EFFECTS OF AN APPLICATION OF *Lactobacillus* ON THE QUALITY AND SENSORY CHARACTERISTICS OF BEEF AND PORK

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CHAPTER I

INTRODUCTION

Food safety is an issue of increasing importance to the consumer. With each new outbreak of a foodborne illness, consumer concern increases dramatically. In 1999, Mead et al. estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year. Many of the pathogens of greatest concern today (e.g., *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Cyclospora cayetanensis*) were not recognized as causes of foodborne illness 20 years ago (Mead et al., 1999). The causes of foodborne illness are constantly changing as new pathogens are discovered and old pathogens are phased out through effective use of antimicrobials and food safety guidelines.

With this in mind, the food industry continues to develop and examine new ways to control and reduce pathogens in the food supply. Methods to reduce or inhibit Gram-negative bacteria by food-grade compounds are of interest to the food industry due to economic and public health concerns (Belifore et al., 2007). Suitable strains of lactic acid bacteria must be identified for use in bio-preservation strategies for fresh meat (Jones et al., 2008). Acid decontamination of meat surfaces may reduce pathogens and spoilage bacteria, thereby increasing shelf-life and reducing potential for foodborne illness (Acuff et al., 1987). Compounds that can inhibit pathogens at refrigerated

temperatures are important. If these inhibitory compounds can be produced during refrigerated storage, pathogens can be reduced or eliminated after the food product reaches the supermarket (Brashears and Durre, 1999).

CHAPTER II

REVIEW OF LITERATURE

Antimicrobial: *Lactobacillus*

Lactobacillus has been used for food preservation since ancient times due to the production of a range of antimicrobial metabolites (Castellano et al., 2008). Lactic acid bacteria have been shown to reduce the populations of foodborne pathogens such as *E. coli* O157:H7 and *Salmonella.* In 2005, Smith et al. showed that selected strains of lactic acid bacteria reduced *E. coli* O157:H7 populations by 4-5 logs following 8 and 12 d of storage. This study also showed an almost 4-log reduction in *Salmonella* by the same strains of lactic acid bacteria. These lactic acid bacteria create a competitive environment by producing hydrogen peroxide, bacteriocins, and weak acids. Also, lactic acid bacteria are capable of growing under a variety of conditions because they do not require oxygen for growth, are resistant to carbon dioxide, and tolerate lower pH values than the gram negative bacteria found on meats (Egan, 1983). Addition of lactic acid bacteria to refrigerated meat could inhibit pathogen growth due to the production of inhibitory substances (Smith et al., 2005). This could also alert consumers to temperature abuse of refrigerated foods, since the lactic acid bacteria will cause spoilage. During conditions of temperature abuse, lactic acid bacteria will grow and produce acid that will alter the food product (Brashears and Durre, 1999).

Lactic acid bacteria are approved for use by the U.S. Food and Drug Administration (FDA) in fresh and processed meat and poultry products (Hoyle et al., 2009). Since these bacteria are generally regarded as safe for use in food products, this creates an opportunity for lactic acid bacteria to be utilized as an antimicrobial agent. *Lactobacillus* can be used as an added natural antimicrobial compound. These cultures can be directly added to ground beef and other meat and poultry products as a food safety intervention as defined by the FSIS (Hoyle et al., 2009). The FDA does not have a limit on the concentration of lactic acid that can be used; however, the USDA only allows it to be used at the lowest concentration necessary for the intended purpose (Kotula and Thelappurate, 1994).

Isolation: *Lactobacillus*

Due to the presence of lactic acid bacteria on multiple food products, various methods of isolation have been used for *Lactobacillus*. Isolation from fresh meat products consists of a similar process regardless of the protein used. Samples are ground or cut under sterile conditions and homogenized with a peptone water solution and then allowed to enrich at room temperature for approximately 24 h. Serial dilutions are made in peptone water and aliquots are plated onto MRS agar, and plates are incubated for 48 h at 30^0C (Najjari et al., 2007). After growth, colonies are analyzed, purified, and then stored until later use. *Lactobacillus* can also be isolated from cooked products by a method similar to the fresh meat procedure. A sample is added to *Lactobacillus* selection broth, homogenized, and incubated at 32°C for 18 to 24 h, followed by cultures being streaked onto *Lactobacillus* selection media and incubated for 48 h at 32°C ((Amezquita and Brashears, 2002). Following this growth period, samples are streaked until pure

cultures are achieved. *Lactobacillus* strains can then be Gram stained and Catalase tested for identification. The fermentation pattern of each isolated strain was also determined by the API system with API 50CHL medium for LAB (Amezquita and Brashears, 2002). The isolated strains can also be identified by ARDRA (amplified ribosomal DNA restriction analysis) and 16S rDNA sequence analysis (Najjari et al., 2007).

Lactobacillus strains can also be isolated from plant products using a method similar to those used on meat products. In 2004, Wilderdyke et al. described a procedure where samples from alfalfa sprouts were stomached in MRS broth and then streaked onto MRS and *Lactobacillus* selective agar and incubated at 37°C for 24 h. After incubation, isolated colonies were inoculated into MRS broth and incubated for 24 h at 37°C; the isolated colonies were cultured repeatedly until uniform in growth and colony size. Following isolation, strains were subjected to Gram stain and catalase testing, and then evaluated using the API with 50CHL medium for lactic acid bacteria (Wilderdyke et al., 2004).

Once *Lactobacillus* strains have been isolated, the selection of the appropriate strain is an important part of the antimicrobial application process. Strains vary in their ability to inhibit microorganisms; some inhibit during growth, others during refrigerated storage, and some do not inhibit microorganisms at all. Brashears and Durre (1999) determined that it is essential that strains be selected that inhibit the pathogen, survive storage, and do not alter the food, except during temperature abuse. Leisner et al. (1995) feels that two criteria should be considered when using lactic acid bacteria to extend the storage life of beef: strains must be able to grown and inhibit unwanted microorganisms and strains should never cause spoilage of the product.

Application

Lactobacillus bacteria can be applied to meat products in various ways. Spraying and dipping are two of the main methods of inoculation. Laboratory sprayers, handheld sprayers, paint sprayers, and spray cabinets have all been utilized to apply *Lactobacillus*. In 1987, a study by Dixon et al. used a Chromist laboratory sprayer to apply 1.0 mL of acid to a steak placed on sterile foil. Distance and time were measured to ensure a consistent amount of bacteria was sprayed. *Lactobacillus* cells diluted in saline were applied to steaks using a hand-operated spray bottle in a study performed by Castellano et al. in 2008. This hand spray application applied a final concentration of 10^6 cfu/g on the steak surface. Pressurized paint spray guns have also proven effective as an application method. Acuff et al. (1986) used stainless steel paint spray guns powered by a 50 psi nitrogen cylinder to apply *Lactobacillus* to whole strip loins. Commercial antimicrobials have also been tested against *Salmonella* and *E. coli* on bottom sirloin butts. A solution of 2.5% Beefxide was applied to beef tips placed in a sanitizing spray cabinet at a rate of 0.305 m/2.5 s (Laury et al., 2008).

