THESIS

# EFFECTS OF VARIOUS PROCESSING TECHNIQUES AND INTERVENTIONS ON BEEF SAFETY AND SHELF-LIFE


Submitted by

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ABSTRACT

Two experiments were conducted; the first evaluated decrease in log survival of pathogenic bacterial populations using three antimicrobial interventions (Peroxyacetic acid – PAA; Lactic Acid – LA; lactic/citric acid blend – LCA) applied at a spray cabinet used just before carcass chilling. Efficacy was evaluated using a Shiga-toxin producing *Eschericia coli* (STEC) inoculation cocktail that incorporated two strains of *E. coli* O157:H7 and 12 non-O157 STEC strains. In addition, this study was intended to validate the use of non pathogenic *E. coli* to serve as surrogates for the aforementioned STEC cocktail in plant operations. Influence of the carcass interventions on color stability of beef subprimals over a 30-day storage period was included to simulate effects on storage and display life. Each day, for three sampling days, 90 hot tissue samples from the plate subprimal were obtained immediately following slaughter. The tissue samples were evenly split into two inoculation groups (n = 45 samples/group): 1) STEC, or 2) surrogate. Within each inoculation group, samples were assigned randomly to one of nine treatments: i) 200 ppm PAA; ii) 1% LCA; iii) 1.5% LCA; iv) 2.5% LCA; v) 5% LA; vi) 8% LA; vii) 10% LA; viii) potable water; or ix) untreated control. Samples assigned to the surrogate inoculation group were further portioned into two equal sections for evaluation of the treatment influence on microbiological decrease in log survival and color. Samples were subjected to treatment using a custom-built, laboratory-scale spray cabinet to apply the intervention. Lightness ($L^*$), redness ($a^*$), and yellowness ($b^*$) was evaluated before and immediately following spray application using a portable spectrophotometer. Following assessment of color immediately post-treatment application, the sample was further divided into three subsections that were vacuum packaged and stored for color evaluation at 10, 20, and 30 d. Among samples inoculated with STEC, log survival means with potable water and control were greater ($P < 0.05$)

when compared to all other spray treatment groups. Likewise, the lower ($P < 0.05$) log survival means were observed for 8 and 10% LA treatment groups. No differences ($P > 0.05$) were observed among PAA, 1.5 and 2.5% LCA. Pairwise comparisons of surviving populations of STEC and surrogates revealed that the non-pathogenic strains could be effectively used as surrogates for the STEC cocktail. Color measures of $L^*$ values for samples spray treated with 8 or 10% LA were lower ($P < 0.05$) than for all other treatments, and declined over the 30 d storage period—indicating that the product darkened due to LA exposure and dark storage. Following 10 d dark storage, $a^*$ values were greater ($P < 0.05$) for untreated control samples than for samples sprayed with 1.5 or 2.5% LCA or for samples treated with any level of LA. Spectrophotometric $b^*$ values increased during dark storage ($P < 0.05$) suggesting product discoloration; however, no noticeable trends were observed among or between treatments.

The second experiment monitored spoilage microorganisms, panelist and instrument color, and lipid oxidation changes during retail case display for three ground beef batches individually. After 7, 14, 18 or 21 d of vacuum-sealed, dark refrigerated storage (4°C), three 73/27 ground beef batches (conventional - control; 25% inclusion of advanced meat recovery (AMR) product from plant one – BBFT 1; 25% inclusion of AMR product from plant two – BBFT 2) were separately fine ground, portioned into 454g loaves, and overwrapped with polyvinyl chloride (PVC) film for retail case display (4°C) for 72 h. Sampling for aerobic plate count (APC), lactic acid bacteria (LAB) and 2-thiobarbituric acid reactive substances (TBAR) assay occurred every 24 h during retail case storage. Trained panelist-determined lean color, discoloration and redness intensity values, along with instrument L* (lightness), a* (redness) and b*(yellowness) measurements occurred every 12 h during retail case display. For each of the three products, neither least squares means for APC nor LAB exceeded 7 log CFU/g until after

21 d dark storage. Throughout retail case display, for all products, following all dark storage times, tan/brown discoloration means remained below 3%. With some exceptions, least squares means for panelist-determined lean color and redness intensity declined ($P < 0.05$) predictably, with greater retail case storage time. The L* means increased inconsistently depending on product or dark storage time; however, in several instances, L* values were highest ($P < 0.05$) toward the end of retail case storage. Conversely, a* values generally declined ($P < 0.05$) with increased storage timesindicating a shift from bright red to dull blue color. Few noticeable trends among product and dark storage time were observed for CIE b* values throughout display. Least squares means for TBAR analyses were either similar ($P > 0.05$) or increased ($P < 0.05$) with retail case storage time.

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TABLE OF CONTENTS

ABSTRACT .......... ii

ACKNOWLEDGMENTS .......... v

LIST OF TABLES .......... ix

Chapters

1. REVIEW OF LITERATURE .......... 1

1.1. Fresh Beef Safety .......... 1

1.11. *Escherichia coli* 0157:H7 and non-O157 STEC .......... 2

1.12. *Salmonella enterica*. .......... 5

1.13. Beef Carcass Contamination .......... 6

1.14. Non-Chemical Beef Carcass and Cut Intervention .......... 8

1.15. Organic Acid Interventions .......... 11

1.16. Other Chemical Interventions .......... 14

1.2. Ground Beef Shelf-Life .......... 15

1.21. Beef Color .......... 15

1.22. Lipid Oxidation and Rancidity .......... 18

1.23. Spoilage Microorganisms .......... 19

1.24 Managing Beef Shelf-Life .......... 20

2. VALIDATION OF VARIOUS ANTIMICROBIAL INTERVENTIONS FOR USE IN A BONE DUST CABINET IN A COMMERCIAL BEEF HARVEST FACILITY ........................ 24

Introduction ........................................................................................................ 24

Materials and Methods ........................................................................................ 25

Results and Discussion ........................................................................................ 28

3. SHELF-LIFE TRIAL OF 73/27 GROUND BEEF WITH ADVANCED MEAT RECOVERY (AMR) PRODUCT INCLUSION ........................................................................ 38

Introduction ........................................................................................................ 38

Materials and Methods ........................................................................................ 38

Results and Discussion ........................................................................................ 41

References ............................................................................................................ 58

LIST OF TABLES

**Table 2.1.** Mean values (± SD) for pH of the intervention solution and meat sample following spray-treatment application[1] ....................33

**Table 2.2.** Surviving populations (log CFU/cm$^2$) of Shiga toxin-producing *Escherichia coli*[1] (STEC) and *E. coli* surrogates[2] on hot beef tissue following spray-treatment with various interventions[1] in a custom-built laboratory-scale spray cabinet. ....................34

**Table 2.3.** Comparison (*P*-values) of the survival of a Shiga toxin-producing *Escherichia coli* (STEC) cocktail[1] versus the non-pathogenic *E. coli* surrogate inoculum[2]. ....................35

**Table 2.4.** Instrumental *L** values (lightness) of lean, beef tissue prior to and immediately following antimicrobial application[1] and following 10, 20 and 30 days of vacuum-packaged dark storage (4°C) ....................36

**Table 2.5.** Instrumental *a** values (redness) of lean, beef tissue prior to and immediately following antimicrobial application[1] and following 10, 20 and 30 days of vacuum-packaged dark storage (4°C) ....................37

**Table 2.6.** Instrumental *b** values (yellowness) of lean, beef tissue prior to and immediately following antimicrobial application[1] and following 10, 20 and 30 days of vacuum-packaged dark storage (4°C) ....................38

**Table 3.1.** Simples means (± SD) for crude fat (%) of the ground beef sample following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C).[1]....................48

**Table 3.2.** Least squares means[1] of aerobic plate counts (APC; Log CFU/g) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C) ....................49

**Table 3.3.** Least squares means[1] of lactic acid bacteria counts (LAB; Log CFU/g) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C) ....................50

**Table 3.4.** Least squares means[1] of trained panelist-determined lean color values (0-100%; – tan/brown - bright, cherry-red) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C). .51

**Table 3.5.** Least squares means[1] of trained panelist-determined tan/brown discoloration values (0-100% of lean surface) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C) ....................52

**Table 3.6.** Least squares means[1] of trained panelist-determined redness intensity values (0-100%; pale - intense) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C) ..................53

**Table 3.7.** Least squares means[1] of L* (lightness) values of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C) ..........................................................................................................54

**Table 3.8.** Least squares means[1] of a* (redness) values of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C). .........................................................................................................55

**Table 3.9.** Least squares means[1] of b* (yellowness) values of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C). ...........................................................................................56

**Table 3.10.** Least squares means[1] of 2-thiobarbituric acid reactive substances analysis (TBAR; mgMDA/kg) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C). ...............................57

# CHAPTER 1

## Review of Literature

### 1.1. Fresh Beef Safety

The Centers for Disease Control and Prevention (CDC) reports 48 million illnesses, 128,000 hospitalizations and 3,000 deaths are caused by foodborne pathogens annually in the United States (Scallan et al., 2011). Because of their protein-dense composition, near-neutral pH and high water activity, meat products, in general, serve as an excellent medium for bacterial growth (Aymerich et al., 2008; Mani-López et al., 2012). The consequences of a 1993 *E.coli* O157:H7 outbreak in the Pacific Northwest linked to undercooked ground beef catalyzed reform of intervention protocols, improved outbreak monitoring and increased concern regarding meat safety among consumers (Wheeler et al., 2014).

Subsequent to the aforementioned *E.coli* O157:H7 outbreak, in 1994, the United States Department of Agriculure's Food Safety Inspection Service (USDA-FSIS) deemed components of ground beef manufacture contaminated with *E.coli* O157 adulterated, or unfit for human food () Also, the USDA-FSIS (USDA-FSIS, 1996) implemented the Pathogen Decrease in log survival, Hazard Analysis and Critical Control Points System Final Rule (PR/HACCP), with the intention of addressing "…the serious problem of foodborne illness in the United States associated with meat and poultry products…[and clarifying] the respective roles of government and industry in food safety". The PR/HACCP required removal of visible fecal matter, milk or ingesta (FMI) on beef carcasses (USDA-FSIS, 1998). Additionally, the PR/HACCP required that HACCP systems, Sanitary Standard Operating Procedures (SSOP), microbial testing for generic *E.coli* and performance standards for *Salmonella* be implemented in red meat production (USDA-FSIS, 1998). Furthermore, the regulation mandated thorough inspector training and strict

enforcement of non-compliance, thought to be paramount in protection of consumers from foodborne disease (USDA-FSIS, 1998).

Also, in light of the devastating 1993 *E.coli* 0157:H7 outbreak in the Pacific Northwest, it became increasingly evident that industry improvements in pathogen sourcing and outbreak monitoring was essential to prevent future outbreaks (CDC, 2016). In 1996, PulseNet reformed pathogen detection and outbreak mapping methods with pulse field gel electrophoresis (PFGE) bacterial "fingerprinting" (CDC, 2016). Today, the CDC, USDA, Food and Drug Administration (FDA) and other food companies and organizations make continuous effort through the Partnership for Food Safety Education's (2015) "Fight BAC" health network to inform consumers of proper meat selection and handling techniques.

### 1.11. *Escherichia coli* O157:H7 and non-O157 STEC

*E.coli* is a natural constituent of gut microflora, and most strains are harmless to human health (CDC, 2015; FDA, 2012). However, pathogenic *E.coli* strains are known to cause foodborne illness upon consumption of contaminated food or water (Brooks et al., 2005; Carney et al., 2006; CDC, 2015; FDA, 2012; Mead & Griffin, 1998). Pathogenic *E.coli* strains are categorized within enterotoxigenic, enteropathogenic, enteroaggregative, enteroinvasive, diffusely adherent and enterohemorrhagic pathotypes (CDC, 2015; FDA, 2012). Enterohemorrhagic *E.coli* (EHEC) serotypes, also known as verocytotoxin-producing (VTEC) or shiga toxin-producing *E.coli* (STEC), are of greatest concern in raw, non-intact beef products (CDC, 2015; USDA-FSIS, 2011). Such pathogens include *E.coli* O157:H7 and the non-O157 or "Big 6" STECs (O26, O45, O103, O111, O121 and O145), along with other shiga toxin-producing strains (CDC, 2015; USDA-FSIS, 2011).

The STEC serotypes are gram-negative, rod-shaped, facultative anaerobes classified within the *Enterobacteriaceae* family named based on their somatic (O) and flagellar (H) antigens (FDA, 2012; Gould et al., 2009). The STEC strains are characterized by presence of Shiga-toxin 1 and/or 2 (stx1; stx 2), intimin gene (eae) and enterohemolysin virulence factors (Brooks et al., 2005; Gould et al., 2009). Infections with STEC occur through the fecal-oral route with a low infective dose and result in symptoms including acute, bloody diarrhea and abdominal cramps, collectively referred to as hemorrhagic colitis (Brooks et al., 2005; FDA, 2012; Gould et al., 2009); they can also lead to hemolytic uremic syndrome (HUS).

