# EFFICACY OF LACTIC ACID AS A PATHOGEN INTERVENTION FOR DRY- AND WET-AGED BEEF SUBPRIMALS

A Senior Scholars Thesis

by

JESSICA RENEE STEGER

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

May 2012

Major: Animal Science

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# ABSTRACT

Efficacy of Lactic Acid as a Pathogen Intervention For Dry- and Wet-Aged Beef Subprimals. (May 2012)

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USDA Select carcasses (n=6) were selected, and paired boneless (n=12) strip loins were cut in half (n=24) and were assigned randomly to be dry-aged (unpackaged) or wet-aged in a vacuum package bag. Strip loin sections were inoculated with a bacterial cocktail containing three non-pathogenic surrogates for *Escherichia coli* O157:H7 and *Salmonella* and were vacuum packaged and stored for 3 d before treatments were applied. These three *E. coli* strains were used to test the efficacy of a 5% L-lactic acid spray whether applied before or after a 28 d dry- or wet-aging period. Microbiological samples were collected from each section after inoculation (to determine the initial attachment of the surrogates), after being removed from the vacuum packaging at 3 d, and after treatment with lactic acid either before or following the dry- or wet-aging period. Dry-aged sections had a greater ($P < 0.05$) log reduction than those sections that were wet-aged. There was no impact ($P > 0.05$) on microbiological counts whether the lactic acid was applied before or after dry- or wet-aging. In addition, there was no significant interaction ($P > 0.05$) between aging and the application time. Dry-aging

reduced pathogens on meat surfaces, and lactic acid appeared to be equally effective as a pathogen intervention when applied before or after dry- or wet-aging.

# DEDICATION

I dedicate this paper to my parents, Gary and Susan Steger. Without their love and support throughout all of my endeavors, I would not have been able to accomplish many things including this project.

# ACKNOWLEDGEMENTS

I begin by thanking my research advisors Dr. Jeff Savell and Dr. Kerri Harris for this opportunity to work on this research project to further my knowledge on this subject. Thanks also to Lisa Lucia for all of her guidance through this project. I also thank Lindsey Mehall for all of her help and advice throughout this project. Lindsey played a huge role in guiding me through this project, and I truly appreciate the time that she has put into helping me in any way she possibly could. Also, thanks to Ashley Haneklaus and Cody Labus for helping in the lab as well as Carson Ulbrich and Russell McKeith for helping me procure my product.

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# CHAPTER I

# INTRODUCTION

The aging of meat, whether it is dry-aged or wet-aged, is implemented to produce a more desirable taste and to increase tenderness. The dry-aging process allows the natural enzymatic and biochemical processes to take place; therefore, dry-aging will improve tenderness and develop a unique flavor (Savell, 2008). Dry-aging is less common in the United States than wet-aging, but is used by a select group of establishments to distinguish their product as gourmet (Savell, 2008). Dry-aging is most commonly used to obtain a product with a unique flavor that as been described as buttery, rich and nutty. Wet-aged beef is known more for having a strong, sour and/or bloody/serumy taste to the meat. The dry-aging process involves the meat, most commonly subprimals, being stored without protective packaging in refrigerated temperatures with strict controls on humidity and airflow. Wet-aging is the more commonly used means of aging meat in the United States due to the implementation of the vacuum package (Laster et al., 2008). The vacuum package or sealed barrier packages along with refrigerated temperatures are needed for the wet-aging process (Smith, 2007), humidity and airflow are not factors considered in wet-aging. There are some advantages to wet-aging, especially when evaluating retail yield of the final aged product. Wet-aged meat products tend to have a much higher retail yield than those subprimals that have been dry-aged (Smith, 2007).

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This thesis follows the style of *Meat Science*.

