is used to recover malonadehyde, which is a by-product of lipid oxidation. Malonaldehyde recovery is often used to establish a measure of lipid oxidation as the test for it is very sensitive and it can be detected early in the oxidation process (Cesa, 2004). After a sample has been distilled down to the TBARS, the sample is analyzed using a spectrophotometer at a wavelength of 538 nanometers (Tarladgis et al., 1960). A higher spectrophotometer reading shows a greater amount of lipid oxidation.

CHAPTER 3

EVALUATION OF EXOGENOUS PLANT BASED ENZYMES IN A LOW-COST FOODSERVICE BEEF MODEL

ABSTRACT

Choice beef hanging tenders (n=32) were randomly assigned to 1 of 4 treatments; control, antioxidant, enzyme (Bromelain), and antioxidant+enzyme. The enzyme treatment consisted of a 15% pump of a quarter concentration of the manufacturers labeled recommendation. Samples were injected, placed in a vacuum bag, and stored at 4℃ for 3 days. Samples were evaluated for Slice Shear Force (SSF), Myofibrillar Fragmentation Index (MFI), lipid oxidation, cook loss, and sensory panel evaluation. All treatment groups had a greater amount of cook loss ($P = 0.0098$) over the control group with the antioxidant+enzyme group yielding the highest percent cook loss. Samples from the enzyme treatments yielded lower SSF values ($P = <0.0001$) compared to samples from treatments not containing the enzyme. Myofibrillar fragmentation was the greatest in treatment groups containing the enzyme ($P = <0.0001$) over the control and antioxidant groups. Sensory panelists indicated the enzyme treated groups yielded samples with more desirable tenderness scores compared to samples that were not treated with the enzyme ($P = <0.0001$). Samples from treatment groups containing the antioxidant showed a decreased rate of lipid oxidation ($P = 0.0043$) over samples that were not treated with the antioxidant. This indicates beef hanging tenders treated with bromelain can increase the tenderness of hanging tenders with little effect on other quality attributes in a 3-day storage system.

INTRODUCTION

Tenderness has been referred to by most customers as the trait that most affects the palatability of a meat product (Huffman et al., 1996; Savell et al., 1989). Tenderness can be described as a trait made up of three primary components: actomysoin effect of myofibrillar proteins, the background effect of connective tissue, and the bulk density of intramuscular fat (Savell & Cross, 1988). Considerable interest exists in the evaluation and development of methods to produce meat with consistent tenderness and to improve the tenderness of tougher red meat cuts, while still maintaining meat quality (Koohmaraire, 1996). Different methods exist that allow producers and packers to manipulate these attributes of tenderness, with the end goal of producing a consistently tender product.

The drought from 2011 to 2012, the cattle industry saw an accelerated decrease in the already shrinking US cattle numbers (Leister et al., 2013), thus presenting a setback to help meet this growing demand for meat products. However, despite high beef prices consumer demand for beef has remained relatively stable, but there is a large portion of frugal consumers that have found non-ground beef cuts to be priced out of their budget. Thus, there is a need to find alternative ways to provide consumers with whole muscle beef cuts at a more budget friendly price. The beef hanging tender is a whole muscle cut that has the potential to be a low cost, whole muscle cut for both consumers and the foodservice market. The hanging tender is a two-pillared muscle, that weighs approximately 2 to 3-pounds. Due to its constant use as an anchor for the diaphragm to the backbone, the hanging tender has a higher connective tissue content, thus perceivably lacking in tenderness. Exogenous plant based enzymes offer the ability to improve the perceivable tenderness through the proteolytic effect these enzymes have on both myofibrillar proteins and connective tissue (Miller et al., 1995; Sullivan & Calkins, 2010).

Currently, when they are traded, the price per pound of beef hanging tenders is on the low end of the spectrum compared to other popular whole muscle beef cuts, thus there exists room for additional processing to be added through enzyme application to improve tenderness, while remaining at a competitive price point. The objective of this study was to determine the effects of an exogenous enzyme on the tenderness of beef hanging tenders over a 3-day storage period.