Complications of hemorrhagic colitis can result in thrombotic thrombocytopenia pupura (TTP), or hemolytic uremic syndrome (HUS), known to cause hemolytic anemia and subsequent renal failure (Brooks et al., 2005; FDA, 2012; Gould et al., 2009). Incidence of HUS is most common in children less than five years of age and is most often a result of *E.coli* O157:H7 infection (Gould et al., 2009). Those STEC strains which produce stx2 are most often associated with HUS when compared with those that produce just stx1 or both stx1 and stx2 (Brooks et al., 2005; Gould et al., 2009).

*E.coli O157:H7*

Following two separate outbreaks of hemorrhagic colitis involving *E.coli* O157:H7 contamination of ground beef, the serotype was first recognized as pathogenic to humans in 1982 (Mead & Griffin, 1998; Rangel et al., 2005). One year later, in 1983, a correlation was made between *E.coli* O157:H7 and the onset of Hemolytic Uremic Syndrome (HUS) (Mead & Griffin, 1998). In 1993, a nationally devastating, multi-state *E.coli* O157:H7 outbreak (causing more than 400 illnesses and four deaths) linked to undercooked ground beef catalyzed reform of the United

States' approach to food safety, diagnosis of foodborne disease and outbreak monitoring (Rangel et al., 2005; Wheeler et al., 2014).

*E.coli* O157:H7 outbreaks have been traced to raw meat from ruminant animals, fruits and vegetables, unpasteurized milk and juice, and unchlorinated water (CDC, 2015; FDA, 2012; Mead & Griffin, 1998). The CDC suggests that bacterial shedding of ruminant animals is most common in warm climates, consequently, *E.coli* O157:H7 infections are most common during summer seasons (Mead & Griffin, 1998).

*Non-O157 STEC*

Similarly to *E.coli* O157:H7, infection from the "Big 6" non-O157 STEC serotypes have been found to cause bloody diarrhea and, in some cases, HUS (Brooks et al., 2005; CDC, 2015; FDA, 2012). Brooks et al. (2005) utilized 940 non-O157 STEC isolates collected from humans across 43 states between 1983-2002 to confirm serotypes and characterize virulence factors. Seventy-one percent of the non-0157 STEC infections were found to be associated with *E.coli* O26, O45, O103, O111, O121 and O145, collectively (Brooks et al., 2005). In fact, because of the virulent nature of the "big 6" STEC, the USDA-FSIS declared the serotypes adulterants (USDA-FSIS, 2011). As of June 4, 2012, the USDA-FSIS began enforcing implementation of routine testing for the "Big 6" in raw beef trimmings (USDA-FSIS, 2011). It may also be noted that PCR screening by facilities with negative results for intimin (eae) and shiga toxin (stx) are accepted as non-compliance with adulterant testing according to the Code of Federal Regulations (USDA-FSIS, 2011). Since 2006, the CDC has reported seven non-O157 STEC outbreaks associated with clover sprouts, frozen food products, flour, human contact and other unknown sources (CDC, 2015).

### 1.12. *Salmonella enterica*

Over 100 million years ago, *Salmonella* evolved from non-pathogenic *E. coli* K-12 by horizontal gene transfer (Morgan, 2007). As described in the FDA's "Bad Bug Book" (2012), *Salmonella* species (*Salmonella* spp.) are gram-negative, rod-shaped, non-spore forming, facultative anaerobes classified within the *Enterobacteriacea* family. *Salmonella* spp. are categorized within two species: *S.* enterica and *S.* bongori. *Salmonella* enterica is further divided into six subspecies. Most notably, and detrimental to human health, are serovars within *S.* enterica subspecies enterica (supspecies I). More than 2,500 serovars have been identified within subspecies I, but a very small percentage of those identified have been associated with human and domesticated animal illnesses (FDA, 2012; Morgan, 2007).

*Salmonellosis* occurs as a result of cytoskeletal modifications to the membrane surface of epithelial cells (Chopra et al., 1999; FDA, 2012). *Salmonella* Typhimirium, specifically, has been found to emerge from the cell membrane, become engulfed by macrophages and delivered to mesenteric lymph nodes, where the pathogen is circulated to other organs (Chopra et al., 1999). This process is in part caused by presence of flagellin, and *Salmonella* enterotoxin gene (stn), virulence factors that induce host inflammation at the epithelial layer (Chopra et al., 1999; Coburn et al., 2007). Virulence genes, stn, are carried on what scientists call *Salmonella* Pathogenicity Islands (SPI); there have been 14 SPIs identified in *Salmonella* enterica spp. (Morgan, 2007). Foodborne infections by *Salmonella* spp. within subspecies I result in two types of illnesses: typhoid fever and intestinal disease/enterocolitis (Coburn et al., 2007; FDA, 2012).

Nontyphoidal *Salmonellosis* is disease caused by all *Salmonella* enterica subspecies I. serotypes, except *Salmonella* Typhi and Paratyphi (FDA, 2012). Nontyphoidal *Salmonellosis* is a self-limiting illness which typically requires a high infective dose and is characterized by

abdominal pain and diarrhea occurring 6-72 hours following exposure (Coburn et al., 2007; FDA, 2012). The USDA-FSIS estimates 1.2 million illnesses, 23,000 hospitalizations, and 400 deaths occur (estimated costs of $3.6 billion) annually as a result of nontyphoidal infections (USDA-FSIS, 2015).

Because *Salmonella* is the most prevalent enteric foodborne pathogen in meat and poultry, the USDA-FSIS (1998) established a verification of system controls program for the pathogen in the PR/HACCP rule. Performance standards were designated as means of process verification to ensure manufacturers were making progress toward limiting contamination by *Salmonella* (USDA-FSIS, 1998). *Salmonella* serotype testing under the PR/HACCP verification program in 2014 revealed the 10 current most common *Salmonella* serovars: *Salmonella* Kentucky, Enteritidis, Montevideo, Typhimirium, Infantis, Dublin, Heidelberg, Newport, Anatum and Agona (USDA - FSIS, 2015).

The USDA-FSIS (2015) reported a 1.6% prevalence of *Salmonella* among 7,000 ground beef samples evaluated the previous year. Of the positive samples, *Salmonella* Montevideo and Dublin accounted for 22.4 and 12.1%, respectively (USDA-FSIS, 2015). Although *Salmonella* Dublin most commonly causes infection in cattle, the organism poses tremendous threat in the United States food supply (USDA-FSIS, 2015). *Salmonella* Dublin is a multi-drug resistant (MDR) organism and, consequently, foodborne illness caused by the serovar results in the greatest number of hospitalizations and mortalities among *Salmonella* spp. annually USDA-FSIS, 2015).

**1.13. Beef Carcass Contamination**

Skeletal muscle of beef carcasses is inherently sterile, but becomes contaminated through contact with intestinal contents, fecal material and, particularly, the animal's hide (Huffman,

2002). The STECs and *Salmonella* spp. exist in the gut of cattle and are shed through the animals' feces (Reid, Small, Avery, & Buncic, 2002). Thus, beef hides can become contaminated with the pathogens by contact with the environment during the feeding, transportation, and the slaughter processes (Reid et al., 2002). According to Wheeler et al. (2014), *E.coli* contamination of beef carcasses occurs primarily during the hide-pulling and skinning phases of beef slaughter. Presence of pathogens on hides serves as the major source of pathogen contamination of beef carcasses, particularly along the midline, where the hide is first separated and detached from the carcass (Baird et al., 2006; Reid et al., 2002; Wheeler et al., 2014). More specifically, the brisket primal is found to be most susceptible to *E.coli* O157:H7 and *Salmonella* contamination (Baird et al., 2006; Reid et al., 2002). This is likely a result of repeated contact of the hide with the ground or floor, where fecal shedding is greatest (Reid et al., 2002). Reid et al. (2002) reported that one in ten briskets samples tested for *E.coli* O157:H7 contamination and one in five sampled positive for *Salmonella* immediately following hide removal.

Further, ground beef from both fed and cull cattle are also subjected to *Salmonella enterica* contamination via the presence of the pathogen within peripheral lymph nodes (PLN; Gragg et al., 2013; Li et al., 2015; Vipham et al., 2015). Because beef fat containing PLN is inevitably part of beef trim, *Salmonella* may become incorporated into ground beef. Because carcass interventions can not influence the internal surfaces of PLN, the beef industry is challenged with controlling this source of contamination pre-harvest (Vipham et al., 2015).

Post-harvest interventions are aimed at removing contamination transferred from the hides before bacterial cell attachment can occur and discussed that there are three major concerns to consider when controlling contamination of fresh beef: " 1) level of pathogens contaminating hides of animals; 2) proficiency in hide removal that minimizes transfer of contamination from

the hide to the carcass; and 3) efficacy of antimicrobial interventions applied at various steps in the process." (Sofos & Smith, 1998)

**1.14. Non-Chemical Beef Carcass and Cut Interventions**

Meat and poultry systems employ a "hurdle technology", or a sequential combination of antimicrobial intervention techniques, for microbial decrease in log survival (Sofos & Smith, 1998; Wheeler et al., 2014). This "multiple-hurdle" concept involves managing intervention technologies, antimicrobial solutions, temperature, pH and packaging techniques throughout the manufacturing process (Sofos & Smith, 1998; Belk, 2001; Huffman, 2002; Mani-López et al., 2012).

*Pre-Harvest Interventions*

Pre-harvest intervention technologies are meant to manage pathogen contamination of the hide and feces (Ransom & Belk, 2003; Woerner et al., 2006). According to Huffman (2002), diet changes (namely increase in roughage prior to harvest), probiotics, sulfate treatment of drinking water and vaccines can be implemented as part of a "multiple-hurdle" system. Ionophores have shown not only to increase feed efficiency in feedlot cattle, but also reduce fecal shedding of *E.coli* 0157:H7 (Woerner et al., 2006). When compared to a control group, Ransom and Belk (2003) found decrease in log survival in *E.coli* O157:H7 isolates on the hides and in the feces of animals subject to neomycin sulfate and *Lactobacillus acidophilus* feed additives and a *E.coli* O157:H7 bacterin vaccine. Individually, each of the additive and vaccine treatments significantly reduced *E.coli* O157:H7, but the most significant decrease in log survivals occurred when treatments were combined (Ransom & Belk, 2003; Woerner et al., 2006).

*Post-Harvest Interventions*

A series of decontamination treatments have proven effective against spoilage and pathogenic microorganisms as part of a synergistic, multiple-hurdle intervention concept in beef production (Aymerich et al., 2008; Belk, 2001; Huffman, 2002). Among the most commonly used treatments are chemical dehairing, hot water treatment, and steam pasteurization/vacuuming (Aymerich et al., 2008; Belk, 2001; Huffman, 2002; Sofos & Smith, 1998).

Nou et al. (2003) found that pre-evisceration carcasses subjected to chemical dehairing showed reduced prevalence of surviving *E.coli* O157:H7 when compared to untreated pre-evisceration carcasses. The chemical dehairing process consists of using a sodium sulfide solution exposure followed by hydrogen peroxide solution and water wash neutralization steps (Nou et al., 2003). This technique was shown effective in removing hair, environmental materials and *Enterobacteriacea* from the hide of beef carcasses (Belk, 2001; Huffman, 2002; Nou et al., 2003). However, sizeable equipment and chemical costs prevent widespread use of chemical dehairing in harvest facilities (Huffman, 2002).

Studies have shown that hot water rinses and/or washes, especially at temperatures exceeding 74-75°C, are effective at reducing bacterial populations on beef tissue (Delmore et al., 1997; Belk, 2001; Huffman, 2002;). In a study performed by Gorman et al. (1995), a combination of hand-trimming and hot-water washing (16-74°C) on surrogate-inoculated brisket adipose tissue resulted in 1.41 – 2.5 log bacterial decrease in log survivals. It was noted that decrease in log survivals increased with increased water temperature and pressure (Gorman et al., 1995). Another study used a hot water rinse (77°C) to achieve a 1.4 log decrease in log survival in inoculated coliforms on inside rounds, along with a combined hand-trimming and hot water (77°C) rinse rinse treatment to attain a 1.8 log coliform decrease in log survival (Delmore et al., 1997).