The dipping method of inoculation has been utilized in several studies. Steaks were dipped in either 0.6% or 1.2% lactic acid at a temperature of 1 to 2°C for 20 s or 120 s and samples were allowed to drip for 1 min to remove excess solution, and then packaged into freezer bags (Kotula and Thelappurate, 1994). Dipping has also been used on smaller pieces of meat. Leisner et al. (1995) utilized sterile wire hooks to suspend beef slices and immerse them in a bacterial solution containing a bacterial density of log 2 CFU/cm^2 for 15 s. This study did not specify a time allowed for excess solution to drip

off the meat surface, and samples were immediately placed into packaging for further analysis.

Packaging and Storage

Fresh meat is packaged in a variety of ways from vacuum packaging to PVC overwrap. Studies have utilized a variety of these methods to analyze the effects of *Lactobacillus*. Most samples used for subjective color analysis are overwrapped with a plastic film. Steaks were placed in foam trays and overwrapped with poly-vinyl chloride film with an oxygen transmission rate of 6500cc/m^2/24h and a moisture vapor transmission rate of 341-419g/m^2/24h (Dixon et al., 1987). Some subjective color samples can also be placed in modified atmosphere packaging (MAP). Modified Atmosphere Packaged steaks are placed in rigid plastic trays and covered with oxygen-barrier film with 100% relative humidity and an oxygen transmission rate of less than 20.0mL/24h/m^2 and a moisture vapor transmission rate of less than 00.1g/24h/ 645.2cm^2 (Grobbel et al., 2008).

Vacuum packaging is normally used in product storage for transportation or aging, mostly in the case of whole subprimals. In the study by Acuff et al. (1987), loin sections were placed into a bag with an oxygen transmission rate of 7.8cc/m^2/24h and a moisture vapor transmission rate of 9.3g/m^2/24h, and the bag was then vacuum packaged using a Cryovac® 8300 vacuum packaging machine and a heat shrink tunnel. Ground beef were vacuum packaged by a Koch model 88045 vacuum packaging machine in straight vacuum seal bags in a study by Smith et al. (2005).

Color Evaluation

Subjective color evaluation of steaks is normally used as an indicator of quality. Consumers tend to purchase meat based on color. Consumers use color as an indicator of freshness and the eating potential of the cooked product (Forbes et al., 1974). Applications of *Lactobacillus* have been shown to have little to no effect on the color of meat placed in a retail display. In 1987, Acuff et al. found only minor differences between overall appearance scores of acid treated and control strip steaks with similar results for lean color, fat color, and surface discoloration. Dixon et al. (1987) found no differences in lean color, surface discoloration, fat color, and overall appearance in PVC or vacuum packaged acid treated and control steaks. However, a study done by Kotula and Thelappurate in 1994 resulted in acid treated steaks being lighter in color than the untreated control steaks.

Sensory Evaluation

Treatment with *Lactobacillus* has been shown to have only a marginal effect on the sensory properties of meat. In 1995, Smith et al. determined that panelists could not detect a difference in treated and untreated ground beef after three days of storage. Kotula and Thelappurate (1994) showed that both acid treated samples did not differ from control in tartness, flavor, and overall acceptability. However, both acid treated samples were less juicy than the control, and the lactic acid treated samples were not different from the control in regards to tenderness. Steaks in the Castellano et al. (2010) study showed no flavor differences between lactic acid treated samples and control samples until after 30 d of storage. Control and saline treated samples displayed rancid,

liver, and acid off flavors at storage d 40 and 50. This showed that the acid treated samples performed better at the end of storage than both the control and saline treated samples. Smith et al. (2005) also demonstrated that lactic acid bacteria had no adverse effects on the sensory properties of ground beef.

Aerobic Plate Counts

Plate counts of acid treated and untreated controls have also been shown to have little differences. In 1987, Acuff et al. found that surface bacteriological counts (APC $\log_{10}/ cm^2$) and percent distribution of microbial types on both control and acid sprayed strip loin steaks were not different. Also in 1987, Dixon et al. determined no differences in aerobic plate counts between control and acid treated steaks that were either PVC overwrapped or vacuum packaged. Conversely, Kotula and Thelappurate (1994) discovered a lower total CFU in samples treated with lactic acid when compared to an untreated control and the 1.2% application of lactic acid applied for 120s had the lowest counts with 1.7 log lower than the control. Hoyle et al. (2009) found that lactic acid bacteria inoculated samples had higher total aerobic plate counts than the controls initially, but counts became similar towards the end of the study.

Lactic Acid Bacteria Plate Counts

Lactic acid bacteria have mostly been shown to increase in number during storage. Castellano et al. (2010) discovered that lactic acid bacteria counts increased from 6.10 to 8.40 log CFU/g during storage on steaks inoculated with lactic acid bacteria. Fadda et al. (2008) showed similar results with an increase of 1 and 2 log after storage at 7^0C for 15 days in a vacuum package. Hoyle et al. (2009) noted that the populations of

lactic acid bacteria in the inoculated samples did not show an increase over time in PVC overwrapped trays stored in a retail case.

TBARS and pH Evaluation

Thiobarbituric acid assay (TBA) values do not appear to have been a subject for evaluation in current literature; however, pH is determined in order to compare acid production of the *Lactobacillus* strains. *Lactobacillus* has been shown to have little to no effect on the pH of meat during storage. No significant differences in pH were found between the control and treated vacuum packaged ground beef after 3 d of storage (Smith et al., 2005). Dixon et al. (1987) also showed no differences in pH between the control and treated steaks. Leisner et al. (1995) showed that the surface pH of beef samples inoculated with 2 log CFU/cm^2 were not different from the control in vacuum packaged samples. Once the vacuum was removed and samples were placed in aerobic storage, the samples treated with lactic acid bacteria had a lower pH. Castellano et al. (2010) also showed a decrease in pH of vacuum packaged, lactic acid bacteria treated samples after 60 d of storage.

Shelf Life and *Lactobacillus*

The utilization of lactic acid bacteria as an antimicrobial agent has the potential to aid in the improvement of shelf life for the meat industry. Acid decontamination of vacuum packaged subprimal cuts had little or no effect on the shelf life of the resulting steaks (Acuff et al., 1987). *Lactobacillus* strains have been shown to contribute to meat aging through small peptides and free amino acid release (Fadda et al., 2008). Lactic acid bacteria cause flavor and texture changes with a preservative effect which results in an

increase in the shelf life of the product (Hugas, 1998). In 1995, Leisner et al. found that storage life of vacuum packaged samples inoculated with log 2 CFU/cm^2 was in excess of 10 weeks, regardless of the strain used to inoculate the samples. Castellano et al. (2010) determined that *Lactobacillus curvatus* CRL705 could be utilized as an additional hurdle to improve storage life of vacuum packaged beef without affecting sensory qualities of the meat. However, little research has been done to determine shelf life in meat that is PVC overwrapped for a retail display.