MATERIALS AND METHODS

Sample Preparation

Thirty-two whole beef hanging tenders from Choice, USDA inspected steers and heifers were used to prepare samples for this study. All hanging tenders were trimmed of external fat and then separated into the two muscle pillars that make up each hanging tender by removing the layer of connective tissue that divides the two pillars of muscle. The separated hanging tenders were then sorted to assure that there would be little variation in size and thickness amongst the samples. After the most uniform pieces were chosen from each of the thirty-two hanging tenders, the thirty-two single pillar hanging tenders were randomly assigned to one of four treatments: control, antioxidant, enzyme, and antioxidant+enzyme. Each treatment contained eight replicates. The enzyme solution that was used in this study was Ribeye Tenderizer with Bromelain and the food grade antioxidant solution was Fortium®. Both products were provided by Excalibur Seasonings and Marinades. Hanging tender samples assigned to the control treatment received no added ingredients or solutions. The antioxidant treatment consisted of a 10% by weight solution of the Fortium®. The enzyme treatment utilized the Ribeye Tenderizer with Bromelain at 15% pump using a quarter concentration of the manufacturers recommended instructions. The antioxidant+enzyme treatment consisted of a 10% by weight solution of the

Fortium® combined with the Ribeye Tenderizer with Bromelain at 15% pump using a quarter concentration of the manufacturer's instructions. The use of a quarter concentration of the manufacturers recommended amount of enzyme was determined for this study through pre-study trials, during which it was found that a quarter concentration provided an optimum time/texture relationship. Hanging tenders assigned to the antioxidant, enzyme, and antioxidant+enzyme treatments were weighed and then injected with the proper amount of their treatment solution as determined by their weight. Samples were injected using a single needle injection syringe and were injected randomly throughout the muscle. Samples were injected over a clean container to capture any of the treatment solution that dripped from the hanging tender. Any excess treatment solution captured in the container would be placed in the package with the associated hanging tender. All samples were placed in a clear, non-permeable vacuum seal bag and then sealed to remove all oxygen. All samples were then placed in a sealed cardboard box and placed in a 4C cooler to simulate a storage and transportation environment. Samples were kept in refrigerated storage for 3 days before removal for testing.

Determination of Cook Loss

Hanging tenders from all treatments were removed from refrigerated storage and allowed to rest at room temperature for fifteen minutes prior to cooking. A 2-inch-long portion was cut from the center of each hanging tender sample and then weighed to provide a raw weight. Thermocouples where then placed in samples prior to cooking to allow for temperature monitoring throughout the cooking process. All hanging tenders were seared on a flat top grill for one minute per side (top and bottom) for a total time of two minutes. After searing, hanging tenders were transferred to an oven pan and placed in a gas oven to finish cooking to an internal

temperature of 70C. Once samples had reached an internal temperature of 70°C, the hanging tenders were removed from the oven and immediately weighed to determine a final cooked weight for each sample. Thermocouples were removed before weighing. The cook loss percentage was determined for each sample using the formula below.

Percent Cook Loss = (Cooked Weight\Raw Weight)*100

Determination of Slice Shear Force Value

Hanging tenders from all treatments were removed from refrigerated storage and allowed to rest at room temperature for fifteen minutes prior to cooking. Thermocouples where placed in samples prior to cooking to allow for temperature monitoring throughout the cooking process. All hanging tenders were seared on a flat top grill for one minute per side (top and bottom) for a total time of two minutes. After searing, hanging tenders were transferred to oven pan and placed in a gas oven to finish cooking to an internal temperature of 70℃. Once samples had reached an internal temperature of 70°C, the hanging tenders were removed from the oven and wrapped in plastic wrap, then samples were allowed to cool for 24 hours at 4°C before testing was conducted. A modified method for Slice Shear Force (SSF) used in Shackelford et al. (1999) was used to measure mechanical tenderness. A 1-cm-thick, 3.81-cm long slice was removed from the center portion of each sample hanging tender parallel to the muscle fibers. Each slice was sheared once with a flat, blunted blade (Shackelford et al., 1999) attached to a United STM Smart-1 Test System SSTM-500 (United Calibration Corp., Huntington Beach, CA). Settings for SSF test speed was 500 mm/min.