In a study performed by Nutsch et al. (1997), 46.4, 37.9 and 16.4% of 140 freshly-harvested beef sides tested positive for *Enterobacteriacea,* coliforms, and generic *E.coli*, respectively. Following steam pasteurization treatment of each of the 140 sides, *Enterobacteriacea*-positive carcasses were reduced to 2.9%, coliform-positive carcasses to 1.4%, and generic *E.coli*-positive carcasses to 0% (Nutsch et al., 1997). Steam pasteurization utilizes hot water to effectively reduce pathogens (Belk, 2001; Huffman, 2002). In several facilities, hand-held, steam vacuums are most commonly used as a spot treatment in beef harvest; hot water steam kills bacteria, and vacuum removes physical contaminants (Belk, 2001; Huffman, 2002). It is important to recognize that efficacy of steam vacuuming against contamination and bacterial populations is largely dependent on the thoroughness of the person employing the technology (Belk, 2001).

Aymerich et al. (2008) discussed utilization of alternative, heat-free intervention technologies to avoid alterations to the shelf-life or sensory characteristics of fresh meat, including irradiation, high pressure pasteurization (HPP) and natural antimicrobial agents (lactic acid bacteria LAB). Decrease in log survivals of STEC and *Salmonella* can be achieved with ionizing irradiation by destruction of DNA bonds in the microbes (Aymerich et al., 2008). This process, however, has not gained widespread acceptance in fresh meat processing facilities as a result of implementation costs and fear of employee exposure to radioactive materials (Aymerich et al., 2008). High pressure pasteurization involves applying isostatically transmitted water pressure to packaged meat products in order to denature proteins and inactivate enzymes (Aymerich et al., 2008). Damage to the cell membrane and altered substrate-enzyme interactions have been found effective against gram-negative cell function (Aymerich et al., 2008). Lactic acid bacteria can be used as chemical additive in meat products, because LAB by-products,

including ethanol, hydrogen peroxide, acetic acid and lactic acid, have been found effective against bacterial growth (Aymerich et al., 2008).

**1.15. Organic Acid Interventions**

The FDA deems organic acids "generally recognized as safe" (GRAS) for use in meat processing systems (Mani-López et al., 2012). As a result of their convenience, cost-efficiency and efficacy against food borne pathogens, acetic, lactic and citric acids are frequently used as part of "multiple-hurdle" pathogen intervention (USDA-FSIS, 2017; Mani-López et al., 2012).

Organic acids' mode of action against bacterial cells occurs in two parts: cell membrane disruption and cytoplasmic acidification (Mani-López et al., 2012; Theron & Lues, 2007). Because of their simple molecular structure and relatively small molecular mass, organic acids easily penetrate bacterial cells (Theron & Lues, 2007). Classified as weak acids, organic acids depend on the pH of their environment to determine the rate at which they dissociate (Theron & Lues, 2007). When organic acid molecules contact meat surfaces and existing bacterial contamination, they occur at a low pH (2.0-4.0; undissociated), which allows them to permeate and disrupt the cell membrane (Mani-López et al., 2012; Theron & Lues, 2007). Once the acids are inside bacterial cells, they encounter high pH and dissociate (Theron & Lues, 2007). Then dissociated, the acid releases protons and charged ions, which accumulate to toxic levels in the cell, inhibiting metabolic function (Mani-López et al., 2012; Theron & Lues, 2007).

*Acetic Acid*

Acetic acid is one of the oldest-known antimicrobial chemicals for food preservation (Theron & Lues, 2007). Acetic acid is used in the production of vinegar and has an overpowering flavor and odor, posing risk for adverse sensory alterations (Mani-López et al., 2012; Theron & Lues, 2007). When applied directly to meat surfaces, acetic acid can cause irreversible color,

odor and flavor changes (Theron & Lues, 2007). In the meat industry, acetic acid is allowed to be applied at up to 4% in solution to dried and fermented sausages (USDA-FSIS, 2017).

Among scientific studies using varying antimicrobial-application techniques, acetic acid reduced *Salmonella* Typhimirium populations more than 2.5 log CFU/cm$^2$ and *E.coli* O157 populations by more than 4.5 log CFU/cm$^2$ on carcass tissues (Stivarius et al., 2002). Efficacy of acetic acid against *Enterobacteriacea* increases with application temperature, suggesting that acid penetration of bacterial cells is enhanced by higher temperatures (Mani-López et al., 2012). Stivarius et al. (2002) observed that a 5% acetic acid in solution demonstrated bactericidal and bacteriostatic effects against *E.coli* O157 and *Salmonella* Typhimirium when applied to beef trimmings. However, the acid demonstrated unfavorable effects on odor and color characteristics (Stivarius et al., 2002).

*Lactic Acid*

In 2000, Vold et al. (year?) concluded that an increase in lactic acid bacteria (LAB), as part of background microflora in inoculated ground beef samples, had bacteriostatic effects on *E.coli* O157. Lactic acid is a derivative of LAB fermentation or anoxic respiration and is proven effective at limiting pathogenic bacteria (Mani-López et al., 2012). The USDA-FSIS (2017) defines application of lactic acid within the following parameters: 1) up to 5%  in solution to carcasses prior to fabrication, variety meats and offal; 2) 2-5% in solution at less than 55°C to beef and pork subprimals and trimmings; 3) 2- 2.8 % in solution to beef heads and tongues.

Laboratory-based studies involving use of lactic acid and/or LAB are conducted using diverse solution-application and microbiological sampling techniques, resulting in varied reported decrease in log survival efficacy (Harris et al., 2012). Elebracht et al. (2005) performed a distilled water dip followed by a LA dip (2% LA in solution; 43°C; 15s) on chilled beef trim

and observed a 1.6 and 2.3 log CFU/cm$^2$ decrease in log survival in Rifampicin-resistant *E.coli* O157 and *Salmonella* Typhimirium, correspondingly. Echeverry et al. (2010) observed a 0.60 log CFU/cm$^2$ decrease in log survival of an inoculated 3-strain mixture of *E. coli* O157:H7 immediately following application of 3% LA at 22°C in a custom-built spray cabinet (1.59 lpm at 1.38 bar across 6 nozzles) for subprimals intended for mechanical tenderization. However, Harris et al. (2012) applied LA used at 2% in solution via a laboratory-scale spray cabinet (2% LA in solution; 25°C; 6 nozzles; 0.42 l/min; 10s) and only reported up to a 0.36 log CFU/cm$^2$ decrease in log survival of *E.coli* O157 and up to a 0.45 log CFU/cm$^2$ of *Salmonella* Typhimirium.

*Citric Acid*

Citric acid can be naturally derived from citrus fruit plants or processed from certain species of molds (Mani-López et al., 2012). The weak, water-soluble acid is known for its ability to chelate metal ions which complements its ability to inhibit pathogen growth and survival (Mani-López et al., 2012). Citric acid is considered a safe ingredient for use in meat production, and its use an antimicrobial is approved up to 5% in solution on beef trimmings and supbrimals, as well as application up to 3% in solution on further-processed meat products (USDA-FSIS, 2017). Although citric acid is not commonly used alone as an intervention for pre-rigor beef, Kalchayanand et al. (2015) achieved a 0.7-2.0 log CFU/cm$^2$ decrease in log survival of STEC on chilled beef trim following citric acid spray (3 nozzles; 1.38 bar; 15s) and a 48 h cold storage time (4°C).

*Lactic and Citric Acid Blends*

Lactic and citric acid blends are approved for use on beef, pork, lamb and poultry carcasses, supbrimal cuts, trimmings, offal and variety meats up to 5% in solution (Inspection.,

2017). Seo et al. (2013) observed a synergistic effect of LAB and citric acid, by direct application of a LAB and 2% citric acid solution, on *E.coli* O157 and *Salmonella* Typhimirium cells, which caused 2 and 6 log CFU/cm$^2$ decrease in log survival, respectively. Laury et al. (2009) tested the efficacy of a lactic and citric acid blend against beef tips inoculated with *E.coli* O157 or a *Salmonella* cocktail. Application of the lactic and citric acid blend at 1.5% in solution to the beef tips resulted in a 1.4 log decrease in log survival of *Escherichia coli* O157 and a 1.1 log decrease in log survival in *Salmonella* spp. (Laury et al., 2009). Similar results were achieved by Scott et al. (2015), where a lactic and citric acid blend applied in a custom spray cabinet (1.9% in solution; 50-51°C; 2.07-3.45 bar; 10s) reduced STEC and *Salmonella* populations 0.9-1.5 log CFU/cm$^2$ on beef brisket tissue. The study by Scott et al. (2015) also demonstrated no differences ($P > 0.05$) in efficacies between acid concentrations or application temperatures.

### 1.16. Other Chemical Interventions

*Peroxyacetic Acid (PAA)*

Peroxyacetic acid is considered an organic peroxide and is known to achieve lysis of pathogens by releasing oxygen into cells and disrupting chemiosmotic function (Kitis, 2004). Approved up to 400 ppm in beef, PAA is commonly used at 200-400 ppm in solution in beef carcass intervention systems (USDA-FSIS, 2017; Wheeler et al., 2014).

In 2005, Ellebracht et al. (2005) tested efficacy of PAA against Rifampicin-resistant *E.coli* O157:H7 and *Salmonella* Typhimirium populations on fresh beef trim. Submerging trim pieces into 200, 500 and 100 ppm PAA solutions (43°C; 15s) reduced the target pathogen populations up to 1.0 log CFU/cm$^2$ (Ellebracht et al., 2005). Kalchayanand et al. (2012) utilized a spray cabinet with oscillating nozzles to determine efficacy of 4% LA and 200 ppm PAA (at 22-25°C) at reducing a 5-strain non-O157 STEC and 3-strain O157:H7 STEC mixture on pre-rigor

mortis beef flanks. The researchers observed a 1.6 to 3.1 log CFU/$cm^2$ decrease in log survival of inoculated non-O157 STEC populations following treatment with 4% LA. Conversely, King et al. (2005) observed a minimal (0.7 log CFU/$cm^2$) decrease in log survival of *E. coli* O157:H7 using PAA applied at 200, 600 and 1000 ppm.

**1.2. Ground Beef Shelf-Life**

Ground beef accounts for more than 40% of domestic, fresh beef sales (Suman et al., 2014). Giminez et al. (2012) described "shelf-life" as the duration for which food products maintain microbiological, nutritional and sensory quality. Because ground beef is labile, the industry is presented a challenge to maintain shelf-life quality, and mechanisms affecting ground beef shelf-life throughout retail storage are complex (Suman et al., 2014). Several scientific studies have shown that beef products can be effectively managed via innovative pre-harvest, antimicrobial, packaging and antioxidant technologies, but that process hygiene and storage temperature are paramount in preventing microbiological and sensory deterioration (Mancini & Hunt, 2005; Suman et al., 2014).

**1.21. Beef Color**

Perceived meat color is the most significant factor influencing consumer purchasing decisions at the retail level (AMSA, 2012). Consumers associate the cherry-red color of aerobically packaged beef products and the purplish-red color of vacuum packaged beef products with freshness or wholesomeness (Suman et al., 2014). Discarding whole packages and grinding whole-muscle cuts as a result of discoloration results in approximately $1 billion in profit loss annually (Suman et al., 2014). Thus, the beef industry has a concerted effort to manage color changes in pre and post-harvest systems (Suman et al., 2014).

Meat color is partially dependent upon the chemical form and concentration of myoglobin (FAUSTMAN et al., 1989; Savell, 2015). According to the AMSA Meat Color Guidelines (2012), myoglobin is a water-soluble, sarcoplasmic protein involved in binding, storing, and transporting oxygen to mitochondria within muscle cells. This protein is identified by a polypeptide chain backbone linking eight alpha-helices, along with a heme-iron ion in the protein's hydrophobic center of a porphyrin ring, known to interact with six ligands. The valence state of the heme iron, coupled with the compound interacting with the sixth—or "free"—ligand, determines the chemical form of myoglobin. Although other chemical forms exist, the four major forms of myoglobin related to meat color are deoxymyoglobin (DMb), oxymyoglobin (OMb), carboxymyoglobin (COMb) and metmyoglobin (MMb). Deoxymyoglobin, OMb and COMb exist when myglobin's heme iron is in the ferrous ($Fe^{2+}$) state, while MMb is formed when the iron is oxidized to a ferric ($Fe^{3+}$) state (Faustman et al., 1989; AMSA, 2012;)

Deoxymyoglobin is formed when there is no compound bound to the sixth ligand; in this state, muscle appears purplish-red in color. This state is maintained in uncut meat surfaces that are not exposed to oxygen, and by vacuum packaging techniques. In some cases, from DMb, the sixth ligand may form an exceptionally stable covalent bond with carbon monoxide and form COMb and, subsequently, a desirable cherry-red color. Otherwise, oxygenation of DMb occurs when oxygen covalently bonds to the sixth ligand, and the ligand interacts with distal-histidine 64. This reaction, also known as "bloom", results in formation of OMb and generation of a bright, cherry-red surface color (AMSA, 2012).