Pathogens, particularly *Escherichia coli* O157:H7 and *Salmonella* can be of great concern in the meat industry. Previous research has shown an increase in the prevalence of *E. coli* O157:H7 in recent years (Hussein & Bollinger, 2005). Food safety concerns have increased because there have been a large number of outbreaks and human illness that has been associated with the consumption of undercooked meat that was contaminated with *E. coli* O157:H7. *Salmonella* can also be a major food safety concern for beef carcasses because of the risk for cross contamination (McEvoy, Doherty, Sheridan, Blair, & McDowell, 2003). *Salmonella* is one of the leading causes of foodborne illnesses that lead to hospitalization (Centers for Disease Control and Prevention, 2012)

There are many different antimicrobial interventions to help combat the prevalence of *E. coli* and *Salmonella* by reducing the number of pathogens on the meat. Some of the interventions used to reduce pathogens include lactic acid, lactic acid bacteria, and acidified sodium chlorite (Echeverry et al., 2010). Lactic acid is a very commonly used pathogen intervention in the meat industry. There are different concentrations of lactic acid sprays used for pathogen interventions, most common are 2% and 5% lactic acid. To comply with regulatory limits, lactic acid should be near but not exceed 55 °C when applied to meat (USDA, 2012)

Surrogate microorganisms are non-pathogenic and respond to specific treatments in the same manner the target microorganism would respond (Cabrera-Diaz et al., 2008).

Surrogates microorganisms can be used to determine efficacy of pathogen intervention without using the actual target microorganism. Surrogates must have growth that is equivalent to that of the target microorganism, and Cabrera et al. (2008) found there were no significant differences in growth between surrogate *E. coli* strains and *E. coli* O157:H7. If surrogates are being used to validate lactic acid interventions, the surrogate microorganisms must have similar acid resistance to that of the target microorganism (Cabrera-Diaz et al., 2008). Surrogate microorganisms must accurately mimic the response that the target microorganism would display in a given situation.

The objective of this study was to determine if the efficacy of a lactic acid spray for pathogen reduction was greater when applied before or after dry- or wet-aging.

# CHAPTER II

# METHODS

## Selection of product and storage before aging

Hot-boned USDA Select strip loins were obtained from cattle slaughtered at a beef processing plant located in Texas. The cattle were typical of those entering the U.S. meat supply and were slaughtered following USDA Food Safety and Inspection Service procedures. The strip loins were collected immediately from both sides of the carcass (n=6) upon entering the blast chill, were placed in an insulated container, and were transported to the Center for Food Safety Food Microbiology Lab at Texas A&M University. Upon arrival, the strip loins were cut in half (n=24) in order to enable each carcass to receive the two aging methods (dry- vs. wet-aging) and the two lactic acid application times (before or after aging). The strip loin pieces then were inoculated with a cocktail containing three non-pathogenic surrogates for *E. coli* O157:H7 and *Salmonella*, were vacuum packaged individually using a Koch, X180 vacuum packager (Ultrasource, Kansas City, MO), and were stored for 3 days. Because beef is traditionally vacuum packaged before shipping, all strip loins were vacuum packaged before aging to simulate industry practices.

## Aging conditions

The four strip loin sections from each carcass were assigned to the aging treatments as well as lactic acid application times. Two strip loin sections from each carcass were

assigned to be dry-aged with one receiving the pathogen intervention before the aging process and the other receiving the pathogen treatment after dry-aging. The other two strip loin sections from the same carcass were assigned to be wet-aged with one receiving the pathogen intervention before the aging process and the other receiving the pathogen treatment after wet-aging. All products were stored in a refrigerated (1 °C) cooler. Microbiological samples were taken after the product was inoculated and before vacuum packaging. After the strip loin sections were stored for 3 d, the packages were opened, and microbiological samples were taken to determine if vacuum packaging had any effect on the inoculation level.

The sections that received the pathogen intervention after the aging process were immediately re-vacuum packaged (wet-aging treatment) or stored without packaging (dry-aging treatment). The strip loin sections that received the pathogen intervention before the aging process were treated with a 5% lactic acid spray. The lactic acid spray was prepared using 88% L-lactic acid (Birko Corporation, Henderson, CO) and water. All product was aged for 28 d. The strip loin sections that were designated to receive the pathogen intervention after the aging period also were treated with the 5% lactic acid spray.