Determination of Myofibrillar Fragmentation Index

Myofibrillar Fragmentation Index was determined using a modified method described by Sams et al. (1991). Circular 250 μm nylon screens were cut to fit the 550 ml Buchner funnels used for this analysis and place in a drying oven at 100˚C for 24 hours prior to analysis. A homogenization solution was freshly prepared prior to analysis and was made up of 0.25 M sucrose, 0.002 M potassium chloride, 0.005 M sodium iodoacetate, and mixed to a final pH of 7.0 using potassium hydroxide and acetic acid. Samples were analyzed in duplicate. 4 to 5 grams of sample was recorded and cut into 2 x 2 mm cubes. Samples were transferred to a 50 ml centrifuge tube and 30 ml of homogenization solution was added, followed by homogenization using a Polytron tissue homogenizer for 30 seconds. A Buchner funnel was placed on top of a vacuum aspirator flask and a weighed nylon screen was placed in the funnel. Screens were weighed prior to vacuum aspirating and each screen weight was recorded with a corresponding sample. A vacuum was applied to the flask and the homogenized sample was pour into the funnel. Next, 20 ml of homogenization solution was used to rinse the centrifuge tube and then poured into the funnel giving a total of 50 ml of homogenization solution used per sample. The screen containing the captured homogenate was aspirated to dryness and then removed and placed on a piece of coarse filter paper, in which the screen and residue was enclosed by folding the ends of the filter paper together and stapling closed. The filter paper containing the screen and residue were placed in a drying oven overnight or 18 hours at 100˚C. Samples were then removed from the drying oven and the screens with residue were removed from the filter paper and weighed and recorded. The Myofibrillar Fragmentation Index was calculated using recorded weights for the raw sample, screen, and screen/residue in the formula below.

Myofibrillar Fragmentation Index = ((1000)*(residue weight/original sample weight))

A lower value for Myofibrillar Fragmentation Index indicates a greater amount of fragmentation within a sample.

Sensory Panel Analysis

A group of 8 consumers were gathered to form an untrained sensory plan. Consumers were eligible to participate if they were 18 years of age or older and were regular beef consumers. Panelists evaluated samples using a 150 mm line scale for the attributes of tenderness (0 = extremely tough, 15 = extremely tender), flavor (0 = extremely intense, 15 = extremely bland), juiciness (0 = extremely juicy, 15 = extremely dry), and overall liking (0 = extremely dislike, 15 = extremely like). Hanging tenders from all treatments were removed from refrigerated storage and allowed to rest at room temperature for fifteen minutes prior to cooking. Thermocouples where placed in samples prior to cooking to allow for temperature monitoring throughout the cooking process. All hanging tenders were seared on a flat top grill for one minute per side (top and bottom) for a total time of two minutes. After searing, hanging tenders were transferred to an oven pan and placed in a gas oven to finish cooking to an internal temperature of 70℃. Once samples had reached an internal temperature of 70°C the hanging tenders were removed from the oven and cut into approximately 1-centimeter by 1-centimeter cube samples. Each panelist received two plates with four samples per plate. Of the four samples on each plate, one came from each of the four treatments. Every replicate of each treatment was sampled twice by a different panelist and samples from replicates were randomly chosen per treatment. Panelists were provided water and crackers to cleanse their palates between samples.

Upon completion of their sample evaluations, panelists were asked to complete a survey about how much they would be willing to pay, over or under a base price, for a guaranteed tender product.

Determination of Lipid Oxidation

Lipid oxidation was measured using the method described by Tarladgis et al. (1960) with modifications from Fernando et al. (2003). Duplicate 5 gram samples of each hanging tender were blended for 2 minutes with 25 ml of distilled water using a Hamilton Beach hand blender. Following homogenization, the cup containing the sample was rinsed with an additional 25 ml of distilled water and transferred into a Kjeldahl flask. Next, 2.5 ml of HCl was added to the flask to balance the pH between 1.5 – 1.6 along with two drops of antifoam solution. Then, 25 ml of each sample was distilled through a water-cooled distillation apparatus. Following distillation, 5 ml of each sample was pipetted into a glass tube followed by 5 ml of TBA (0.02 M thiobarbituric acid in 90% acetic acid) reagent. Samples were then placed in a boiling water bath for 35 minutes and immediately transferred to an ice bath for 10 minutes to stop the chemical reaction. Color absorbance was measured at 538 nm using a Spectronic 20 (Bausch & Lomb, Rochester, NY) spectrophotometer. Values of each reading were recorded and averaged for further calculation. Lipid oxidation was expressed in mg/kg of malonaldehyde recovered and calculated using the recorded spectrophotometer averages and the give equation below.

mg/kg of malonaldehyde = 7.8 * spectrophotometer reading

Statistical Analysis

Statistical analysis for percent cook loss, Slice Shear Force, Myofibrillar Fragmentation Index, sensory evaluation, and lipid oxidation were performed using the GLM procedure of SAS (SAS Institute, Inc., Cary, NC, U.S.A.) to obtain LS means and SE estimates. $P < 0.05$ was used to determine significance for all tests. The model included the fixed effects of treatment. Data from panelists surveys for price of guaranteed tender was analyzed using Microsoft Excel through the creation of a graphical representation of the price data.