Prolonged exposure to oxygen promotes oxidation of ferrous iron to its ferric valence state and the binding of water to the sixth ligand, forming MMb and resultant subsurface and surface discoloration (AMSA, 2012; Mancini & Hunt, 2005). Metmyoglobin reducing activity

(MRA) and oxygen consumption rate (OCR) of myoglobin are among the most studied phenomena relative to MMb formation (Mancini & Hunt, 2005). The MRA is thought to be inversely related with heme iron oxidation and is influenced by myoglobin's oxygen scavenging efficiency and presence of proton donors (i.e., NADH; Mancini & Hunt, 2005; Suman et al., 2014). Decrease in log survival of oxygen tension can convert OMb back to DMb in a two-step reaction, where heme iron is first oxidized (forming MMb), then reduced (Mancini & Hunt, 2005). Not surprisingly, OCR has been shown to have a direct relationship with heme iron oxidation and color stability (Tang et al., 2005). Tang et al. (2005) found that increased concentrations of mitochondria in cardiac muscle cells increased OCR of myoglobin, affirming that species and muscle type affect color stability and subsequent shelf life.

*Quantifying Color*

In 1976, the Commission Internationale de l'Eclairage (CIE) developed a three-dimensional color model, known as the CIE L*a*b* color space (AMSA, 2012). This spherical, solid model comprehensively described color as perceived by the human eye, with a* (+60 = red; -60 = green) on its X-axis, b* (+60 = yellow; -60 = blue) on its Y-axis and L* (100 = white; 0 = black) on the Z-axis, or third dimension (AMSA, 2012). Deviations from the X-axis via incident angles determine the hue (color) of sample, while distance from the origin of X, Y and Z axes determine the chroma, or saturation of color (AMSA, 2012).

Although there are several spectrophotometers with varying illuminant light sources, portable spectrophotometers using illuminant A are popular for use in objective meat color measurement (Tapp III, Yancey, & Apple, 2011). Long, red wavelengths associated with illuminant A are known to result in values that most highly correlate with visual color assessments (Tapp III et al., 2011). In instrumental color analysis, L*, a* and b* are not used as

absolute measures of color, but rather relative measures observed for correlation with visual color assessment (AMSA), 2012; Tapp III et al., 2011).

According to the AMSA Meat Color Guidelines (2012), panels using consumers and rigorously trained personnel may serve as significant sources of color stability information. Properly trained color panelists will evaluate color attributes on anchored scales, omitting personal preference and bias. Training serves as a means for uniform interpretation and communication of different color characteristics, and values obtained from trained panels are considered an objective measurement (comparable to instrument color analysis) when performing meat research (AMSA, 2012).

Mancinin and Hunt (2005) explain that, while L* (lightness) and a* values can be strongly related to visual color analysis, training to correlate color perception with b* values has proven challenging. Although yellow and blue are not directly associated with red meat products, studies have shown a direct relationship between glycogen content, pH values and b* value. In other words, meat products which retain a higher ultimate pH and subsequent darker color result in greater yellowness than those products with lower ultimate pH (Mancini & Hunt, 2005).

**1.22. Oxidation and Rancidity**

According to Labuza (1971), by-products of unsaturated fatty acid oxidation cause rancidity in food products. Phospholipids (high in polyunsaturated fatty acids) present in the mitochondria and cell membrane of muscle cells are especially susceptible to oxidation (Campo et al., 2006). Lipid oxidation occurs through a free-radical cascade mechanism known as auto-oxidation, where environmental influences, such as heat and oxygen, encourage pro-oxidants to interact with unsaturated fatty acids and form free-radical substances (Campo et al., 2006). First, oxygen is bound to carbon atoms adjacent to an unsaturated carbons, and hydroperoxides are

formed as reaction intermediates (Watts, 1954). Labile hydroperoxides, further break down to form products that contribute to off-flavors and odors (Watts, 1954).

Malonaldehyde, a secondary by-product of polyunsaturated fatty acid oxidative degradation, is extracted in meat samples to quantify the amount of lipid and pigment oxidation (Guillén-Sans & Guzmán-Chozas, 1998). The 2-thiobarbituric acid reactive substances (TBAR) assay uses distillation and acid-extraction methods to measure malonaldehyde concentration as a means to compare lipid and pigment oxidation occurring in meat samples (Guillén-Sans & Guzmán-Chozas, 1998). When evaluating effects of additives on MRA, Hutchins et al. (1967) observed a positive correlation between MMb formation and malonaldhyde concentration.

**1.23. Spoilage Microorganisms**

Ercolini et al. (2009) explained that organoleptic changes in meat products are caused, in part, by microbiological presence and growth (Ercolini et al., 2009). Spoilage microorganisms actively consume nutrients present in meat products, including sugars and free amino acids. In this consumption process, microorganisms release objectionable volatile metabolites. Propensity of a given meat product to express microbiological deterioration is related to pH, water activity, oxygen availability, packaging type (aerobic vs, anaerobic) and temperature (Ercolini et al., 2009).

Common meat spoilage microorganisms include *Lactobacillus, Psuedomonas, Acinetobacter, Aeromonas, Altermonas, and Brochothrix* spp. (Huffman, 2002). Ercolini et al. (2009) described that growth of *Pseudomonas* is most prevalent in high-oxygen packaging, such as traditional polyvinyl overwrap, while *Lactobacillus* has the highest potential for proliferation in vacuum packaged meat products.

### 1.24. Managing Beef Shelf Life

Shelf-life improvement technologies are important in order to manage pigment oxidation, lipid oxidation and microbiological deterioration, and these strategies will be reviewed further below. However, as previously discussed, likelihood of consumers making the fresh meat purchase-decision in a retail setting is largely dependent on visual color appraisal(Mancini & Hunt, 2005; Suman et al., 2014; Zerby et al., 1999). Thus, most interventions aimed at improving meat shelf life are, by default, aimed at improving color. Several antemortem and postmortem factors influence myoglobin and color chemistry, including genetics, nutrition, chilling rate and subsequent pH decline, seam fat and marbling, additives and packaging techniques (AMSA, 2012).

*Pre-Harvest*

Shelf-life and color stability are primarily contingent on intrinsic factors, such as animal species, genetics and age, as well as individual muscle type and function (AMSA, 2012). Reagan et al., (1977), Mancini and Hunt (2005) and Suman et al. (2014) reported variation in shelf-life characteristics of whole muscle beef cuts from grass versus grain-fed cattle. Further, alpha-tocopherol (Vitamin E) concentrations are inherently higher in lean tissue from grass-fed animals, inhibiting free radical-induced peroxidation of polyunsaturated fatty acids (PUFA; Faustman et al., 1989; Suman et al., 2014; Zerby et al., 1999). Still, these positive attributes regarding shelf-life of grass-fed beef are offset by elevated levels of PUFA (Reagan et al., 1977; Suman et al., 2014). Specifically, grass-fed beef contained more alpha-Linoleic (Omega-3) acid, a labile fatty acid prone to accelerated lipid oxidation, than grain-finished beef (Mancini & Hunt, 2005; Reagan et al., 1977; Suman et al., 2014).

Diet supplementation techniques have been explored to improve color (Mancini & Hunt, 2005; Suman et al., 2014). Vitamin E supplementation to grain-finished cattle has shown to increase lipid stability and increase beef shelf-life (Zerby et al., 1999). Also, Vitamin A restriction in cattle diets are known to increase lean tissues redness (Suman et al., 2014). Additionally, Mancini and Hunt (2005) discussed that studies have shown soy hulls improve overall muscle color.

*Post-Harvest*

Current efforts made to improve shelf-life primarily consist of application of antimicrobial solutions and implementation of various packaging techniques (Mancini & Hunt, 2005; Suman et al., 2014). Logically, application of antimicrobial solutions to subprimals and retail meat products can be used to reduce initial loads of spoilage microflora and prevent microbial growth throughout retail display (Mancini & Hunt, 2005). Concerted effort has been made to evaluate effects of lactic acid treatment on instrumental and panelist ratings for color and sensory characteristics of beef trimmings and ground beef (Semler et al., 2013; Stivarius et al., 2002). Some studies have shown 2-4% lactic acid in solution increased L* (lightness) and decreased a* (redness) of beef samples throughout refrigerated retail case storage (Stivarius et al., 2002; Semler, 2013). Similarly, hot water plus lactic acid treatment has shown to decrease panel-determined redness and increase lightness (Mancini & Hunt, 2005). Conversely, Jimenez-Villarreal et al. (2003) and Harris (2013) observed no difference in color values between control and lactic acid-treated samples. In other studies, 5% acetic acid reduced redness and overall color stability on fresh beef trim and resultant ground beef (Mancini & Hunt, 2005).

Modified atmosphere packaging (MAP) systems are achieved by replacing atmospheric oxygen with specified mixtures of gasses; they have proven an effective method for managing

myoglobin chemistry and color stability (Suman et al., 2014). High oxygen MAP (Hi-Ox MAP; perhaps the most common case-ready packaging technique) provides an environment made up of 80% oxygen and 20% carbon dioxide (Mancini & Hunt, 2005; Suman et al., 2014). Its high oxygen tension promotes and maintains oxymyoglobin formation, but promotes lipid oxidation (Mancini & Hunt, 2005; Suman et al., 2014). Inclusion of carbon monoxide in MAP systems (CO-MAP) at levels up to 0.4% have proven immensely effective at stabilizing desirable, red color in retail meats, even under abusive temperatures (Mancini & Hunt, 2005; Rogers et al., 2014; Suman et al., 2014). One common concern regarding CO-MAP is its potential to mask spoilage, but some have observed that off-odors should still be present in microbiologically or oxidatively spoiled products (Mancini & Hunt, 2005; Suman et al., 2014).

Vacuum packaging systems are effective at preventing myoglobin oxidation because of the low oxygen tension vacuum sealing imposes on lean product (Suman et al., 2014). However, consumers prefer bright, cherry-red beef color of OMb to the purple associated with DMb (Suman et al., 2014). Use of films which incorporate nitrite into the polymer layers for vacuum packaging materials have been shown to be effective in promoting formation of Nitric oxide myoglobin (NOMb) and its resultant red color, but consumer concerns for nitrite levels have hindered popular use of this technology (Suman et al., 2014). Furthermore, as described by Martin et al. (2013), "…consumers are steadfast in their acceptance of polyvinyl chloride (PVC) packages." Despite advantages associated with color stability when Hi-Ox MAP or CO-MAP packaging has been used, via the master-bag concept, PVC packaging in the retail case remains prevalent (Martin et al., 2013). Techniques to improve shelf-life and prevent purchase discrimination of beef products in PVC packaging and involve carefully managing storage temperatures, along with use of master-packaging technology. Low oxygen (Lo-Ox) MAP

involves replacing oxygen in a “mother bag” (containing PVC packages) with a gaseous mixture usually devoid of oxygen (Suman et al., 2014). By this means, color stability of overwrapped packages can be prolonged (Suman et al., 2014).

Interventions to inhibit free-radical oxidation exist in the meat industry in the form of synthetic and natural antioxidant compounds, often applied to trimmings before grinding (Suman et al., 2014). Synthetic compounds, including butylated hydroxyanisole (BHA) and buylated hydroxytoluene (BHT), when applied at 0.2%, are proven largely effective at maintaining a* values and preventing lipid oxidation throughout retail case storage (Ahn et al., 2004). Naturally antioxidant compounds, such as grape seed extract, chitosan, olive leaf extract and rosemary extract are in some instances equally, or exceedingly, effective at preserving shelf-life as synthetic antioxidants (Suman et al., 2014).

Other interventions, such as potassium lactate, succinate, malate and pyruvate can be directly applied to meat surface to increase MRA by competing with Mb for oxygen and introducing reducing agents, such as NADH (Suman et al., 2014). These interventions darken, but further stabilize beef color (Suman et al., 2014).

# CHAPTER 2

# Validation of Various Antimicrobial Interventions for use in a Bone Dust Cabinet in a Commercial Beef Harvest Facility

## Introduction

As a result of their convenience, cost-efficiency and efficacy against foodborne pathogens, organic acids and other chemicals are frequently used as part of beef industry pathogen interventions (Belk, 2001; Mani-López et al., 2012). Previous research has shown that peroxyacetic acid (PAA), lactic acid (LA), and acid blends applied at the spray cabinet and as submersion treatments are effective against *Enterobacteriacea* (Ellebracht et al., 2005; Kalchayanand et al., 2015; Laury et al., 2009; Mani-López et al., 2012). Although significant data regarding the efficacy of variation interventions exists, validation of new and emerging antimicrobial interventions is paramount in order to assemble the most effective, convenient and economical constituents of a multiple-hurdle system (Pohlman et al., 2002).