Summary

Food safety will continue to be a concern to the public, meat producers and retailers. Lactic acid bacteria have been proven to reduce microbial contamination in meat and meat products. Applications of *Lactobacillus* are approved for use by both the USDA and FDA, and should be utilized to protect the food supply. Prior research suggests that applications of *Lactobacillus* have little to no effect on the sensory characteristics of meat, but have an added benefit of improving shelf life. However, a majority of these studies have been performed on ground product and whole subprimals. Therefore, research on the impacts of an application of *Lactobacillus* to retail wrapped product is needed in the future.

CHAPTER III

EFFECTS OF AN APPLICATION OF *Lactobacillus* ON THE QUALITY AND SENSORY CHARACTERISTICS OF BEEF AND PORK

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ABSTRACT

Food safety is an ever increasing concern for consumers and producers alike. Therefore, the objective of this study was to determine the effects of an application of *Lactobacillus* on the sensory and quality characteristics of both beef and pork. Beef strip loins (n = 10) and boneless pork loins (n = 10) were obtained and aged for approximately one week. Steaks/chops were cut and treated with a solution containing approximately 10^8 cfu/ml of a *Lactobacillus* bacterium. Samples designated for d 0 analysis were either frozen or analyzed immediately. Samples for d 3 and d 6 were placed into the retail display case. Steaks/chops were evaluated for subjective color, sensory, pH, thiobarbituric acid reactive substances (TBARS), aerobic and *Lactobacillus* plate counts. No significant differences ($P > 0.05$) for treatment effects were found for subjective color analysis in beef and pork. Sensory, pH, and TBARS also did not display a significant treatment effect in beef and pork. Aerobic and *Lactobacillus* plate counts showed a treatment effect ($P < 0.05$); however, plate counts for d 3 and d 6 were determined to be the same in both control and treated steaks. Plate counts from d 6 were shown to be significantly ($P < 0.05$) different from both d 0 and d 3 in pork chops. In this study, an

application of *Lactobacillus* by dipping was proven to have no significant treatment effect on the quality and sensory characteristics of beef strip steaks and pork loin chops.

INTRODUCTION

Food safety is an issue of increasing importance to the consumer. With each new outbreak of a foodborne illness, consumer concern increases dramatically. In 1999, Mead et al. estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year. The causes of foodborne illness are constantly changing as new pathogens are discovered and old pathogens are phased out through effective use of antimicrobials and food safety guidelines. With this in mind, the food industry continues to develop and examine new ways to control and reduce pathogens in the food supply. Suitable strains of lactic acid bacteria must be identified for use in bio-preservation strategies for fresh meat (Jones et al., 2008). Acid decontamination of meat surfaces may reduce pathogens and spoilage bacteria, thereby increasing shelf-life and reducing potential for foodborne illness (Acuff et al., 1987). Therefore, the objectives of this study were to determine what effects an application of *Lactobacillus* bacteria would have on the quality and sensory characteristics of beef and pork products.

MATERIALS AND METHODS

Isolation of *Lactobacillus*

The *Lactobacillus* isolated from beef was found in modified atmosphere packaged (MAP) ground beef that had been refrigerated for approximately two weeks. Following this period of refrigeration, samples were prepared using the following procedure:

following sterile removal from package, 50 g samples were placed into a stomacher bag and 450 ml of peptone water was added. Samples were then homogenized in a stomacher unit for 2 min (Contents of this bag are $1:10^1$). Following homogenization, 1 ml was removed from the side of the filter that did not contain the meat and placed onto a plate containing either deMan, Rogosa, and Sharp (MRS) or *Lactobacillus* selective (LBS) media. Also, 1 ml of solution was removed from the stomacher bag to begin a serial dilution. This 1 ml was added to 99 ml of peptone water and 0.1 ml was then plated. From this initial bottle, 1 ml was removed and added to a new bottle containing 99 ml of peptone water and 0.1 ml was plated from this second dilution bottle. Plates were shaken with glass beads to distribute the sample, were taped together, and incubated anerobically for 48 h at 37^oC.

Following incubation, a colony from the MRS plates was transferred into tubes containing Trypticase Soy Broth (TSB) media. The tubes were then refrigerated overnight at 37°C. The following day, a sterilized loop was used to streak MRS and LBS onto Trypticase Soy Agar (TSA) plates in a zigzag pattern. Plates were then incubated for 48 h at 37°C. After incubation, a colony was removed from each plate and placed into TSB tubes. Tubes were incubated at 37°C. Following this, a sample was removed from the tubes using a sterile loop and streaked onto plates. Plates were incubated 37°C for 48 h. After this final incubation on plates, a sterile needle was used to select a colony from the plates and the needle was stabbed into a tube containing TSA stab media. The inoculated stab media were refrigerated until regrowth for the Api test to identify the type of *Lactobacillus*.

Isolation of *Lactobacillus* from ground pork was conducted in a similar manner; however, the amount of sample and the enrichment media used were changed to accommodate pork products and isolates. Approximately, 100 g of ground pork and 200 ml of MRS broth were placed into a stomacher bag, and samples were homogenized in a stomacher unit for 2 minutes. After homogenization, 0.1 ml was removed from the side of the filter that did not contain the meat and placed onto a plate containing MRS agar. Also, 1 ml was removed from the stomacher bag and added to 9 ml of peptone water to create a 1:10 dilution. An additional 1:10 dilution was made using the first dilution tube, and both dilutions were plated on MRS agar in duplicate using 0.1 ml of solution. Plates were shaken with glass beads to distribute the sample and were taped together and aerobically incubated for 48 h at 37^{o}C. The stomacher bags containing the ground pork and MRS broth mixture were also allowed to enrich overnight. The following morning a dilution scheme was conducted using seven tubes each containing 9 ml of peptone water. For the first tube, 1 ml of solution was transferred directly from the bag into the tube, the tube was then vortexed, and 1 ml was removed to be placed into the second tube. Serial dilutions were carried out through the 10^{-7} dilution. All dilution tubes were plated onto MRS agar using 0.1 ml, and initial plating was done using the enriched solution. Plates were then aerobically incubated for 48 h at 37^{o}C.

Following incubation, a colony from the MRS plates was transferred into tubes containing TSB. The tubes were then refrigerated overnight at 37°C. The following day, a sterilized loop was used to streak MRS and LBS onto TSA plates in a zigzag pattern. Plates were then incubated for 48 h at 37°C. After incubation, a colony was removed from each plate and placed into LBS or MRS tubes. Tubes were incubated at

37°C. Following this, a sample was removed from the tubes using a sterile loop and streaked onto plates. Plates were incubated 37°C for 48 h. After this final incubation on plates, a sterile needle was used to select a colony from the plates and the needle was stabbed into a tube containing TSA stab media. The inoculated stab media were refrigerated until regrowth for the Api test to identify the type of *Lactobacillus*.

Lactobacillus Identification

Identification of *Lactobacillus* was conducted in the same way for beef and pork. To prepare samples for the Api test, a sterile loop was used to remove bacteria from the stab. The bacteria were then streaked onto MRS plates and incubated anerobically at 37^{o}C overnight. The following day colonies were selected from the plates, Gram stain and Catalase tests were performed. *Lactobacillus* bacteria have been shown to be Gram positive rods and catalase negative. Colonies were selected from each plate and placed into three different test tubes containing MRS media. One test tube was incubated at 42^{o}C, one in a water bath at 15^{o}C, and one in a water bath at 45^{o}C. Following overnight incubation, all tubes were observed for growth. Samples that grew under all three temperatures were then used for Api testing.