PRE-STUDY TRIALS TO DETERMINE PROPER ENZYME APPLICATION

A series of pre-study trials were conducted to establish the amount of commercial tenderizer to apply to the hanging tenders, as well as the best method for application of the tenderizing solution. Furthermore, different cooking methods were used over the trials to find the most optimum cooking method for this whole muscle product.

Trial 1

The initial trial compared hanging tenders that had been treated with a full concentration tenderizing solution, per manufacturer's instructions and half concentration of the tenderizing solution. Samples were individually placed into vacuum bags with 15% pump worth of each tenderizing solution poured in to the bag. The bags were then sealed allowing the hanging tenders to marinade in one of the two solutions. All hanging tenders were allowed to marinade for 3 days. After marinating, samples were cooked in a moist environment using crockpots. Samples were cooked to 71℃, at which time they were removed from the crockpots. Samples were then to be placed in refrigeration for 24 hours and then analyzed for tenderness by Slice

Shear Force, however samples from both treatments experienced too high of levels of myofibrillar proteolysis and they lacked the structural integrity to undergo testing. Results from trial 1 lead to the use of different preparation methods and cooking methods of hanging tender samples in trial 2.

Trial 2

The second trial experiment compared an untreated control group of hanging tenders to a group that had been treated with the same half concentration tenderizing solution, per manufacturer's instructions, as seen in trial 1. This time, hanging tenders in the treated group were injected with the solution at 15% pump rather than just allowing the solution to be poured over the hanging tenders. Samples were still individually placed into vacuum bags and store in refrigerated conditions for 3 days. Following the 3-day storage period, samples were cooked on a flat top griddle to a final internal temperature of 71°C. Samples were then removed from heat and placed in refrigeration for 24 hours and analyzed for tenderness by Slice Shear Force. Injecting the tenderizing solution allowed for more uniform tenderization and kept the hanging tenders intact; however, the enzyme treatment still yielded less than desirable slices for shearing and there was a detectable mushiness to the texture of the treated hanging tenders. Slices from the treated tenders were able to be sheared and yielded lower Slice Shear Force values compared to the control group. Trial 2 results indicated that a lower concentration of enzyme solution needed to be tested.

Trial 3

The third trial experiment compared three treatment groups: an untreated control group, a group that had been treated with the same half concentration tenderizing solution per manufacturer's instructions as seen in trial 2, and a group treated with a quarter concentration per manufacturer's instructions. All hanging tenders treated with an enzyme solution were injected with their treatment solution at 15% pump. Samples were still individually placed into vacuum bags and stored in refrigerated conditions for 3 days. Following the three-day storage period, samples were cooked on a flat top griddle to a final internal temperature of 71°C. Samples were then removed from heat and placed in refrigeration for 24 hours and then analyzed for tenderness by Slice Shear Force. Injecting the tenderizing solution still showed a more uniform tenderization effect in all groups of treated hanging tenders and the half concentration treatment yielded similar results to those found in the second trial experiment. Slices were obtained from all three groups and were successfully sheared. Both enzyme treatments yielded hanging tenders with lower Slice Shear Force values compared to the control group. However, it was found that the Slice Shear Force values from the quarter concentration treatment were extremely close in value to shear force values from the half concentration treatment. Hanging tenders from the quarter concentration treatment also lacked the crumbly texture found on the outer surface of hanging tenders from the half concentration treatment group. Results from this trial indicated that a quarter concentration of the commercial enzyme treatment can successfully produce noticeable differences in mechanical tenderness, while still maintaining structural integrity.

RESULTS AND DISCUSSION

Percent Cook Loss

Results for the percent of cook loss from samples can be found in Table 1. Results showed a significant difference among treatments for cook loss percentage ($P = 0.0098$) with treatments ranking from the highest cook loss percentage to least being antioxidant+enzyme > antioxidant > enzyme > control with means of 33.39, 30.50, 28.18, and 25.69 respectively. A significant difference was found between all treatments, except between the antioxidant and enzyme treatments. These results are to be expected for the most part as increased proteolysis caused by the enzyme found in the enzyme treatments, which should result in greater water loss due to degradation of the protein matrix that holds water through physical binding and ionic interactions. Shin et al. (2008) showed similar results for percent cook loss with enzyme treated beef samples having a greater percentage of cook loss verses a non-treated control.