Therefore, the objective of this study was to evaluate survival of pathogenic bacterial populations subjected to three antimicrobial interventions (200 ppm peroxyacetic acid – PAA; three concentrations of lactic acid – LA; and three concentrations of a lactic/citric acid blend – LCA) applied in a custom-built laboratory-scale spray cabinet simulating carcass application parameters. Efficacy of antimicrobial interventions were evaluated using a seven-serogroup Shiga toxin-producing *Escherichia coli* (STEC) cocktail (O157:H7, O26, O45, O103, O111, O121, and O145). In addition, this study served to validate the utility for non-pathogenic *E. coli*, specifically a 5-strain mixture, to serve as surrogates for the aforementioned STEC cocktail. Lastly, the influence of carcass interventions on the color stability of beef tissue over a 30-day storage period was evaluated

## Materials and Methods

*STEC and Surrogate Bacterial Strains and Inoculum Preparation*

The STEC inoculum was comprised of two strains of *E. coli* O157:H7 and two strains each of the "Big Six" non-O157 STEC serogroups (i.e., O26, O45, O103, O111, O121, and O145). The non-pathogenic *E. coli* biotype I inoculum included strains ATCC BAA-1427, ATCC BAA-1428, ATCC BAA-1429, ATCC BAA-1430, and ATCC BAA-1431 (Cabrera-Diaz et al., 2009; Niebuhr et al., 2008). All strains used in this study were resistant to 100 µg/ml rifampicin. Rifampicin-resistant strains were used to facilitate selective enumeration of the inoculum from microbial populations naturally associated with fresh beef tissue. Before each replication, STEC and *E. coli* biotype I strains were individually cultured and subcultured (35°C, 24 ± 2 h) in 10 ml of tryptic soy broth (TSB) (Difco, BD; Sparks, MD) supplemented with 100 µg/ml of rifampicin (Sigma; St Louis, MO). Broth cultures (10 ml) of all strains (per inoculum type) were combined and cells were harvested by centrifugation (4000 rpm, 20 min at 4°C). Resulting cell pellets were washed with 10 ml of phosphate-buffered saline (PBS; pH 7.4) (Sigma), re-centrifuged, and then resuspended to the original inoculum volume in PBS to obtain an inoculum population of approximately 8 to 9 log CFU/ml.

*Beef Tissue Collection and Inoculation*

Each day, for four sampling days, 90 hot beef tissue samples from the plate region were obtained from carcasses select randomly immediately following slaughter. Tissue samples were transported to the Center for Meat Safety & Quality at Colorado State University (Fort Collins, CO) in an insulated cooler and maintained at ambient temperature. Upon arrival, the tissue samples were evenly split into two inoculation groups (n = 45 samples/group): 1) STEC, or 2) surrogate. Within each inoculation group, samples were randomly assigned to one of nine

treatments: i) 200 ppm PAA; ii) 1% LCA; iii) 1.5% LCA; iv) 2.5 LCA; v) 5% LA; vi) 8% LA; vii) 10% LA; viii) potable water; or ix) untreated control. Samples assigned to the surrogate inoculation group were further portioned into two equal sections for evaluation of the treatment influence on microbiological plate counts and post-spray color.

Inoculation. Edible carcass ink was used to designate a 50 $cm^2$ area on the external fat surface for inoculation of sample tissues. The external fat surface of each 50 $cm^2$ section was spot inoculated (targeted inoculation of 6 log CFU/$cm^2$) with 100 µl of the STEC or surrogate inoculum using methods described by Pittman et al. (2012). The inoculum was then spread over the marked area using a sterile plastic spreader. After inoculation, samples were held for 20 minutes to allow for bacterial cell attachment. Following attachment, pre-treatment bacterial populations were assed from the control treatment group, while all other treatment groups were subjected to spray treatments as described below.

*Beef Tissue Treatment and Sampling Frequency*

*Intervention Formulation.* Intervention treatments were formulated each day according to manufacturer's instructions using potable water. The pH of the intervention solution was recorded for every replication and are reported in Table 2.1.

*Intervention Application.* Within each inoculum type, samples were subjected to their respective treatment using a custom-built laboratory-scale spray cabinet which used eight floodjet spray nozzles (Spraying Systems Co.; Wheaton, IL) to apply the intervention solution at a rate of 0.53 lpm at 1.38 bar. The spray cabinet was flushed and cleaned between intervention applications. Individual sample weights were obtained from those in the surrogate group before and following spray treatment application. Although not reported, post-spray weight was approximately 0.01% greater than the initial weight (Table 2.1).

*Microbiological Analysis*

Microbiological samples (within the pre-marked 50 $cm^2$ section) were obtained approximately 10 min after spray-treatment application using a sampling sponge hydrated with 10 ml of Dey/Engley (D/E) neutralizing broth (Difco, BD). Samples were obtained by vigorously sponging the interior of a 50 $cm^2$ section on the external surface of the sample. After sampling, 15 ml of D/E neutralizing broth was added to all sponge samples to formulate a total of 25 ml diluent volume (10 ml D/E + 15 ml D/E). Afterwards, sponge samples were pummeled (Masticator, IUL Industries, Barcelona, Spain) for 2 min at 230 rpm before formation of serial (10-fold) dilutions in 0.1% buffered peptone water (Difco, BD). Appropriate dilutions were plated in duplicate on tryptic soy agar (Acumedia, Neogen Corp.; Lansing, MI) with rifampicin (100 µg/ml; TSA+rif) to enumerate rifampicin-resistant STECs and surrogate *E. coli*. Agar plates were incubated at 35°C for 24 h before manual enumeration of colonies. Surviving populations on duplicate plates were averaged and converted to log CFU/$cm^2$ values before statistical analysis.

*Color Evaluation and pH Analysis*

<u>Color Evaluation</u> Within the surrogate inoculum group, a subsection of lean tissue was reserved for evaluation of lean color following spray intervention treatment. International Commission on Illumination (CIE) lean tissue color was evaluated before and immediately following spray application using a portable spectrophotometer (Hunter MiniScan XE Plus; Reston, VA) with illuminant A, a standard observer angle of 10°, d50 setting, and a 5 mm aperture and following guidelines provided by the American Meat Science Association (AMSA, 2012). Specifically, lean lightness (CIE $L*$), redness (CIE $a*$), and yellowness (CIE $b*$) were evaluated in triplicate for each sample. Following assessment of color immediately post-spray,

the sample was further divided into three subsections that were vacuum packaged and stored (2 to 4 °C) for color evaluation at 10, 20, and 30 d. At each storage interval, the sample was removed from the vacuum package and allowed to bloom for 20 min prior to color assessment as described above. The three scans from each sample piece were averaged to form a single observation value before statistical analysis.

*pH Analysis* From each of the designated day 0 color samples, a 10 g sample was obtained for pH evaluation using the methods described by Luque et al. (2011). For pH measurement, samples were diluted (1:5 dilution) with deionized water and stomached for 2 min at 230 rpm. The pH was measured with a calibrated pH meter fitted with a glass electrode (Denver Instruments, Arvada, CO). pH was measured in triplicate and results values were averaged prior to analysis.

*Statistical Analysis*

This experiment was conducted as a complete, randomized block design, using treatment day as block. Separate analyses of microbiological results were performed for STEC and surrogate inoculation groups. Data were evaluated using the MIXED procedure of SAS (v9.2; Cary, NC). Further, STEC and surrogate comparisons for validation were evaluated using the MIXED procedure of SAS with the microbial population of the untreated control samples used as a covariate. Microbial populations evaluated following spray intervention application are expressed as least squares means of log $CFU/cm^2$ and differences were determined using an $\alpha$ of 0.05. The color portion of the experiment was conducted as a longitudinal repeated measure design and data were evaluated using the MIXED procedure of SAS. CIE colorimeter values ($L^*$, $a^*$ and $b^*$) were expressed as least squares means and an $\alpha$ of 0.05 was used for mean separation.

## Results and Discussion

*Microbiological Results*

Effects of the various antimicrobial intervention treatments on log survival of STEC and surrogate bacteria on hot beef tissue are shown in Table 2. Among samples inoculated with STECs, the remaining plate counts subsequent to treatment with potable water were lower ($P < 0.05$) when compared to all other spray treatment groups. Likewise, the greatest decrease in log survival ($P < 0.05$) were observed for 8 and 10% LA treatment groups. Within the 10% LA treatment group decrease in log survival were higher ($P < 0.05$) than the 8% LA treatment group. No differences in log survival of STEC of surrogate populations ($P > 0.05$) were observed among PAA, 1.5 and 2.5% LCA. Similarly, plate counts for remaining STEC and *E. coli* surrogates were greater ($P < 0.05$) for samples treated with water when compared to all other treatment groups. In agreement with the STEC inoculum, the greatest log survival ($P < 0.05$) in surrogate populations was observed for samples sprayed with 10% LA. Additionally, regardless of level, LA treatments were more effective ($P < 0.05$) than PAA or any of the tested concentrations of LCA. Further, not surprisingly, as LA concentration increased, surviving STEC and surrogate populations decreased ($P < 0.05$).

Pairwise comparisons of surviving populations of STECs and surrogates revealed that the non-pathogenic strains could be effectively used as surrogates for the seven-serogroup STEC cocktail (Table 3).

Previous research has demonstrated efficacy of LA as a surface intervention for beef tissues. Echeverry et al. (2010) observed a 0.60 log CFU/cm$^2$ decrease in log survival of an inoculated 3-strain mixture of *E. coli* O157:H7 immediately following application of 3% LA at 22°C in a custom-built spray cabinet (0.42 lpm at 1.38 bar across 6 nozzles) for subprimals

intended for mechanical tenderization. The spray cabinet parameters in the Echeverry et al. (2010) study were comparable to those used in the current experiment (0.53 lpm at 1.38 bar across 8 nozzles), and results reported by Echeverry et al. (2010) were consistent with remaining plate counts obtained in our study for the STEC inoculum (0.58 log CFU/$cm^2$) using 5% LA. However, cold tissues were utilized by Echeverry et al. (2010) versus the pre-rigor tissues utilized in the current study. Regardless, the current study and the study conducted by Echeverry et al. (2010) both suggested that the efficacy against STEC with low-levels of LA is less than 1.0 log CFU/$cm^2$. Conversely, Heller et al. (2007) observed a 0.9 to 1.1 log CFU/100 $cm^2$ decrease in log survival of a 3-strain cocktail of *E. coli* O157:H7 using 2.5 and 5.5% LA at 55°C. Although spray cabinet parameters were not specified in the Heller et al. (2007) study, heated acids (55°C) have been shown to be more effective at reducing *E. coli* O157:H7 populations (Kalchayanand et al., 2012). As suggested in a study performed by Praisai et al. (1997), bacterial cell lysis by LA increases with time. Lactic acid at 1.5% in solution applied to beef subprimals demonstrated increased efficacy sequentially at 14, 28, 56, 84 and 126 d vacuum packaged, refrigerated dark storage (Praisai et al., 1997).

Kalchayanand et al. (2012) utilized a spray cabinet with oscillating nozzles to determine efficacy of 4% LA and 200 ppm PAA (at 22-25°C) at reducing a 5-strain non-O157 STEC and 3-strain O157:H7 STEC mixture on beef flanks. The researchers observed a 1.6 to 3.1 log CFU/$cm^2$ decrease in log survival of inoculated non-O157 STEC populations following treatment with 4% LA. Furthermore, 0.9 to 1.5 log CFU/$cm^2$ decrease in log survivals of surviving populations of STEC populations were obtained with 200 ppm PAA (Kalchayanand et al., 2012). However, in agreement with the current study, King et al. (2005) observed minimal decrease (0.7

log CFU/$cm^2$) in surviving populations of *E. coli* O157:H7 using PAA applied at 200, 600 and 1000 ppm.

In a previous study performed at Colorado State University, Scott et al. (2015) observed a 0.9-1.1 log CFU/$cm^2$ decrease in log survival of *E. coli* O157:H7 (5-strain mixture), non-O157 STEC (12-strain mixture; two strains each of O26, O45, O103, O111, O121, O145 and O157) and the *E. coli* biotype I mixture evaluated in this study (ATCC BAA-1427, ATCC BAA-1428, ATCC BAA-1429, ATCC BAA-1430, and ATCC BAA-1431) on hot beef tissue using a 1.9 and 2.5% lactic and citric acid blend (LCA). In their study, Scott et al. (2015) applied the LCA at 43 and 60°C for 5 s in a custom-built spray cabinet (0.42 Lpm at 1.03 bar across 2 nozzles). Similarly, Laury et al. (2009) observed a 1.4 log CFU/$cm^2$ decrease in log survival of *E. coli* O157:H7 on inoculated beef tips (5.5 log CFU/100$cm^2$) using 2.5% LCA applied at 2.79 bar in a custom-built spray cabinet. The decrease in survving populations of *E.coli* O157:H7 observed by Scott et al. (2015) and Laury et al. (2009), when compared to the current study, may have been due to distribution of pressure across fewer nozzles or the greater spray pressure, respectively.