**Preparation of inoculum**

Three strains of rifampicin resistant *E. coli* (American Type Culture Collection: BAA-1427, BAA-1428, and BAA-1430) were obtained for the preparation of inoculum. One

loop of the working culture was transferred to a tryptic soy agar (TSA; Becton Dickinson, Sparks, MD) slant and incubated for 18 h at 37 °C. One loop then was transferred to tryptic soy broth (TSB; Becton Dickinson, Sparks, MD) and incubated for 18 h at 37 °C. The TSB then was centrifuged at 1620 x *g,* the supernatant was poured off and the pellet was resuspended in 10 ml of phosphate-buffered saline (PBS). This step was repeated a total of three times with the last resuspension being in 2 ml of PBS to make a concentrated inoculum. This was performed for all three of the surrogates, and the final product was a 30 ml cocktail.

**Inoculation of the meat**

The strip loins were inoculated with 1 ml of the cocktail of surrogate organisms. The cocktail was spread over the surface of the meat by using a sterile disposable bacterial cell spreader (VWR International, Radnor, PA). After the inoculum was spread over the surface, the strip loin sections were allowed a dwell time of 30 min to allow for attachment of the surrogates to the product. The strip loin sections were inoculated with 8.6 log CFU/$cm^2$ of the surrogate organisms.

**Microbiological sampling and analysis**

Rifampicin-resistant organisms were used and plated using serial dilutions on rifampicin-resistant tryptic soy agar (rif-TSA). The rif-TSA was prepared by adding .1 g of rifampicin (Sigma-Aldrich, St. Louis, MO) dissolved in 5 ml of methanol to 1 liter of autoclaved and cooled (55 °C) TSA. Before inoculation, three 10-$cm^2$ samples were

taken from random strip loin sections using a sterile stainless steel borer, scalpel, and forceps to determine if any of the surrogates were naturally present on the product. On Day 0, after the strip loins were cut in half and inoculated, two 10-$cm^2$ samples were excised from each strip loin section using a flame sterilized borer, scalpel and forceps. Buffered peptone water (99 ml) was added to each sterile stomacher bag that the samples were placed in, and the samples were pummeled in the stomacher (A. J. Seward, London, UK) for one minute at 260 rpm. Decimal dilutions were plated on rif-TSA and allowed to incubate for 24 h. After the incubation period, the samples were enumerated and counts were recorded as $\log_{10}$ CFU/$cm^2$. After microbiological samples were taken, the strip loin sections then were vacuum packaged and stored for 3 d until samples were taken again. On Day 3, samples were taken immediately after all 24 sections were removed from the vacuum packages. The lactic acid was applied to 12 sections, then samples were collected from all 12 sections before vacuum packaging 6 sections for wet-aging and placing 6 sections on trays for dry-aging. After the strip loin sections were aged for 28 d, samples were taken from all 24 sections. Lactic acid intervention was applied to the remaining 12 untreated strip loin sections, and microbiological samples were taken.

**Pathogen intervention**

A Flo-Master garden pump sprayer (Root-Lowell Manufacturing Company, Lowell, MI) was used to apply the 5% lactic acid spray. Preliminary tests showed the flow rate for the sprayer was approximately 6ml/sec. The sprayer was filled with approximately

5% lactic acid solution that had a pH of 1.8. To ensure the temperature of the lactic acid solution was maintained at greater than 50 °C and less than 55 °C, temperature was taken before spraying, midway through spraying, and at the end of spraying. Pieces were held with meat hooks and sprayed for 5 sec per side (10 sec total).

**Statistical analysis**

All microbiological data were converted into logarithms before obtaining means and performing statistical analyses. In the case of counts below the minimal level of detection, the lowest level (0.5) of detection was used to facilitate data analysis. Data were analyzed using JMP® Software (JMP® Pro, Version 9.0.0, SAS Institute Inc., Cary, NC 1989-2010). Reduction of log counts for aging method (dry- vs. wet-aging), lactic acid application (before vs. after aging), and their interaction were evaluated using Fit Model Least Squares Means.