Slice Shear Force

Results for Slice Shear Force can be found in Table 1. Analysis showed a significant difference ($P = <0.0001$) among treatments for Slice Shear Force with antioxidant+enzyme < enzyme < control < antioxidant with mean values of 7.288, 8.417, 18.388, and 19.399 respectively. The results from this test show that the treatments that included the commercial enzyme had significantly lower and more desirable Slice Shear Force values. These lower values indicate that the enzyme containing treatments should yield a more tender final product when compared to products not treated with the enzyme solution. There was no significant difference ($P > 0.05$) shown between the antioxidant+enzyme and enzyme treatments, as well as

between the control and antioxidant treatments, thus indicating that the addition or exclusion of the antioxidant had no effect on Slice Shear Force determined tenderness.

Myofibrillar Fragmentation Index

The results for myofibrillar fragmentation index are shown in Table 1. Anaylsis of data shows a significant difference ($P = <0.0001$) in the amount of myofibrillar fragmentation between treatment samples. The enzyme and antioxidant+enzyme treatment showed the lowest myofibrillar fragmentation indexes, followed by the control treatment and then the antioxidant treatment with mean values of 15.62, 23.21, 47.24, and 115.73 respectively. A lower myofibrillar fragmentation index indicates a greater amount of myofibrillar fragmentation. While a value difference existed between the enzyme and antioxidant+enzyme treatments, there was no significant difference ($P > 0.05$) between these treatments. Nonetheless, the results show the enzyme treatments yielded a greater degree of myofibrillar fragmentation over the control and antioxidant treatments. A greater amount of myofibrillar fragmentation in beef samples treated with bromelain verses a non-treated control was also see in a study performed by Zhao et al. (2012). However, the control treatment did still display far greater levels of myofibrillar fragmentation over the antioxidant treatment. It was no surprise to find that the enzyme treatments showed greater levels of myofibillar fragmentation, as it is the function of the enzyme used in these treatments to disrupt and breakdown myofibrils. While it is not fully understood why there is such a large difference in value between the control and antioxidant treatments, however this difference may be the result of the antioxidant reducing the proteolytic ability of the endogenous calpain system. This could be caused by the antioxidant blocking the release of calcium that is needed to drive the calpain system.

Sensory Panel Analysis

Table 2 shows the results for tenderness, juiciness, flavor, and overall likeability scores. Significant differences ($P = <0.0001$) were found between treatments for the attribute of tenderness, with panelists results showing a preferred order for tenderness (0 = extremely tough, 15 = extremely tender) being antioxidant+enzyme = enzyme > control = antioxidant with mean values of 12.8, 11.6, 8.4, and 7.5 respectively. Results from the sensory panel for tenderness reveal that consumer panelists were able to pick up on the improved tenderness found in treatments prepared with the enzyme solution. Both Qihe et al. (2006) and Sullivan and Calkins (2010) found similar results with beef samples treated with exogenous enzyme solutions yielding more desirable tenderness scores compared to the non-treated control. There were no significant differences ($P > 0.05$) found between treatments for the attributes of juiciness, flavor, and overall likability. A lack of significant difference among treatments for overall likability can signify that the treatments containing the enzyme tenderizing solution were not over tenderized to the extent that panelists would experience a mushy or undesirable texture, while at the same time not producing any significant off-flavors or decrease in juiciness. The results for juiciness vary from what would be expected as it would be expected that muscles undergoing an increased rate of proteolysis should have increased purge, thus resulting in a less juicy eating experience. When looking back at the results for the percent cook loss the control treatment showed significantly less cook loss as to be expected, thus this should correlate to a potentially juicier product. However, panelists disagree with this, which is most likely due to different saliva production rates by panelists, or even a mental perception of similar juiciness due to less bite resistance found in the enzyme solution treatments. Sullivan and Calkins (2010) also found no significant difference in off-flavor between enzyme treated samples and the control, thus indicating that the

exogenous enzyme preparations did not give samples an undesirable flavor to consumers.

Lipid Oxidation

Results for lipid oxidation as indicated by levels of malonaldehyde recovered can be found in Table 1. A significant difference (P = 0.0043) was found between sample treatments. Mean values and their corresponding treatments ranging from lowest amount of malonaldehyde recovered to the most were 0.217 for the antioxidant treatment, 0.259 for antioxidant+enzyme, 0.382 for the enzyme treatment, and 0.386 for the control, respectively. The significant difference (P = 0.0043) between treatments was found between the treatments that contained the antioxidant, the antioxidant and antioxidant+enzyme treatments, and the treatments that did not contain the antioxidant, the enzyme and control treatments. No significant difference existed between the antioxidant treatment and the antioxidant+enzyme treatments, as well as, between the enzyme treatment and the control. Results for this test were to be expected as the antioxidant should reduce the rate of lipid oxidation over time, thus increasing shelf life.