Api testing was done following the procedure in the Api Ch 50 manual. The MRS tube containing the sample was vortexed, and then poured into a sterile centrifuge tube. Tubes were centrifuged for 5 min at 4000 rpm and 4^{o}C. The supernatant was removed and 10 ml of peptone water were added to the tube to wash the pellet and remove MRS media. Tubes were centrifuged again for 5 min at 4000 rpm and 4^{o}C. Supernatant was again removed, making sure to leave the pellet in the tube. Next, 10 ml

of Api CHL 50 media was poured into the tube with the pellet and vortexed to mix thoroughly. To prepare the tray for the Api strips, about 10 ml of deionized water was added to the wells in the bottom of the tray to create a moist environment. Using a sterile pipette, the suspension was dispensed into the tubes in the Api strip. Once filled, the lid was put on the tray and the tray was incubated anerobically at 37^{o}C for 24 and 48 h. The trays were observed for changes in color (yellow +, green -) at both 24 and 48 h. Also, if the number 25 well is positive it will turn black in color.

The ground beef samples were positively identified using the Api identification software as *Lactobacillus plantarum*. The ground pork samples were identified as *Lactobacillus brevis* using the Api software, in addition to a positive gram stain and negative catalase test.

Preparation of Treatment Solutions

Treatment solutions for both beef and pork were prepared using the following method: a sterile loop was used to remove bacteria from the stab. The bacteria were then streaked onto MRS plates and incubated anerobically at 37^{o}C overnight. Following incubation, a single colony was selected using a sterile loop and used to inoculate a 10 ml tube containing MRS broth. This tube was then incubated overnight at 37^{o}C. The next day, 1 ml was removed from the 10 ml tube and added to 100 ml of MRS broth which was then incubated overnight at 37^{o}C. Following this last incubation, 50 ml of inoculated MRS was added to 450 ml of 0.1% buffered peptone water to make the *Lactobacillus* treatment solution, which had an initial inoculation level of 10^{8} cfu/ml. The solution was inverted to thoroughly mix it, and was then placed on ice until sample inoculation. The

control solution was made by sterilizing 500 ml of deionized water, which was also placed on ice until product inoculation.

Inoculation of Product

United States Department of Agriculture (USDA) Choice Strip Loins IMPS # 180 (n=10) were obtained from Creekstone Farms in Arkansas City, KS, transported to the OSU Food & Agricultural Products Center (FAPC), and were aged for approximately 1 week. Each strip loin was then fabricated into 12 - 2.54 cm steaks with the first 6 steaks receiving the *Lactobacillus* treatment and the last 6 steaks receiving the control. Treatment and control steaks were each placed on separate tables to receive treatment. Treatment and control steaks were inoculated using the same procedure. Either the *Lactobacillus* solution or sterile water was added to a clean MAP tray. Sterile tongs were used to lift the steak and then release it into the solution for a standard time of 5 sec. Steaks were then removed from the solution and allowed to drip for 10 sec before being randomly placed into a pre-labeled styrofoam tray. Trays were labeled with day (0, 3, or 6), and method of analysis (Thiobarbituric Acid Reactive Substances (TBARS) and pH, aerobic and lactic acid bacteria plate counts (APC/LAB), sensory evaluation (SEN), or subjective color evaluation (Color). All d 0 samples designated for pH, TBARS, and sensory were vacuum packaged and frozen until further analysis. Plate count samples, both APC and LAB, samples were taken to the microbiology labs in FAPC for analysis. Sensory d 6 samples were also used for subjective color analysis.

United States Department of Agriculture (USDA) boneless pork loins IMPS # 413 (n = 10) were obtained from Ralph's Packing Company in Perkins, Ok, transported to

the OSU Food & Agricultural Products Center (FAPC), and were aged for approximately 1 week. After aging, the pork loins were fabricated into 12 - 2.54 cm chops and treated in the same manner as the strip loin steaks.

Simulated Retail Display

Following treatment, all d 3 or d 6 samples were placed on a styrofoam tray with a soaker pad and were over-wrapped with a polyvinyl chloride film (PVC). Trays were placed into the coffin style display case which was maintained at 2°C ± 1°C, under lighting conditions (Philips Delux Warm White Florescent lamps) for 24 h per d. The meat surface was exposed to 900 to 1365 lux as recommended by AMSA (1991). Steaks and chops designated for color evaluation were subjectively evaluated for color attributes at 12 h intervals until 6 d of retail display. Samples designated d 3 were pulled after 3 d of retail display and d 6 were pulled after 6 d of display.

Subjective Color Evaluation

A trained panel of five Oklahoma State University personnel evaluated subjective color. Panelists were trained using Munsell color tiles and were required to receive a passing score before being used as a panelist. A set of 20 steaks and chops were evaluated for subjective color, and were vacuum packaged and frozen after 6 d of display. Panelists assigned scores to each steak/chop for muscle color, surface discoloration, and overall appearance at every evaluation time. Muscle color (8 = extremely bright cherry-red; 1= extremely dark red), surface discoloration (7 = total discoloration (100%); 1 = no discoloration (0%)), and overall appearance (8 = extremely desirable; 1 = extremely undesirable) were described as outlined by the Guidelines for Meat Color Evaluation

(AMSA, 1991). For pork, a 6-point scale (6 = very dark purplish-red; 1 = very pale) was utilized to describe muscle color, with the scales for surface discoloration and overall appearance remaining the same as those for beef.

Sensory Analysis

Sensory analysis and preparation followed the AMSA guidelines (AMSA, 1995). Samples were assigned a randomized number for sensory sessions. Steaks /chops were allowed to temper in a cooler at 4°C for 24 h before cooking. Steaks/chops were cooked on an impingement oven (XLT Impinger, Model 3240-TS, BOFI Inc., Wichita, KS) at 204.4° C to a final internal temperature of 70°C determined by an Atkins AccuTuff 340 thermocouple (Atkins Temtec, Gainesville, FL) as the steaks/chops exited the oven. Samples were then sliced into approximately 2.54 cm × 1.27cm × 1.27cm cubes and served warm to panelists. Preparation of pork samples followed the same procedure as the beef samples.

Sensory attributes were evaluated by a five member trained panel consisting of Oklahoma State University personnel. Panelists were trained for tenderness, connective tissue, and juiciness. Panelists were also trained to evaluate beef and pork flavor, as well as painty/fishy and livery/metallic off flavors. Sensory sessions were performed three times over a period of two days and contained 20 samples per session. Samples were evaluated using a standard ballot provided by the AMSA guidelines (AMSA, 1995). Panelists were asked to evaluate each sample in duplicate to determine initial juiciness (IJ) and sustained juiciness (SJ), initial tenderness (IT), amount of detectable connective tissue (CT), and overall tenderness (OT), all using an 8-point scale. For the juiciness

factors the scale was 1= extremely dry and 8= extremely juicy. The scale for IT and OT was 1= extremely tough and 8= extremely tender, and the score for CT was 1= abundant and 8= none. Beef/pork flavor and off flavors were evaluated using a 3 point scale (1 = not detectable; 3= strongly detectable).