*Color Evaluation Results*

The influence of spray intervention treatment on the color of beef tissue samples is shown in Tables 4, 5, and 6. CIE $L^*$ values (lightness) for samples spray treated with 8 or 10% LA were lower ($P < 0.05$) than for all other treatments and declined over the 30 d storage period—indicating that the product darkened due to LA exposure and dark storage.

Following 10 d dark storage, $a^*$ values were greater ($P < 0.05$; more red) for untreated control samples than for samples sprayed with 1.5 or 2.5% LCA or for samples treated with any level of LA. Untreated control and samples sprayed with 200ppm PAA had similar $a^*$ values ($P > 0.05$). These results are consistent with research performed by Stivarius et al. (2002) and

Semler (2013), where *a** values decreased subsequent to application of 2 or 4% LA to beef trimmings. Similarly, hot water plus lactic acid treatment has shown to decrease panel-determined redness (Mancini & Hunt, 2005). In the current study, *a** values were similar ($P > 0.05$) for untreated control samples and samples sprayed with 200ppm PAA until 30 d dark storage. A study performed by Quilo et al. (2009) actually observed redness protection effects compared to untreated control samples when beef trimmings were sprayed with 200 ppm PAA. *b** values increased during dark storage ($P < 0.05$) suggesting product discoloration; however, no noticeable trends were observed among or between treatments.

**Table 2.1.** Mean values (± SD) for pH of the intervention solution and meat sample following spray-treatment application[1].

| Treatment | pH Values | |
|---|---|---|
| | Solution pH[2] | Sample pH[3] |
| Untreated Control | N/A | 6.05±0.27 |
| Water | 7.95±0.70 | 5.96±0.32 |
| 200 ppm PAA | 4.18±0.06 | 5.66±0.41 |
| 1.0% LCA | 2.91±0.12 | 5.22±0.26 |
| 1.5% LCA | 2.79±0.09 | 5.08±0.31 |
| 2.5% LCA | 2.68±0.77 | 4.60±0.95 |
| 5% LA | 2.77±0.77 | 4.78±0.21 |
| 8% LA | 2.19±0.10 | 4.41±0.21 |
| 10% LA | 2.13±0.13 | 4.36±0.15 |

[1]Average product uptake: 0.01%
[2]Averages of samples for each solution (n=8/solution)
[3]Averages of samples for each treatment (n=20/treatment), except untreated control (n=12)

**Table 2.2.** Surviving populations (log CFU/cm$^2$) of Shiga toxin-producing *Escherichia coli*[1] (STEC) and *E. coli* surrogates[2] on hot beef tissue following spray-treatment with various interventions[1] in a custom-built laboratory-scale spray cabinet.

| Treatment | Microorganism | |
|---|---|---|
| | STEC[2] | Surrogate[3] |
| Untreated Control | 5.51$^{bc}$ | 5.67$^{a}$ |
| Water | 5.70$^{a}$ | 5.66$^{a}$ |
| 200 ppm PAA | 5.39$^{c}$ | 5.45$^{b}$ |
| 1% LCA | 5.55$^{ab}$ | 5.38$^{b}$ |
| 1.5% LCA | 5.51$^{bc}$ | 5.40$^{b}$ |
| 2.5% LCA | 5.46$^{bc}$ | 5.33$^{b}$ |
| 5% LA | 4.93$^{d}$ | 4.96$^{c}$ |
| 8% LA | 4.85$^{d}$ | 4.73$^{d}$ |
| 10% LA | 4.59$^{e}$ | 4.22$^{e}$ |
| SEM[4] | 0.13 | 0.13 |

[a-e] Within each inoculum type (STEC and Surrogate), LSmeans with different superscripts are different ($P < 0.05$).

[1]Except for untreated control samples, all samples were subject to spray treatment application (0.53 lpm at 1.38 bar over 8 nozzles) using a custom-built laboratory-scale spray cabinet.

[2]STEC cocktail: two strains of *E. coli* O157:H7 and two strains each of the "Big Six" non-O157 STEC serogroups (i.e., O26, O45, O103, O111, O121, and O145)

[3]Surrogate cocktail: Five strain mixture of *E. coli* biotype I strains (ATCC BAA-1427, ATCC BAA-1428, ATCC BAA-1429, ATCC BAA-1430, and ATCC BAA-1431)

[4] Pooled standard error of the mean.

**Table 2.3.** Comparison (*P*-values) of the survival of a Shiga toxin-producing *Escherichia coli* (STEC) cocktail[1] versus the non-pathogenic *E. coli* surrogate inoculum[2].

| Treatment | STEC vs. Surrogate |
|---|---|
| Water | 0.33 |
| 200 ppm PAA | 0.86 |
| 1.0% LCA | 0.05 |
| 1.5 % LCA | 0.09 |
| 2.5 % LCA | 0.18 |
| 5% LA | 0.08 |
| 8% LA | 0.36 |
| 10% LA | 0.32 |

[1] Two strains each of O157:H7, O26, O45, O103, O111, O121 and O145
[2] Cocktail of ATCC BAA-1427, 1428, 1429, 1430 and 1431

**Table 2.4.** Instrumental *L** values (lightness) of lean, beef tissue prior to and immediately following antimicrobial application[1] and following 10, 20 and 30 days of vacuum-packaged dark storage (4°C).

| Treatment | Time | | | | | |
|---|---|---|---|---|---|---|
| | Pre-Treatment | Post-Treatment[2] | Day 10 | Day 20 | Day 30 | SEM[3] |
| Untreated Control | 45.38[a] | N/A | 43.42[bc] | 39.64[c] | 40.45[bc] | 2.26 |
| Water | 43.98[bc] | 49.33[a] | 43.14[bc] | 42.21[bc] | 40.81[bc] | 1.60 |
| 200 ppm PAA | 49.48[a] | 48.20[a] | 42.28[bc] | 40.70[bc] | 41.41[bc] | 1.60 |
| 1% LCA | 46.65[a] | 47.03[a] | 42.92[bc] | 41.03[bc] | 41.77[bc] | 1.60 |
| 1.5% LCA | 42.72[bc] | 43.98[bc] | 42.48[bc] | 44.28[bc] | 41.42[bc] | 1.60 |
| 2.5% LCA | 47.62[a] | 46.36[a] | 44.06[bc] | 42.74[bc] | 41.31[bc] | 1.60 |
| 5% LA | 47.25[a] | 46.40[a] | 44.60[b] | 44.33[bc] | 39.89[c] | 1.60 |
| 8% LA | 49.65[a] | 42.49[bc] | 41.14[bc] | 37.31[cd] | 33.63[d] | 1.60 |
| 10% LA | 47.29[a] | 47.58[a] | 40.10[c] | 36.94[c] | 34.00[d] | 1.60 |

[a-d]LSmeans with different superscripts are different ($P < 0.05$).

[1]Except for untreated control samples, all samples were subject to spray treatment application (0.53 lpm at 1.38 bar over 8 nozzles) using a custom-built laboratory-scale spray cabinet.

[2]All samples except untreated control were subject to post-treatment instrumental color values.

[3]Pooled standard error of the mean.

**Table 2.5.** Instrumental *a** values (redness) of lean, beef tissue prior to and immediately following antimicrobial application[1] and following 10, 20 and 30 days of vacuum-packaged dark storage (4°C).

| Treatment | Time | | | | | |
|---|---|---|---|---|---|---|
| | Pre-Treatment | Post-Treatment[2] | Day 10 | Day 20 | Day 30 | SEM[3] |
| Untreated Control | 11.38[c] | N/A | 17.48[a] | 16.58[ab] | 15.21[ab] | 1.27 |
| Water | 12.35[bc] | 12.35[cd] | 15.43[ab] | 15.03[ab] | 10.88[cd] | 0.91 |
| 200 ppm PAA | 11.09[cd] | 11.09[cd] | 15.59[ab] | 14.93[ab] | 11.03[cd] | 0.91 |
| 1% LCA | 11.57[c] | 12.60[bc] | 14.69[ab] | 14.56[ab] | 10.10[cd] | 0.91 |
| 1.5% LCA | 12.03[bc] | 11.70[bc] | 14.14[b] | 13.45[bc] | 8.68[d] | 0.91 |
| 2.5% LCA | 11.42[c] | 11.90[bc] | 14.14[b] | 14.10[b] | 10.07[cd] | 0.91 |
| 5% LA | 12.02[bc] | 12.48[bc] | 12.93[bc] | 12.53[bc] | 8.89[d] | 0.91 |
| 8% LA | 12.00[bc] | 12.57[bc] | 11.90[bc] | 10.86[cd] | 8.82[d] | 0.91 |
| 10% LA | 13.47[bc] | 12.51[bc] | 11.61[c] | 10.75[cd] | 8.48[d] | 0.91 |

[a-d]LSmeans with different superscripts are different ($P < 0.05$).

[1]Except for untreated control samples, all samples were subject to spray treatment application (0.53 lpm at 1.38 bar over 8 nozzles) using a custom-built laboratory-scale spray cabinet.

[2]All samples except untreated control were subject to post-treatment instrumental color values.

[3]Pooled standard error of the mean.

**Table 2.6.** Instrumental *b** values (yellowness) of lean, beef tissue prior to and immediately following antimicrobial application[1] and following 10, 20 and 30 days of vacuum-packaged dark storage (4°C).

| Treatment | Time | | | | | |
|---|---|---|---|---|---|---|
| | Pre-Treatment | Post-Treatment[2] | Day 10 | Day 20 | Day 30 | SEM[3] |
| Untreated Control | $6.99^{c}$ | N/A | $10.45^{ab}$ | $11.27^{ab}$ | $10.24^{ab}$ | 1.16 |
| Water | $6.52^{c}$ | $7.42^{c}$ | $10.27^{ab}$ | $10.82^{ab}$ | $9.69^{b}$ | 0.94 |
| 200 ppm PAA | $7.66^{c}$ | $7.25^{c}$ | $10.26^{ab}$ | $10.64^{ab}$ | $10.17^{ab}$ | 0.94 |
| 1% LCA | $7.23^{c}$ | $8.16^{bc}$ | $9.90^{ab}$ | $9.81^{ab}$ | $9.09^{bc}$ | 0.94 |
| 1.5% LCA | $6.33^{c}$ | $6.49^{c}$ | $9.36^{bc}$ | $10.40^{ab}$ | $8.60^{ab}$ | 0.94 |
| 2.5% LCA | $6.71^{c}$ | $7.58^{c}$ | $10.18^{ab}$ | $11.31^{ab}$ | $11.00^{ab}$ | 0.94 |
| 5% LA | $7.98^{bc}$ | $8.19^{bc}$ | $10.87^{ab}$ | $11.08^{ab}$ | $10.90^{ab}$ | 0.94 |
| 8% LA | $7.44^{c}$ | $7.66^{c}$ | $10.64^{ab}$ | $11.65^{a}$ | $10.12^{ab}$ | 0.94 |
| 10% LA | $7.94^{bc}$ | $8.56^{bc}$ | $11.20^{ab}$ | $11.22^{ab}$ | $9.59^{b}$ | 0.94 |

$^{a\text{-}c}$LSmeans with different superscripts are different ($P < 0.05$).

[1]Except for untreated control samples, all samples were subject to spray treatment application (0.53 lpm at 1.38 bar over 8 nozzles) using a custom-built laboratory-scale spray cabinet.

[2]All samples except untreated control were subject to post-treatment instrumental color values.

[3]Pooled standard error of the mean.

# CHAPTER 3

## Grind trial of 73/27 ground beef with advanced meat recovery (AMR) product inclusion

### Introduction

An advanced meat recovery system (AMR) is any machine technology used to separate lean from bone, without crushing, grinding or pulverizing bone materials (NAMI, 2015). Lean captured via AMR recovers an estimated $100 million in ground beef and pork each year, and does not require special labeling (NAMI, 2015). In a study performed by Calhoun et al. (1999), AMR-derived pork, when included up to 15% in ground pork, was found to have little effect on lipid oxidation and color stability throughout retail case display when compared to an untreated control. Maintaining microbiological quality, color stability, and delaying lipid oxidation in ground meat products is paramount to satisfy retail customers and prevent food waste. Objectives of this study were to individually observe spoilage microorganisms, color, and lipid oxidation in three different ground beef batches following four separate vacuum-sealed dark storage times during retail display.