Consumer Valuation of Guaranteed Tender

Results from consumer surveys for how they value guaranteed tender products can be found in Figure 1. Panelists responses ranged from $0.20 per pound more to >$1.00 per pound more for a guaranteed tender steak. Furthermore, panelists' responses from the guaranteed tender surveys showed that the consumer panelists would be willing to pay an average of $0.55 per pound more for a guaranteed tender steak. When comparing the average premium of $0.55 per pound back to the $5.50 per pound value of the example cut provided to the consumers, this tells us that these consumers would be willing to pay an average of 10 percent more per pound

for a guaranteed tender beef product. Results from the consumer survey can be supported by studies performed by Wheeler & Koohmaraie (1994), Boleman et al. (1997), Miller et al. (2001), and Platter et al. (2005), all of which determined that consumers are willing to pay premiums for guaranteed tender products.

CONCLUSION

The addition of an exogenous enzyme to beef hanging tenders can increase the perceivable tenderness of beef hanging tenders and can be successfully adapted to a 3-day storage system. Not only did samples treated with a commercial tenderizing solution containing bromelain show increased levels of myofibrillar fragmentation and improved tenderness through mechanical methods of measurement, but panelists from a non-trained consumer panel were also able to perceive the improved tenderness found in the enzyme treated samples. Furthermore, the addition of a food grade antioxidant can help slow the rate lipid oxidation, thus aiding in increasing the shelf life, while at the same time not causing any significant detriment to the functionality of the enzyme tenderizing solution. The application of an exogenous plant based enzyme in this study could be utilized with other low cost beef cuts, thus increasing carcass utilization and carcass value. Further study is needed to increase the storage life of enzyme injected products to allow for longer storage time without over tenderization, therefore providing more cushion for distribution schedules and giving more flexibility in preparation time.

Table 1. Measurement of properties of meat quality.

	Control	Antioxidant	Enzyme	A+E[2]	SEM	p-value
Cook Loss %[1]	25.69[c]	30.50[ab]	28.18[ab]	33.39[a]	1.53	0.0098
Slice Shear Force[1]	18.39[a]	19.40[a]	8.42[b]	7.29[b]	1.89	<0.0001
Myofibrillar Fragmentation Index[1]	47.24[b]	115.73[a]	15.62[c]	23.21[c]	11.93	<0.0001
Lipid Oxidation (TBARS)[1,3]	0.386[a]	0.217[b]	0.382[a]	0.259[b]	0.061	0.0043

[1]Means within the same row followed by a different letter differ significantly ($p<0.05$)
[2]Anitoxidant+Enzyme
[3]Thiobarbutaric Acid Reactive Substances

Table 2. Consumer sensory panel evaluation of quality attributes.

	Treatment					
Sensory Attribute[1]	Control	Antioxidant	Enzyme	A+E[2]	SEM	p-value
Tenderness	8.4[b]	7.5[b]	11.6[a]	12.8[a]	0.587	<0.0001
Juiciness	5.0	6.6	7.5	6.5	0.805	0.2042
Flavor	6.4	6.3	5.9	5.5	0.805	0.8541
Overall Likability	9.2	8.0	9.5	9.7	0.716	0.3762

[1]Means within the same row followed by a different letter differ significantly ($p<0.05$)
[2]Anitoxidant+Enzyme