Panelists were randomly seated in individual booths in a temperature and light controlled room. During serving the panelists were under red filtered lights as suggested by AMSA (AMSA 1995). The samples were served randomly to each panelist. The panelists were provided distilled, deionized water and crackers with unsalted tops to cleanse the palate.

Thiobarbituric Acid Reactive Substances

Steaks and chops were tempered in a cooler at 4°C for 24 h before evaluation. Lipid oxidation was evaluated by TBARS using the modified method of Buege and Aust (1978). A 10 g sample was placed in a blender (model 51BL31, Waring Products, INC., Torrington, CT) and homogenized with 30 ml of cold deionized water and then poured into a disposable tube. Each tube was centrifuged at 7°C and 3000 rpm for 10 min. Two ml of supernatant was pulled from the tube and placed in a glass test tube in duplicates. Prior to the addition of the supernatant, 4 ml of thiobarbituric acid/trichloroacetic acid (TBA/TCA) and 100 µl of butylated hydroxyanisol (BHA) were added to each tube. Tubes were vortexed and incubated in a boiling water bath for 15 min followed by a 10 min cold-water bath. Tubes were then centrifuged for 10 min at 25°C at 3000 rpm. The absorbance was read at 531 nm. Standard curves were replicated using 1,1,3,3-tetra-ethoxypropane (TEP). The amount of lipid oxidation was measured in duplicate for each

steak and the average absorbance reading was used for each sample. Results were conveyed as mg of malonaldehyde per kg of sample.

pH

The pH for each sample was measured using the same steak or chop used for TBARS analysis. The pH was measured using a Model IQ150 handheld pH/mv/temperature meter by IQ Scientific Instruments, 2075-E Corte del Nogal, Carlsbad, CA with a PHO5-SS Heavy Duty Meat Handle Stainless Steel pH Probe. The probe was inserted into the sample and then a pH reading was taken. Probe was cleaned with distilled water and wiped dry before each use.

APC and LAB Plate Counts

Plate counts were conducted for all bacteria (APC) and *Lactobacillus* (LAB). Counts were done on d 0, d 3, and d 6. Samples were removed from the retail case and then removed from their package and halved using a sterile knife and cutting board. One half of the sample was then vacuum packaged and frozen for TBARS and pH analysis at a later date and the other half was put into a sterile filter membrane Whirl-Pak bag and placed on ice. Plate count samples were then taken to the microbiology lab in the FAPC. Once in the FAPC lab, 50 ml of 0.1% buffer peptone water was added to each Whirl-Pak bag and the bags were massaged to suspend the bacterial cells. For each set of serial dilutions, 1 ml was removed from the side of the filter bag that did not contain the meat. This 1 ml was then added to a tube containing 9 ml of 0.1% buffer peptone water and vortexed (contents of this tube were labeled as -1). Serial dilutions were carried out through 4 tubes for d 0 in beef and pork, and 0.1 ml was removed from each of the last 3

tubes and placed in duplicate onto plates containing either APC or MRS media. Plates were then taped and incubated for 48 h at 37°C. Following incubation plates were counted using a lighted plate counter. Plate counts were repeated on d 3 using 6 dilution tubes in beef (plating the last 3 tubes) and 4 dilution tubes in pork (again plating the last 3 tubes). On d 6, beef counts were plated using the last 3 tubes from 7 dilutions and pork counts were plated using the last 3 tubes from 6 dilution tubes. Counts were then entered into a spread sheet and the log cfu/cm^2 was determined using each samples specific dilution factors.

Statistical Analysis

Data were analyzed using the MIXED model of SAS (SAS Inst. Inc., Cary, NC). The analysis of variance model for color attributes were analyzed using a repeated measures model with time as the repeated measure, identification number as the subject, and treatment as the fixed effect. The analysis of variance model for sensory, pH, TBARS, aerobic and *Lactobacillus* plate counts were analyzed using a mixed model with sample and panelists (when appropriate) as the random effect, day as the group variable, and treatment as the fixed effect. Aerobic and *Lactobacillus* plate counts were also examined by day using +/− 2 SEM to determine if means overlapped. All models also included primary and secondary interaction effects. The least squares means were separated using a pairwise t-test when the model displayed a treatment effect ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Subjective Color Evaluation

The treatment by hour interaction was not significant ($P > 0.05$) for muscle color, surface discoloration, and overall appearance for strip loin steaks. As shown in Table 1, control steaks has a slightly higher average muscle color over all time periods, but the difference was not significant. Kotula and Thelappurate (1994) also found that acid treated steaks were found to be lighter in color than control steaks. Control and *Lactobacillus* treated steaks showed a similar progression toward an increased area of surface discoloration, with no significance associated with treatment. Overall appearance of control and treated steaks decreased in a similar manner over the observed time. Table 1 also shows that steaks treated with *Lactobacillus* had a numerically higher overall appearance at the end of retail display. This higher overall appearance indicates a greater desirability, but the values were not significantly different ($P > 0.05$). These results are supported by Acuff et al. (1987) who found only minor differences between the overall appearance scores of acid treated and control strip steaks with similar results for lean color, fat color and surface discoloration.

The treatment interaction was also not significant ($P > 0.05$) for subjective color evaluation of pork loin chops. Table 2 shows that control and *Lactobacillus* treated steaks has similar values for muscle color, surface discoloration, and overall appearance. Muscle color of the chops remained close to a 3 (slightly pale) for control and treated steaks for the duration of retail display. All chops displayed no surface discoloration until 120 h, and only showed slight discoloration from 120 h until the end of display.

Control and treated chops also displayed similar values for overall appearance over display time; however, the results were not significant.

Sensory Analysis

Control and *Lactobacillus* treated steaks showed no treatment differences in initial and sustained juiciness ($P > 0.05$). Treated steaks had slightly lower initial and sustained juiciness values than control steaks (Table 3), and this is supported by Kotula and Thelappurate (1994). Treated samples in this study were shown to be less juicy than the control steaks. No significant treatment differences ($P > 0.05$) were seen in first impression and overall tenderness, as well as, connective tissue in the beef strip loin steaks. Table 3 shows that treated steaks had slightly higher values for connective tissue, which means a smaller amount of connective tissue. Control and treated steaks had similar values for first impression and overall tenderness. This was demonstrated by Kotula and Thelappurate (1994) in a previous study, where they found that lactic acid treated samples were not different from the controls in regards to tenderness. Table 4 displays the means for intensity of flavor attributes of strip steaks by treatment. Control and *Lactobacillus* treated steaks were not shown to be significantly different in regards to off flavors ($P > 0.05$). Steaks showed similar values for beef flavor, as well as, painty/fishy and metallic off flavors. A study by Castellano et al. (2010) found that control and treated samples displayed no differences in flavor until after 30 d of storage. Since the steaks for this current study were only stored for approximately two weeks, off flavors do not seem to be an issue.