### Materials and Methods

*Sample Collection and Preparation*

Cases of labeled 73% lean and 27% ground beef product, containing six or eight 454 g, clear plastic chubs were shipped to the Colorado State University Meat Laboratory on six pallets, under refrigeration (4°C). Pallets were sorted by type (Control; 25% brisket-derived advanced meat recovery [AMR] product-inclusion from plant one – BBFT 1; 25% brisket-derived AMR product-inclusion from plant two –BBFT 2 and stored in a holding cooler at 4°C. At seven, 14, 18 and 21 d post-production, three chubs (one each from three randomly selected cases of each ground beef type) were opened, fine ground (Hobart table-top grinder; 3 mm grind plate),

portioned into 20, 454 g loaves on black foam trays, and overwrapped with polyvinyl film for display in a retail case (4°C). Also, during each grind day, a 50 g composite sample of the three chubs for each product were used for folch extraction for crude fat composition analysis. Results of crude fat percentage by dark storage d and product are listed in table 3.1.

*Microbiological Analysis*

Following each dark storage time (7, 14, 18 and 21 d), 50 g of product were aseptically removed from five trays of each ground beef type at 0, 24, 48 and 72 h of retail case display. Ten ml of a peptone saline diluent (maximum recovery diluent; MRD; Difco, BD) were added to all samples, and the samples were pummeled (Masticator, IUL Industries, Barcelona, Spain) for 2 min at 230 rpm before formation of serial (10-fold) dilutions in 0.1% buffered peptone water (BPW; Difco, BD). Appropriate dilutions were plated in duplicate on tryptic soy agar (TSA; Difco, BD) to enumerate aerobic bacteria plate count (APC) and onto Man, Rogosa, and Sharp (MRS; Difco, BD) agar to enumerate lactic acid bacteria plate count (LAB). The TSA and MSR plates were incubated at 35°C for 24 h and 25°C for 72 h, respectively, for visual enumeration of colonies. Before experimentation, it was determined that, if enumerated LAB or APC counts exceeded 6 log CFU/g at 0 h following any given dark storage interval, microbiological and lipid oxidation assay sampling times would be performed every 12 h instead of every 24 h to more closely monitor shelf-life changes.

*Trained Panel Color Analysis*

Five overwrapped packages per product were randomly assigned to remain in the retail case to the completion of retail display time (72 h) following each dark storage interval and were subject to trained panel evaluation. Panelists (trained via AMSA Meat Color Guidelines) were assembled every 12 h of retail case display following specified dark storage time (7, 14, 18 or 21

d) to asses color attributes on a 0-100 scale with verbal anchors: 1) lean color (0 = “tan/brown” – 100 = “bright, cherry-red”); 2) discoloration (0-100% = expressed as percentage of lean surface influenced by brown/tan discoloration); and 3) red color intensity (0 = “pale” – 100=“very intense”).

*Instrument Color Analysis*

After each dark storage interval, the same five overwrapped packages designated for panel evaluation from each product were subjected to instrumental color evaluation every 12 h of retail case display. At each specified display time, International Commission on Illumination (CIE) lean tissue color was evaluated following guidelines provided by the American Meat Science Association (AMSA, 2012) using a portable spectrophotometer (Hunter MiniScan XE Plus) with illuminant A, a standard observer angle of 10°, d50 setting, and a 5mm aperture, to measure CIE $L^*$, $a^*$ and $b^*$. Three scans were obtained from each sample piece and averaged before statistical analysis.

*2-thiobarbituric Acid Reactive Substances (TBAR) Analysis*

A 50 g composite sample from each of the ground beef productss was aseptically removed from packaging, frozen in liquid nitrogen, and homogenized using an industrial food processor (Robot Coupe, U.S.A.) at the same dark storage and display time intervals that were used for microbial sampling. Lipid oxidation values (mg malonaldehyde/kg) were measured via 2-thiobarbituric acid reactive substances (TBAR) assay, using protocol described by Buege and Aust (1978).

*Statistical Analysis*

Analysis for APC, LAB, panel color, instrument color, and TBAR assays were collected for each product in a split-split plot design, and analyzed within subplot dark storage times (7,

14, 18 and 21 d) with assigned repeated measures at retail case display intervals (microbiological and TBAR sampling times – 0, 24, 48 and 72 h; panel and instrument color sampling times – 0, 12, 24, 36, 48, 60 and 72 h). All data were compared using a least squares means approach in the MIXED procedure of SAS (v9.2; Cary, NC). Comparison-wide alpha was selected at 0.05.

**Results and Discussion**

*Microbiological Results (APC Means - Table 3.1; LAB Means - Table 3.2)*

After specified dark storage times, for all productss, APC and LAB means steadily increased throughout retail case display. Jay (1996) concluded that spoilage microorganism thresholds (where off-odors and flavors become unacceptable) exist around $10^7$-$10^8$ log/g. In this study, for all productss, neither APC nor LAB means exceeded 7 log CFU/g until after 21 d of dark storage.

Control Unsurprisingly, after 7 d of vacuum-packaged dark storage, log CFU/g APC means from ground beef sampled at 72 h retail case display were higher ($P < 0.05$) than those sampled at 0 h, and enumerated LAB were highest ($P < 0.05$) at 72 h display. Following 14 d dark storage, APC means increased ($P < 0.05$) with each sampled display time, and LAB values were highest ($P < 0.05$) at 72 h display. APC means increased ($P < 0.05$) sequentially with display time and LAB means were lower ($P < 0.05$) at 0 and 24 h display than those at 48 or 72 h subsequent to 18 d dark storage. Following 21 d dark storage, APC means were lowest ($P < 0.05$) at 0 h display time, and LAB values were lower ($P < 0.05$) at 0 and 24 h retail case display than those at 60 and 72 h.

*BBFT 1* Following 7 d dark storage, enumerated APC were similar ($P > 0.05$) across retail display times, and LAB means were greater ($P < 0.05$) at 72 h sampling time than 0 and 24 h and greater ($P < 0.05$) at 48 h than at 0 h. After 14 dark storage days, samples evaluated at 0 h

produced the lowest ($P < 0.05$) APC values, and APC means were lower ($P < 0.05$) at 24 h than 60 and 72 h. Moreover, LAB values were highest ($P < 0.05$) at 72 h retail case display and greater ($P < 0.05$) at 48 h than at 0 and 24 h succeeding 14 d dark storage. APC and LAB counts increased ($P < 0.05$) with each sampled display time after 18 d dark storage. Following 21 days dark storage, APC values were greater ($P < 0.05$) at 60 h display than 0 or 24 hours display, and lower ($P < 0.05$) at 0 h than 24 and 48 h display, while LAB means were greatest ($P < 0.05$) at the 60 h display time, and greater ($P < 0.05$) at 48 h than 0 and 24 h.

BBFT 2 Enumerated log CFU/g APC values highest ($P < 0.05$) at 72 h display, and LAB means successively increased ($P < 0.05$) with each display hour sampling time after 7 d dark storage. Following 14 d dark storage, least squares means for APC increased ($P < 0.05$) with each display hour sampling time, and LAB counts were highest ($P < 0.05$) at 72 h and higher ($P < 0.05$) at 48 h than 0 or 24 h. Least squares means for APC and LAB increased ($P < 0.05$) with each sampled display time after 18 d dark storage. After 21 d, APC counts were lower ($P < 0.05$) at 0 h than 24 and 60 h display, but similar ($P > 0.05$) at 0 and 48 h. Also, expectedly after 21 d, LAB counts were lower ($P < 0.05$) at 0 h than 72 h.

*Color Results (Trained Panelist-Determined Color - Tables 3.3, 3.4 and 3.5; Instrument Color Values - Tables 3.6, 3.7 and 3.8).*

With some exceptions, least squares means for panelist lean color ratings and redness intensity scores declined ($P < 0.05$) with increased retail case storage time. Least squares means for CIE $L^*$ increased inconsistently depending on product and dark storage time; however, in several instances, $L^*$ values were highest ($P < 0.05$) toward the end of retail case storage. This suggest  Conversely, $a^*$ values generally declined ($P < 0.05$) with increased storage time. This

increase in CIE $L^*$ and decrease in CIE $a^*$ suggested the product became faded (lighter) and less red throughout retail case display.

In a study published by Martin et al. (2013), increased trained panelist ratings for lean color were observed in leaner ground beef product. Although in this study, analyses were not performed to compare batch product, higher numerical values for lean color were observed in BBFT 1 and 969 samples than untreated control samples. While we cannot compare product effects in this particular study, crude fat percentages (Table 3.1) were numerically higher for those batches with BBFT inclusion. One may project that, in a study comparing productss, this lean point difference would at least initially result in increased lean color ratings by panelists and $a^*$ measures (redness).

According to Hood & Riordan (1973), once 20% of a given meat product surface is discolored, customers become less likely to purchase it. Furthermore, customers will altogether reject a beef product at retail when 40 to 60% discoloration is present (Hood & Riordan, 1973). Throughout retail case display, for these particular ground beef batches, at all dark storage times, least squares means for panelist ratings of discoloration remained below the aforementioned percentage values.

*Control* After 7 d dark storage, trained panelist ratings for lean color were highest ($P < 0.05$) at 0 h display and lowest ($P < 0.05$) at 72h for redness intensity. After 14, 18 and 21 d dark storage, least squares means for panelist ratings of lean color were lower ($P < 0.05$) at 60 and 72 h display than at 0, 12, 24 and 36 h. Although there were no remarkable trends in redness intensity values following 18 d dark storage, intensity was lowest ($P < 0.05$) at 72 h or 60 h after 14 d or 21 d dark storage, respectively.

Except for ground beef stored for 21 d in dark storage, where $L^*$ values were similar ($P$ >0.05) at 0 and 72 h, least squares means for CIE $L^*$ values were comparable after dark storage intervals, where values were highest ($P < 0.05$) at 72 h (7 and 14 d) or lowest ($P < 0.05$) at 0 h (18 d). Least squares means for $a^*$ for control samples generally decreased over time ($P < 0.05$). However, after 18 d, $a^*$ values were lower ($P < 0.05$) at 12 h than 0 h, but similar ($P > 0.05$) throughout the rest of display. Few noticeable trends were observed for CIE $b^*$ values.

*BBFT 1* After 7 d dark storage, least squares means for panelist ratings of lean color were lowest ($P < 0.05$) at 72 h display time and greater ($P < 0.05$) at 0 h than 36, 48 and 60 h. Comparable trends for lean color were observed following 14, 18 or 21 d dark storage, where least squares means for panelist ratings of lean color were lowest ($P < 0.05$) at the last two retail display sampling times (60 and 72 h, 60 and 72 h, and 48 and 60h, respectively). Least squares means for panelist ratings of redness intensity were lowest ($P < 0.05$) at the last display interval after 14, 18, or 21 d.

Following 7 or 14 d of dark storage, least squares means for $L^*$ values were highest ($P <$ 0.05) at 72 h display. After 18 d, least squares means for $L^*$ values were lower ($P < 0.05$) at 0 and 12 h than 60 and 72h display. Least squares means for $L^*$ values were higher ($P < 0.05$) at 0 and 12 h than at 36, 48 or 60 h after 21 d. Least squares means for $a^*$ means were lowest ($P <$ 0.05) at 60 and 72 h display, lowest ($P < 0.05$) from 48 h throughout display, lowest ($P < 0.05$) at 72 h, and lower ($P < 0.05$) at 36 h throughout display than preceding sampling times at 7, 14, 18 and 21 d dark storage, respectively. No discernable patterns for least squares means of $b^*$ values within dark storage times were detected in BBFT 1.

*BBFT 2* After 7 d dark storage, least squares means for lean color decreased ($P < 0.05$) and for discoloration means increased ($P < 0.05$) with retail case display. Least squares means

for panelist ratings of lean color were to lowest ($P < 0.05$) at 72 h and for redness intensity were highest ($P < 0.05$) at 0 h following 14 d dark storage. After 18 d, least squares means for panelist ratings of lean color and redness intensity were lowest ($P < 0.05$) at the end of display. Least squares means for panelist ratings of lean color decreased ($P < 0.05$) with time following 21 d dark storage and least squares means for panelist ratings of intensity were highest ($P < 0.05$) at 0 and 12 h.