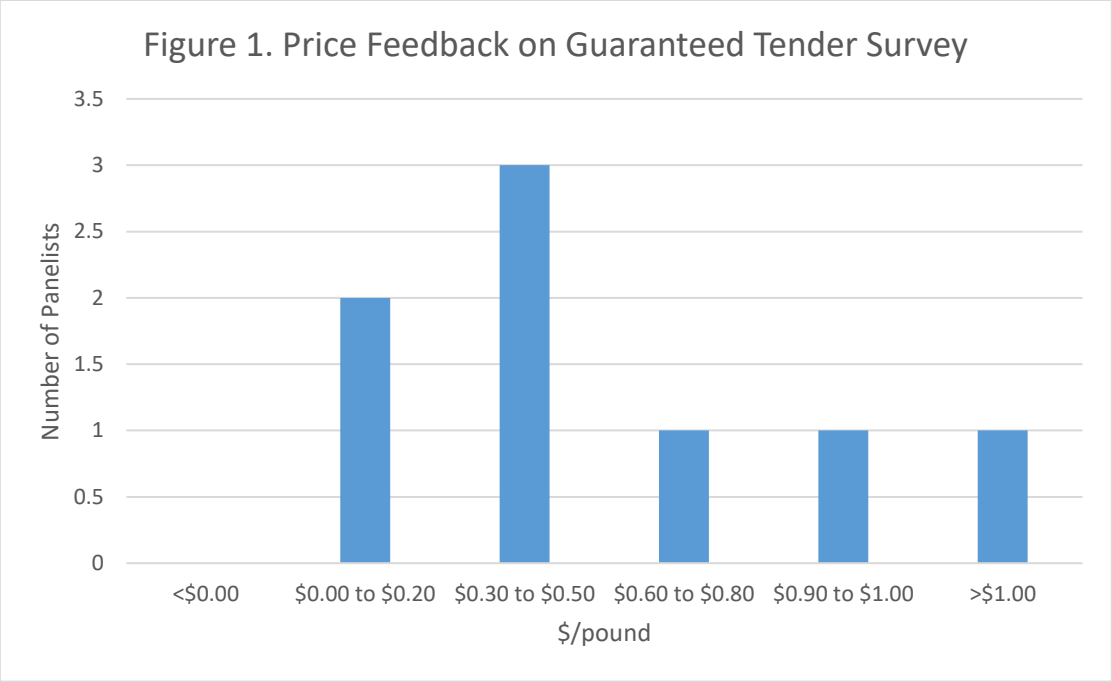
Figure 1. Price Feedback on Guaranteed Tender Survey
Number of Panelists
3.5
3
2.5
2
1.5
1
0.5
0
<$0.00
$0.00 to $0.20
$0.30 to $0.50
$0.60 to $0.80
$0.90 to $1.00
>$1.00
$/pound

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APPENDIX A: MATERIALS AND METHODS

A.1 Myofibrillar Fragmentation Index Determination

Myofibrillar Fragmentation Index was determined using a modified method described by Sams et al. (1991).

Sams, A. R., Birkhold, S. G., and Mills, K. A. (1991). Fragmentation and tenderness in breast muscle from broiler carcasses treated with electrical stimulation and high-temperature conditioning. *Poultry Science*, 70, 1430-1433.

Homogenization Solution:

0.25 M sucrose (FW-342.30)

0.002 M potassium chloride (FW-74.55)

0.005 M sodium iodoacetate (MW-207.93)

Final pH of 7.0 using potassium hydroxide and acetic acid.

Procedure:

1. Cut screens to match size of filter paper. Label and dry screens for 24 hours at 100 C.
2. Weigh screen and record screen weight.
3. Place Buchner funnel onto vacuum flask.
4. Place screen into Buchner funnel.
5. Cut frozen meat into 2x2 mm cube and weigh 4-5 grams of meat. Record weight of meat.
6. Place sample into centrifuge tube with 30 mL of the homogenization solution.
7. Homogenize sample for 30 seconds using the Polytron tissue homogenizer.
8. Pour homogenized muscle solution into Buchner funnel, rinse the centrifuge tube with an additional 20 mL of the homogenization solution for a total of 50 mL.
9. Vacuum aspirate the homogenate to visible dryness.
10. Place screen and residue onto a piece of coarse flow filter paper and stable close. Do not

staple through the screen.

11. Place the sample in a drying oven overnight, or approximately 18 hours, at 100 C.
12. Remove samples form the drying oven and place the samples in a decicator to cool for at least 20 minutes prior to weighing samples.
13. Carefully remove screen and residue from the filter paper and weigh the dried screen and residue and record the weight.

Myofibrillar Fragmentation Index = ((1000)*(residue weight/original sample weight))

A.2 Lipid Oxidation (Thiobarbituric Acid Reactive Substances)

Lipid oxidation was measured using the method described by Tarladgis et al. (1960) with modifications from Fernando et al. (2003).

Tarladgis, B. G., Watts, B. M., Younthan, M. T., and Dugan JR., L. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists Society*, 37, 44-48.

Fernando, L. N., Berg, E. P., and Grun, I. U. (2003). Quantitation of hexanal by automated SPME for studying dietary influences on the oxidation of pork. *Journal of Food Composition and Analysis*, 16, 179-188.

Procedure:

1. Duplicate 5 gram samples of each hanging tender were blended for 2 minutes with 25 ml of distilled water using a Hamilton Beach hand blender.
2. Following homogenization, the cup containing the sample was rinsed with an additional 25 ml of distilled water and transferred into a Kjeldahl flask.
3. 2.5 ml of HCl was added to the flask to balance the pH between 1.5 – 1.6 along with two drops of antifoam solution.
4. 25 ml of each sample was distilled through a water-cooled distillation apparatus.
5. Following distillation, 5 ml of each sample was pipetted into a glass tube followed by 5 ml of TBA (0.02 M thiobarbituric acid in 90% acetic acid) reagent.
6. Samples were then placed in a boiling water bath for 35 minutes and then immediately transferred to an ice bath for 10 minutes to stop the chemical reaction.
7. Color absorbance was measured at 538 nm using a Spectronic 20 (Bausch & Lomb, Rochester, NY) spectrophotometer.