Sensory evaluation of the pork chops showed similar results to the beef steaks. Table 5 shows no significant differences ($P > 0.05$) in initial and sustained juiciness between control and treated chops. Numerical values for both were similar, even when looking at the specific days. As shown in Table 5, tenderness and connective tissue values were also not significant in regards to treatment group ($P > 0.05$). *Lactobacillus* treated chops had slightly higher values for first impression and overall tenderness, and both groups showed similar results for the amount of connective tissue. Least squares means for intensity of flavor attributes are shown in Table 6. Pork flavor values were similar for both control and treated chops; and were more predominant than the described off flavors. Painty/fishy and livery/metallic off flavors were only slightly shown to be present with values of approximately 1 (not detectable).

pH

Data for pH evaluation of strip steaks is displayed in Table 7. No significant differences ($P > 0.05$) were observed in regards to treatment for pH. A study by Dixon et al. (1987) showed no differences in pH between control and treated steaks. Control steaks had slightly higher pH values on d 3 and 6 when compared to treated steaks. This has been previously shown by Leisner et al. (1995). This study found that vacuum packaged samples treated with lactic acid bacteria have a lower pH after removal from the vacuum.

The values for pH evaluation of pork chops are shown in Table 8. The pH evaluation of pork chops showed no significant differences ($P > 0.05$) in treatment effect.

Lactobacillus treated pork chops displayed a slightly higher pH value when compared to control chops on all observed days.

Thiobarbituric Acid Reactive Substances

The TBARS evaluation for steaks and chops showed no significant treatment effect ($P > 0.05$). Steak data is displayed in Table 7 and mg of malonaldehyde per kg of sample are shown to increase with d of display for control and treated steaks. *Lactobacillus* treated steaks showed slightly higher MDA values when compared with the control. This contradicts the results of a study performed by Hoyle-Parks et al. (2011) which looked at lactic acid bacteria and ground beef. This study found that TBARS tended to be lower in treated samples than the control. The TBARS data for chops (Table 8) also displayed an increase; however, it was on a much smaller scale. The mg of malonaldehyde per kg of sample only increased approximately 0.1 over 6 d of display. This increase was also observed in a study by Shrestha and Min (2004) with TBARS values increasing with increased concentrations of lactic acid and increased days of storage.

APC and LAB Plate Counts

Plate count data shown in Table 9 for steaks and Table 10 for chops displayed the only significant differences ($P < 0.05$) in treatment effect for this study. Aerobic plate count and *Lactobacillus* (MRS) plate count data for strip steaks were shown to increase over time in both control and *Lactobacillus* treated samples from d 0 to d 3. The $\log_{10}$ cfu/cm^2 then decreased from d3 to d 6 for all counts except control APC. When plate count evaluation data was compared using +⁄− 2 SEM, d 0 control plates were different

($P < 0.05$) from d 3 and d6 for both control and *Lactobacillus* treated steaks. However, d 3 and d 6 counts were determined to be the same for both APC and MRS in control steaks. For treated steaks, d 0 was found to be the same as d 6 but different ($P < 0.05$) from d 3 in APC and MRS. Plate counts for d 3 and d 6 were determined to be the same in treated steaks. This shows that plate counts did not increase significantly from d3 to d 6. Also, in *Lactobacillus* treated steaks, counts from d 3 and d 6 were the same. The level of bacteria on both the control and treated steaks did not significantly change in the last 3 d of storage. Previous studies contradict the data for aerobic plate counts. Acuff et al. (1987) found that surface counts (APC $\log_{10}/cm^2$) on control and acid treated steaks were not different. Also, Dixon et al. (1987) found no differences in aerobic plate counts between control and treated steaks. Initial counts for APC were higher for the steaks treated with *Lactobacillus*, and this is shown in a similar study by Hoyle et al. (2009). *Lactobacillus* (MRS) plate counts have been shown to increase during storage, as shown in this study from d0 to d 3. Castellano et al. (2010) and Fadda et al. (2008) both found an increase in lactic acid bacteria counts during storage.

Aerobic and *Lactobacillus* plate counts also showed a significant treatment effect in pork chops, as seen in table 10. Counts from both methods significantly ($P < 0.05$) increased from d 3 to d 6, with *Lactobacillus* treated chops displaying a higher concentration of bacteria than the controls. Plate count evaluation data was compared using +/− 2 SEM with no significant differences being found between d 0 and d 3 for both control and treated chops. Plate counts from d 6 were shown to be significantly ($P < 0.05$) different from both d 0 and d 3. These results differ slightly when compared to the results from the strip steak counts found in this study. In pork, the counts were shown to

increase significantly ($P < 0.05$) from d 3 to d 6. In beef, d 3 and d 6 counts were found to be the same.

CONCLUSION

The results from this research showed no adverse effects on sensory and quality characteristics of beef and pork from the application of *Lactobacillus* bacteria. Applications of lactic acid bacteria were shown to have no significant effect on off flavors, TBARS values, and color evaluation of both beef strip loin steaks and pork chops. However, more research is needed to determine the effectiveness of specific strains of *Lactobacillus* on harmful foodborne pathogens. Also, additional research on the prolonged effects of applications of lactic acid bacteria should be conducted.

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Table 1. Least squares means of subjective color evaluation of strip loin steaks (n = 20).

	time, h													
Color Characteristics	12	24	36	48	60	72	84	96	108	120	132	144	156	SEM
Control														
Muscle Color[1]	7.84	6.96	6.40	6.06	5.00	4.98	4.35	4.48	4.64	4.38	4.18	4.18	4.04	0.17
Surface Discoloration[2]	0.00	0.00	0.00	0.00	0.00	1.00	1.25	1.12	1.34	1.44	1.92	2.28	2.24	0.07
Overall Appearance[3]	8.00	7.66	7.08	6.70	6.12	6.12	5.48	5.32	5.18	5.02	3.86	3.98	3.74	0.15
***Lactobacillus* treated**														
Muscle Color[1]	7.80	6.90	6.30	5.92	4.94	4.82	4.33	4.44	4.58	4.38	4.16	3.98	3.84	0.17
Surface Discoloration[2]	0.00	0.00	0.00	0.00	0.00	1.06	1.35	1.08	1.20	1.26	1.72	1.96	1.98	0.07
Overall Appearance[3]	8.00	7.60	7.00	6.68	6.02	6.14	5.27	5.26	5.22	5.20	4.32	4.16	4.00	0.15

[1]Muscle color was measured on an 8-point scale (8 = extremely bright cherry red, and 1 = extremely dark red).
[2]Surface discoloration was measured on a 7-point scale (1 = no discoloration-0%, and 7 = total discoloration-100%).
[3]Overall acceptability was measured on an 8-point scale (1 = extremely undesirable, and 8 = extremely desirable).