# CHAPTER III

# RESULTS

Presented in Table 1 are the log counts for the initial attachment of the rif-resistant *E. coli* on the surface of the unchilled strip loin sections and the counts after vacuum packaging and chilling for 3 d. The attachment was 5.9 $\log_{10}$ CFU/$cm^2$ after the inoculation of 8.6 $\log_{10}$ CFU/$cm^2$. There was a decrease ($P < 0.05$) in counts after the 3 d packaging and chilled storage period. These Day 3 counts served as the beginning levels for the evaluation of the log reductions due to the treatments.

Reductions in least squares means for method of aging and lactic acid application are presented in Table 2. There was a significant ($P < 0.05$) method of aging effect for reduction of microorganisms. Dry-aged sections had a greater ($P < 0.05$) log reduction that those sections that were wet-aged. There was no impact ($P > 0.05$) on microbiological counts whether the lactic acid was applied before or after dry- or wet-aging. In addition, there was no significant interaction ($P > 0.05$) between method of aging and the lactic acid application. Dry-aging reduced pathogens on meat surfaces, and lactic acid appeared to be equally effective as a pathogen intervention when applied before or after dry- or wet-aging.

Research has shown that dry-aging beef carcasses can result in a decreased number of pathogens on the carcass, and that dry-aging could be used as a small plant pathogen

intervention (Buege & Ingham, 2003). Another study found similar results in that dry-aging effectively reduced the pathogens on beef carcasses (Algino, Ingham, & Zhu, 2007). In this study, three different dry-aging periods were investigated and each one resulted in a statistically significant decrease in the prevalence of *E. coli*. Algino, Ingham, and Zhu (2007) utilized beef carcasses for their dry-aging study, but this study is the first known to investigate dry-aging boneless beef subprimals as a possible form of pathogen intervention.

Lactic acid can be very successful at reducing bacterial counts on carcasses (Castillo, Lucia, Mercado, & Acuff, 2000). Research has shown that lactic acid treatment can have a great impact on pathogen reduction on carcass surfaces (Hardin, Acuff, Lucia, Oman, & Savell, 1994). There are some instances where lactic acid treatments are not as effective as in other studies. In a study conducted by Youssef, Yang, Badoni, and Gill (2012), an application 5% lactic acid was applied to different surfaces to determine the effectiveness on the reduction of *E. coli*. The lactic acid treatment produced inconsistent results in pathogen reduction, which was believed to be related to the surface to which it was applied. In this research, there were great differences in the dry-aged and wet-aged surfaces; however, the application of lactic acid was equally effective for both surfaces.

**Table 1**
Least squares means for initial attachment (Day 0) and after chilled, vacuum package storage (Day 3) on counts ($log_{10}$ CFU/$cm^2$) of rifampicin-resistant *Escherichia coli.*

| | Log |
|---|---|
| Day 0 | 5.9[a] |
| Day 3 | 5.5[b] |

Means lacking a common letter (a-b) differ ($P < 0.05$).

**Table 2**
Least squares means for method of aging and lactic acid application on log reductions ($log_{10}$ CFU/$cm^2$) of rifampicin-resistant *Escherichia coli.*

| Treatments | Log reduction |
|---|---|
| Method of aging | |
| Dry | 4.0[a] |
| Wet | 2.7[b] |
| Lactic acid application | |
| Before | 2.9[a] |
| After | 3.8[a] |

Means lacking a common letter (a-b) within a treatment differ ($P < 0.05$).

# CHAPTER IV

# SUMMARY AND CONCLUSIONS

Dry-aging is not a widely used practice in the U.S. meat industry due to the time, cost, and loss of yield that it takes to achieve the final dry-aged product. The limited scientific studies that have been reported on dry-aging have primarily focused on its use in improving the palatability of beef and not on what this process may do to reduce the number of pathogens on meat. An aspect of this work was to determine if the point of application of a pathogen intervention such as 5% lactic acid should be applied at the beginning or end of the aging process. This work showed that applying lactic acid to beef subprimals was equally effective when applied either before or after the aging process. What was surprising was the impact that dry-aging had on pathogen reduction. Findings of this research may not lead to significant changes in how beef is aged before being marketed, but there may be limited opportunities for some foodservice and retail operators to explore dry-aging not only for its use in improving palatability, but also for its potential role in reducing pathogens.

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