8. Values of each reading were recorded and averaged for further calculation.

mg/kg of malonaldehyde = 7.8 * spectrophotometer reading

APPENDIX B: PANELIST BALLOTS

SENSORY BALLOT-FILL OUT THE FOLLOWING INFORMATION BY PLACING AN "X" ALONG THE LINE SCALE.

1. Indicate by placing a mark in the box your **LEVEL OF THE TENDERNESS** of this sample.

Extremely Tough — Extremely Tender

2. Indicate by placing a mark in the box how you feel about the **LEVEL OF THE JUICENESS** of this sample.

Extremely Juicy — Extremely Dry

3. Indicate by placing a mark in the box how you feel about the **LEVEL OF THE BEEF FLAVOR** of this sample.

Extremely Intense — Extremely Bland

4. Indicate by placing a mark in the box how you feel about the **OVERALL LIKE/DISLIKE** of this sample.

Extremely Dislike — Extremely Like

Beef Steak Questionnaire

1. If $5.50 is the average price per pound of a beef top sirloin steak, what is the maximum price difference you would be willing to pay for beef labeled as guaranteed tender?

_____ > $1.00/lb (more)
_____ $1.00/lb
_____ $0.90/lb
_____ $0.80/lb
_____ $0.70/lb
_____ $0.60/lb
_____ $0.50/lb
_____ $0.40/lb
_____ $0.30/lb
_____ $0.20/lb
_____ $0.10/lb
_____ $0.00/lb (no difference)
_____ - $0.10/lb
_____ - $0.20/lb
_____ - $0.30/lb
_____ - $0.40/lb
_____ - $0.50/lb
_____ - $0.60/lb
_____ - $0.70/lb
_____ - $0.80/lb
_____ - $0.90/lb
_____ - $1.00/lb
_____ -> $1.00/lb (less)

APPENDIX C: SAS CODES

Cook Loss Analysis.

```
options ls=95 ps=70;
data cl;
input ID Rawwt CookWeight CookLoss  CookLossPer;
trt=1;
if id>8 then trt=2;
if id>16 then trt=3;
if id>24 then trt=4;
datalines;
proc print;


proc sort; by trt;
proc means n mean std stderr min max; by trt;
proc glm; class trt;
model Rawwt CookWeight CookLoss  CookLossPer=trt;
means trt/lsd lines;
lsmeans trt/stderr pdiff;
run;
```

Lipid Oxidation Analysis.

```
data two;
input trt      ID$ prep      Spec      TBAR;
datalines;
proc print;

proc sort; by trt;
proc means n mean std stderr min max; by trt;
proc glm; class id trt;
model  Spec TBAR =trt id(trt);
test h=trt e=id(trt);
means trt/lsd lines e=id(trt);
lsmeans trt/s p e=id(trt);
run;
```

Slice Shear Force Analysis.

```
data ssf;
```

```
input ID SSF;
trt=1;
if id>8 then trt=2;
if id>16 then trt=3;
if id>24 then trt=4;
datalines;

proc print;


proc sort; by trt;
proc means n mean std stderr min max; by trt;
proc glm; class trt;
model SSF=trt;
means trt/lsd lines;
lsmeans trt/stderr pdiff;
run;
```

Myofibrillar Fragmentation Index Analysis.

```
data two;
input trt      ID$ prep    ScrWt OriWt  ResWt ResScr MFI;
datalines;
proc print;

proc sort; by trt;
proc means n mean std stderr min max; by trt;
proc glm; class id trt;
model  ScrWt  OriWt  ResWt ResScr MFI =trt id(trt);
test h=trt e=id(trt);
means trt/lsd lines e=id(trt);
lsmeans trt/s p e=id(trt);
run;
```

Sensory Panel Analysis.

```
data cl;
input ID Tend Jui       Fla       LikeDisl;
trt=1;
if id>8 then trt=2;
if id>16 then trt=3;
if id>24 then trt=4;
datalines;
proc print;
```

```
proc sort; by trt;
proc means n mean std stderr min max; by trt;
proc glm; class trt;
model Tend Jui          Fla       LikeDisl=trt;
means trt/lsd lines;
lsmeans trt/stderr pdiff;
run;
```