Table 2. Least squares means of subjective color evaluation of pork chops (n = 20).

	time, h													
Color Characteristics	12	24	36	48	60	72	84	96	108	120	132	144	156	SEM
Control														
Muscle Color[1]	3.30	3.20	3.20	3.06	3.12	3.02	3.02	2.98	2.98	2.94	2.98	2.98	2.98	0.10
Surface Discoloration[2]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.04	1.18	1.52	1.46	0.03
Overall Appearance[3]	7.60	7.24	7.00	6.00	6.00	5.96	6.00	5.96	5.96	5.74	5.58	4.94	4.64	0.03
***Lactobacillus* treated**														
Muscle Color[1]	3.30	3.20	3.20	3.06	3.16	3.02	3.04	3.02	3.00	2.88	2.98	2.98	2.90[a]	0.10
Surface Discoloration[2]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.02	1.15	1.54	1.32	0.03
Overall Appearance[3]	7.60	7.20	7.00	6.00	6.00	5.96	6.00	5.98	5.98	5.74	5.63	4.98	4.68	0.03

[1]Muscle color was measured on a 6-point scale (6 = very dark purplish-red; 1 = very pale).
[2]Surface discoloration was measured on a 7-point scale (1 = no discoloration-0%, and 7 = total discoloration-100%).
[3]Overall acceptability was measured on an 8-point scale (1 = extremely undesirable, and 8 = extremely desirable).

Table 3. Least squares means for sensory by treatment and day for juiciness, tenderness and connective tissue on strip loin steaks (n = 60).

Treatment		IJ[1]	SEM	SJ[1]	SEM	FIT[2]	SEM	OT[2]	SEM	CT[3]	SEM
Control											
	Day 0	5.43	0.43	5.33	0.43	5.85	0.31	5.85	0.31	6.98	0.30
	Day 3	5.59	0.40	5.52	0.40	5.93	0.39	5.86	0.36	7.25	0.21
	Day 6	5.55	0.43	5.33	0.49	5.53	0.32	5.60	0.32	7.10	0.31
***Lactobacillus* treated**											
	Day 0	5.45	0.43	5.25	0.43	6.00	0.31	5.90	0.31	7.30	0.30
	Day 3	5.39	0.40	4.97	0.40	5.75	0.40	5.64	0.38	7.00	0.22
	Day 6	5.46	0.45	5.19	0.49	5.72	0.33	5.68	0.32	7.25	0.31

[1]Initial (IJ) and sustained juiciness (SJ) was measured on an 8-point scale (1 = extremely dry; 8 = extremely juicy).
[2]First impression (FIT) and overall (OT) tenderness was measured on an 8-point scale (1 = extremely tough; 8 = extremely tender).
[3]Connective tissue (CT) was measured on an 8-point scale (1 = abundant; 8 = none).

Table 4. Least squares means for sensory by treatment for intensity of flavor attributes on strip loin steaks (n = 60).

Treatment		Beef[1]	SEM	Painty/Fishy[1]	SEM	Livery/Metallic[1]	SEM
Control							
	Day 0	2.33	0.35	1.08	0.05	1.13	0.08
	Day 3	2.32	0.38	1.11	0.12	1.09	0.05
	Day 6	2.25	0.31	1.18	0.14	1.23	0.12
***Lactobacillus* treated**							
	Day 0	2.40	0.35	1.05	0.05	1.13	0.08
	Day 3	2.31	0.38	1.19	0.12	1.03	0.05
	Day 6	2.23	0.31	1.35	0.15	1.18	0.12

[1]Flavor intensity was measured on a 3-point scale (1 = not detectable; 3 = strongly detectable).

Table 5. Least squares means for sensory by treatment and day for juiciness, tenderness and connective tissue on pork chops (n = 60).

Treatment		IJ[1]	SEM	SJ[1]	SEM	FIT[2]	SEM	OT[2]	SEM	CT[3]	SEM
Control											
	Day 0	5.31	0.27	4.88	0.18	5.98	0.39	5.93	0.41	7.24	0.28
	Day 3	5.22	0.37	5.08	0.32	6.03	0.46	6.03	0.44	7.28	0.20
	Day 6	5.11	0.31	4.72	0.25	5.53	0.41	6.12	0.38	7.32	0.24
***Lactobacillus* treated**											
	Day 0	5.14	0.27	4.85	0.18	6.10	0.39	6.11	0.41	7.11	0.28
	Day 3	5.47	0.39	5.13	0.35	6.48	0.46	6.48	0.46	7.31	0.24
	Day 6	5.28	0.31	4.76	0.25	6.19	0.41	6.23	0.38	7.21	0.24

[1]Initial (IJ) and sustained juiciness (SJ) was measured on an 8-point scale (1 = extremely dry; 8 = extremely juicy).
[2]First impression (FIT) and overall (OT) tenderness was measured on an 8-point scale (1 = extremely tough; 8 = extremely tender).
[3]Connective tissue (CT) was measured on an 8-point scale (1 = abundant; 8 = none).

Table 6. Least squares means for sensory by treatment and day for intensity of flavor attributes on pork chops (n = 60).

Treatment		Pork[1]	SEM	Painty/Fishy[1]	SEM	Livery/Metallic[1]	SEM
Control							
	Day 0	2.69	0.17	1.03	0.31	1.03	0.04
	Day 3	2.75	0.15	1.05	0.08	1.10	0.12
	Day 6	2.73	0.16	1.13	0.08	1.04	0.07
***Lactobacillus* treated**							
	Day 0	2.72	0.17	1.02	0.30	1.07	0.04
	Day 3	2.57	0.16	1.15	0.09	1.20	0.12
	Day 6	2.67	0.16	1.02	0.08	1.11	0.07

[1]Flavor intensity was measured on a 3-point scale (1 = not detectable; 3 = strongly detectable).

Table 7. Least squares means for pH and TBARS evaluation by treatment and day on strip loin steaks (n = 60).

Treatment		pH	SEM	MDA[1]	SEM
Control					
	Day 0	5.60	0.06	0.22	0.03
	Day 3	5.74	0.06	0.59	0.12
	Day 6	5.70	0.07	1.30	0.22
***Lactobacillus* treated**					
	Day 0	5.64	0.06	0.31	0.03
	Day 3	5.66	0.06	0.64	0.12
	Day 6	5.63	0.07	1.35	0.22

[1]MDA = mg of malonaldehyde per kg of sample.

Table 8. Least squares means for pH and TBARS evaluation by treatment and day on pork chops (n = 60).

Treatment		pH	SEM	MDA[1]	SEM
Control					
	Day 0	5.78	0.07	0.14	0.04
	Day 3	5.80	0.05	0.10	0.02
	Day 6	5.87	0.04	0.21	0.36
***Lactobacillus* treated**					
	Day 0	5.89	0.07	0.15	0.04
	Day 3	5.87	0.05	0.13	0.02
	Day 6	5.85	0.04	0.20	0.22

[1]MDA = mg of malonaldehyde per kg of sample.

Table 9. Least squares means for aerobic and *Lactobacillus* plate count evaluation by treatment and day on strip loin steaks (n = 30).

Treatment		Day 0	SEM	Day 3	SEM	Day 6	SEM
Control							
	APC[1]	3.00^{a,x}	0.03	6.27^{b,x}	0.40	6.66^{b,x}	0.26
	MRS[2]	3.00^{a,x}	0.30	6.60^{b,x}	0.45	6.00^{b,x}	0.09
***Lactobacillus* treated**							
	APC[1]	7.45^{a,y}	0.03	7.76^{b,y}	0.40	7.61^{ab,y}	0.26
	MRS[2]	7.41^{a,y}	0.30	7.82^{b,y}	0.45	7.48^{ab,y}	0.09

[1]APC = counts performed on aerobic plate count media.
[2]MRS = counts performed on deMan, Rogosa, and Sharp (MRS) media.
[ab]Means, in a row, containing different superscripts, differ ($P < 0.05$).
[xy]Means, within a column and within a plate count method (APC or MRS), containing different superscripts, differ ($P < 0.05$).

Table 10. Least squares means for aerobic and *Lactobacillus* plate count evaluation by treatment and day on pork chops (n = 60).

Treatment		Day 0	SEM	Day 3	SEM	Day 6	SEM
Control							
	APC[1]	3.00[a,x]	0.07	3.48[a,x]	0.16	6.78[b,x]	0.21
	MRS[2]	3.00[a,x]	0.06	3.42[a,x]	0.20	6.09[b,x]	0.26
***Lactobacillus* treated**							
	APC[1]	5.18[a,y]	0.07	6.20[a,y]	0.16	7.94[b,y]	0.21
	MRS[2]	5.19[a,y]	0.06	6.22[a,y]	0.20	8.06[b,y]	0.26

[1]APC = counts performed on aerobic plate count media.
[2]MRS = counts performed on deMan, Rogosa, and Sharp (MRS) media.
[ab]Means, in a row, containing different superscripts, differ ($P < 0.05$).
[xy]Means, within a column and within a plate count method (APC or MRS), containing different superscripts, differ ($P < 0.05$).

APPENDICES

Oklahoma State University Institutional Review Board

Date: Tuesday, February 15, 2011 Protocol Expires: **2/24/2012**

IRB Application No: AG1011

Proposal Title: Food Safety: Farm to Table

Reviewed and Processed as: **Modification/Continuation**

Status Recommended by Reviewer(s) **Approved**

Principal Investigator(s) :

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Stillwater, OK 74078

Amber Sharp
106 ANSI
Stillwater, OK 74078

Approvals are valid for one calendar year, after which time a request for continuation must be submitted. Any modifications to the research project approved by the IRB must be submitted for approval with the advisor's signature. The IRB office MUST be notified in writing when a project is complete. Approved projects are subject to monitoring by the IRB.

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

Signature :

Sheila Kennison, Chair, Institutional Review Board

Tuesday, February 15, 2011
Date

Sensory Evaluation

Informed Consent Form
Hatch: Evaluation of post harvest quality and safety attributes of beef and pork products
Pork & Beef Loin Project

The following document contains important research information concerning your participation in this research study. Please read all the information carefully. Your participation in this project is voluntary and you may, at anytime, stop participating without penalty.

1. This research study is being conducted through Oklahoma State University.
2. The purpose of this research study is to determine palatability differences, if any, in pork and beef products.
3. The pork and beef products were made with ingredients at levels approved by FDA and USDA.
4. The pork and beef product samples will be served to you, and you will be expected to evaluate samples and mark a ballot with your impression of the characteristics listed on the ballot.
5. There are no known risks associated with this project which are greater than those ordinarily encountered in daily life.
6. You will be trained to evaluate tenderness, juiciness and flavor components of pork and beef product.
7. You will be asked to participate in 30 – 15 minute sessions. Session dates and times will be scheduled once all panelists are identified.
8. You are encouraged to ask any questions about procedures.
9. You will not be asked to make any identifying marks on the ballots and efforts are being made to maintain the confidentiality of your responses.
10. Data will be stored on the investigators computer during analysis and report preparation and then stored on a backup drive for three years. Data will be accessible to the investigators listed on the project.
11. In the case of injury or illness resulting from this study, emergency medical treatment will be available. No funds have been set aside by Oklahoma State University to compensate you in the event of illness or injury.
12. You will be provided with candy and/or breath mints upon completion of each session.

For questions about the research study, contact:
Dr. Deb VanOverbeke
104D Animal Science
Stillwater, OK 74078
405.744.6616 office
deb.vanoverbeke@okstate.edu

This research has been reviewed and approved by the Institutional Review Board for Human Subjects in Research at Oklahoma State University. If you have questions about your rights as a research volunteer, you may contact Dr. Shelia Kennison, IRB Chair, 219 Cordell North, Stillwater, OK 74078, 405-744-3377 or irb@okstate.edu.

Deb VanOverbeke, PI

Participant

Department of Animal Science Oklahoma State University Stillwater, OK 74078

VITA

Amber Lee Sharp

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF AN APPLICATION OF *Lactobacillus* ON THE QUALITY AND SENSORY CHARACTERISTICS OF BEEF AND PORK

Major Field: Food Science

Biographical:

Personal Data: Born in Harrison, Arkansas on December 13, 1984, the daughter of Larry Bruce and Rogenia Sharp.

Education: Graduated from Bruno-Pyatt High School in Everton, Arkansas in May 2003; Graduated from Oklahoma State University, Stillwater, Oklahoma, with a Bachelor of Science in Animal Science in 2007; Completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, Oklahoma in December, 2011.

Experience: Employed by Oklahoma State University as a graduate research and teaching assistant.

Professional Memberships: American Meat Science Association

Name: Amber Lee Sharp Date of Degree: December, 2011

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EFFECTS OF AN APPLICATION OF *Lactobacillus* ON THE QUALITY AND SENSORY CHARACTERISTICS OF BEEF AND PORK

Pages in Study: 44 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study: Meat Science

Findings and Conclusions:

The objective of this study was to determine the effects of an application of *Lactobacillus* on the sensory and quality characteristics of both beef and pork. Beef strip loins (n = 10) and boneless pork loins (n = 10) were obtained and aged for approximately one week. Steaks/chops were cut and treated with a solution containing approximately 10^8 cfu/ml of *Lactobacillus* bacteria. Samples designated for d 0 analyses were either frozen or analyzed immediately. Samples for d 3 and d 6 were placed into the retail display case. Steaks/chops were evaluated for subjective color, sensory, pH, Thiobarbituric Acid Reactive Substances, and aerobic and *Lactobacillus* plate counts. No significant differences ($P > 0.05$) for treatment effects were found for subjective color analysis in beef and pork. Sensory, pH, and TBARS also did not display a significant treatment effect in beef and pork. Aerobic and *Lactobacillus* plate counts showed a treatment effect ($P < 0.05$). However, when comparing plate counts by day, d 3 and d 6 were determined to be the same in both control and treated strip steaks. In pork chops, plate counts from d 6 were shown to be significantly ($P < 0.05$) different from both d 0 and d 3 in both control and treated chops. In this study, an application of *Lactobacillus* by dipping was proven to have no significant treatment effect on the quality and sensory characteristics of beef strip steaks and pork loin chops. *Lactobacillus* bacteria displayed no adverse effects on off flavors, TBARS values, or color of beef and pork over 6 d of retail display. More research is needed to determine the effectiveness of specific *Lactobacillus* strains as antimicrobials, and their specific effect on the quality and sensory characteristics of meat products.

ADVISER'S APPROVAL: Dr. Deborah L. VanOverbeke