After 7 d or 21 d dark storage, trends in CIE $L^*$ values were not obvious throughout retail case display; however, least squares means for CIE $L^*$ values were highest ($P < 0.05$) at 72 h after 14 d storage and lower ($P < 0.05$) at 0 h than 36, 48, 60 and 72 h after 18 d. Except for those samples displayed after 18 d dark storage, least squares means for CIE $a^*$ values appeared to decrease steadily throughout retail case storage. No consistent trends were observed in least squares means for $b^*$.

*TBAR Results (Table 3.10)*

*Control* Least squares means for TBAR were highest ($P < 0.05$) at 72 h display following 14 and 18 d dark storage and similar ($P > 0.05$) at each retail display time following 21 d dark storage.

*BBFT 1* After both 7 and 21 d dark storage, least squares means for TBAR did not differ ($P < 0.05$) by retail display time. Expectedly, least squares means for TBAR were greater ($P < 0.05$) at 48 and 72 h than at preceding storage times subsequent to 14 d dark storage. Moreover, least squares means for TBAR were lowest ($P < 0.05$) at 0 h display time following 18 d storage.

*BBFT 2* After 7 or 21 d of dark storage, least squares means for TBAR in ground beef samples were similar ($P < 0.05$) between display times. Least squares means for TBAR values

were lower ($P < 0.05$) at the first two display intervals than the last two following 18 d dark storage. However, there were no trends observed in TBAR means following 14 d dark storage.

**Table 3.1.** Simple Means (± SD) for crude fat (%) of the ground beef sample following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C).

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Product | 7 | 14 | 18 | 21 |
| Control | 22.48±1.03 | 19.36±1.06 | 20.57±1.42 | 20.53±1.21 |
| BBFT 1 | 14.50±1.32 | 14.67±1.03 | 16.10±1.14 | 16.01±1.25 |
| BBFT 2 | 17.38±0.99 | 15.67±0.95 | 16.71±0.85 | 15.76±1.18 |

**Table 3.2.** Least squares means[1] of aerobic plate counts (APC; Log CFU/g) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C).

| Control | | | | |
|---|---|---|---|---|
| | Days Dark Storage | | | |
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 3.75$^{b}$ | 4.51$^{d}$ | 5.68$^{d}$ | 6.16$^{c}$ |
| Hour 24 | 3.94$^{ab}$ | 5.38$^{c}$ | 6.42$^{c}$ | 6.65$^{b}$ |
| Hour 48 | 3.87$^{ab}$ | 5.95$^{b}$ | 7.13$^{b}$ | 7.00$^{a}$ |
| Hour 60 | - | - | - | 7.09$^{a}$ |
| Hour 72 | 4.40$^{a}$ | 6.42$^{a}$ | 7.53$^{a}$ | - |
| SEM[3] | 0.21 | 0.15 | 0.98 | 0.05 |
| **BBFT 1** | | | | |
| | Days Dark Storage | | | |
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 4.13$^{a}$ | 4.98$^{c}$ | 5.60$^{d}$ | 6.31$^{c}$ |
| Hour 24 | 4.30$^{a}$ | 5.45$^{b}$ | 6.25$^{c}$ | 6.76$^{b}$ |
| Hour 48 | 4.40$^{a}$ | 6.11$^{a}$ | 6.91$^{b}$ | 6.97$^{ab}$ |
| Hour 60 | - | - | - | 7.37$^{a}$ |
| Hour 72 | 4.19$^{a}$ | 6.52$^{a}$ | 7.39$^{a}$ | - |
| SEM[3] | 0.11 | 0.15 | 0.11 | 0.15 |
| **BBFT 2** | | | | |
| | Days Dark Storage | | | |
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 4.25$^{b}$ | 4.92$^{d}$ | 5.09$^{d}$ | 6.95$^{b}$ |
| Hour 24 | 4.41$^{b}$ | 5.31$^{c}$ | 6.06$^{c}$ | 7.35$^{a}$ |
| Hour 48 | 4.27$^{b}$ | 6.11$^{b}$ | 6.54$^{b}$ | 7.08$^{ab}$ |
| Hour 60 | - | - | - | 7.28$^{a}$ |
| Hour 72 | 4.77$^{a}$ | 6.45$^{a}$ | 7.14$^{a}$ | - |
| SEM[3] | 0.10 | 0.11 | 0.14 | 0.09 |

[1]LSmeans analyses were performed within each product (Control, BBFT 1, or BBFT 2), within each dark storage time (7, 14, 18, or 21 days), between display times.
[2]Following 21 days dark storage, after exceeding pre-determined spoilage microorganism thresholds at 0 hours display time, the last sampling time was revised to 60 hours to monitor APC changes at a closer interval.
[3]Pooled standard error of the mean within treatment, within pull time.
[a-d] LSmeans with different superscripts within treatment, within column are different ($P < 0.05$).

**Table 3.3.** Least squares means[1] of lactic acid bacteria counts (LAB; Log CFU/g) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C).

Control

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 3.16$^{b}$ | 5.23$^{c}$ | 5.84$^{b}$ | 5.97$^{c}$ |
| Hour 24 | 3.32$^{b}$ | 5.77$^{bc}$ | 6.30$^{b}$ | 6.58$^{b}$ |
| Hour 48 | 3.38$^{b}$ | 6.09$^{b}$ | 7.21$^{a}$ | 7.06$^{a}$ |
| Hour 60[2] | - | - | - | 7.17$^{a}$ |
| Hour 72 | 4.52$^{a}$ | 6.92$^{a}$ | 7.44$^{a}$ | - |
| SEM[3] | 0.19 | 0.25 | 0.19 | 0.13 |

BBFT 1

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 3.36$^{c}$ | 4.96$^{c}$ | 5.46$^{d}$ | 6.08$^{c}$ |
| Hour 24 | 3.68$^{bc}$ | 5.26$^{c}$ | 6.02$^{c}$ | 6.34$^{c}$ |
| Hour 48 | 3.93$^{ab}$ | 5.73$^{b}$ | 6.48$^{b}$ | 7.06$^{b}$ |
| Hour 60[2] | - | - | - | 7.40$^{a}$ |
| Hour 72 | 4.33$^{a}$ | 6.54$^{a}$ | 7.26$^{a}$ | - |
| SEM[3] | 0.14 | 0.15 | 0.12 | 0.15 |

BBFT 2

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 3.15$^{d}$ | 4.77$^{c}$ | 5.17$^{d}$ | 6.66$^{b}$ |
| Hour 24 | 3.44$^{c}$ | 5.07$^{c}$ | 5.71$^{c}$ | 7.05$^{ab}$ |
| Hour 48 | 3.85$^{b}$ | 5.65$^{b}$ | 6.23$^{b}$ | 7.16$^{ab}$ |
| Hour 60[2] | - | - | - | 7.30$^{a}$ |
| Hour 72 | 4.48$^{a}$ | 6.36$^{a}$ | 7.11$^{a}$ | - |
| SEM[3] | 0.07 | 0.13 | 0.08 | 0.17 |

[1]LSmeans analyses were performed within each product (Control, BBFT 1, or BBFT 2), within each dark storage time (7, 14, 18, or 21 days), between display times.

[2]Following 21 days dark storage, after exceeding pre-determined spoilage thresholds at 0 hours display time, the last sampling time was revised to 60 hours to monitor microbial LAB at a closer interval.

[3]Pooled standard error of the mean within treatment, within pull time.

[a-d] LSmeans with different superscripts within treatment, within column are different ($P < 0.05$).

**Table 3.4.** Least squares means[1] of trained panelist-determined color values (0-100%; – tan/brown - bright, cherry-red) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C).

Control

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 88.22$^{a}$ | 82.95$^{a}$ | 90.16$^{a}$ | 78.52$^{a}$ |
| Hour 12 | 80.64$^{b}$ | 76.09$^{b}$ | 85.08$^{ab}$ | 66.03$^{bc}$ |
| Hour 24 | 74.55$^{c}$ | 79.05$^{ab}$ | 88.51$^{a}$ | 71.01$^{b}$ |
| Hour 36 | 74.20$^{c}$ | 77.71$^{ab}$ | 82.71$^{b}$ | 65.00$^{c}$ |
| Hour 48 | 76.13$^{bc}$ | 75.79$^{b}$ | 86.54$^{ab}$ | 52.08$^{d}$ |
| Hour 60 | 73.06$^{c}$ | 67.51$^{c}$ | 76.56$^{c}$ | 50.03$^{d}$ |
| Hour 72 | 71.26$^{c}$ | 67.88$^{c}$ | 74.39$^{c}$ | - |
| SEM[3] | 3.92 | 3.23 | 2.52 | 3.92 |

BBFT 1

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 92.75$^{a}$ | 93.49$^{a}$ | 92.15$^{a}$ | 88.69$^{a}$ |
| Hour 12 | 90.00$^{ab}$ | 87.45$^{cd}$ | 85.57$^{b}$ | 79.43$^{b}$ |
| Hour 24 | 87.19$^{bc}$ | 90.16$^{abc}$ | 92.07$^{a}$ | 78.06$^{b}$ |
| Hour 36 | 86.27$^{c}$ | 91.77$^{ab}$ | 86.96$^{b}$ | 76.91$^{b}$ |
| Hour 48 | 85.82$^{c}$ | 87.84$^{bc}$ | 89.07$^{ab}$ | 64.30$^{c}$ |
| Hour 60 | 85.11$^{c}$ | 80.19$^{e}$ | 79.55$^{c}$ | 63.45$^{c}$ |
| Hour 72 | 82.05$^{d}$ | 85.33$^{d}$ | 77.88$^{c}$ | - |
| SEM[3] | 1.81 | 2.03 | 2.29 | 2.93 |

BBFT 2

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 92.16$^{a}$ | 93.49$^{a}$ | 86.78$^{a}$ | 87.11$^{a}$ |
| Hour 12 | 89.25$^{ab}$ | 87.45$^{cd}$ | 76.54$^{bc}$ | 76.54$^{b}$ |
| Hour 24 | 85.50$^{bc}$ | 90.16$^{abc}$ | 83.81$^{ab}$ | 76.94$^{b}$ |
| Hour 36 | 86.32$^{bc}$ | 91.77$^{ab}$ | 72.05$^{cd}$ | 74.21$^{b}$ |
| Hour 48 | 83.99$^{c}$ | 87.84$^{bcd}$ | 76.23$^{bc}$ | 62.71$^{c}$ |
| Hour 60 | 83.39$^{c}$ | 85.33$^{d}$ | 64.53$^{d}$ | 60.31$^{c}$ |
| Hour 72 | 84.56$^{c}$ | 80.19$^{e}$ | 66.25$^{d}$ | - |
| SEM[3] | 1.99 | 2.03 | 3.75 | 3.13 |

[1]LSmeans analyses were performed within each treatment (Control, BBFT 1, or BBFT 2), within each dark storage time (7, 14, 18, or 21 days), between display times.
[2]Following 21 days dark storage, after exceeding pre-determined spoilage microorganism thresholds at 0 hours display time, the last sampling time was revised to 60 hours to monitor lean color changes at a closer interval.
[3]Pooled standard error (largest) of the mean within treatment, within pull time.
[a-d] LSmeans with different superscripts within treatment, within column are different ($P <$ 0.05).

**Table 3.5.** Least squares means[1] of trained panelist-determined tan/brown discoloration values (0-100% of lean surface) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C).

Control

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 0.00$^{b}$ | 1.37$^{a}$ | 0.17$^{a}$ | 2.00$^{b}$ |
| Hour 12 | 0.00$^{b}$ | 0.00$^{a}$ | 0.93$^{a}$ | 0.03$^{b}$ |
| Hour 24 | 0.43$^{ab}$ | 0.00$^{a}$ | 1.13$^{a}$ | 0.21$^{b}$ |
| Hour 36 | 1.27$^{a}$ | 0.00$^{a}$ | 0.04$^{a}$ | 2.39$^{b}$ |
| Hour 48 | 1.23$^{a}$ | 0.13$^{a}$ | 0.00$^{a*}$ | 0.73$^{b}$ |
| Hour 60 | 0.00$^{b}$ | 1.40$^{a}$ | 0.00$^{a*}$ | 6.94$^{a}$ |
| Hour 72 | 0.43$^{ab}$ | 2.77$^{a}$ | 0.33$^{a}$ | - |
| SEM[3] | 0.38 | 1.13 | 0.75 | 1.17 |

BBFT 1

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 0.01$^{c}$ | 1.49$^{b}$ | 0.97$^{a}$ | 1.23$^{a}$ |
| Hour 12 | 0.00$^{c*}$ | 0.00$^{b}$ | 0.30$^{a}$ | 1.24$^{a}$ |
| Hour 24 | 0.00$^{c*}$ | 0.00$^{b}$ | 1.30$^{a}$ | 0.00$^{a}$ |
| Hour 36 | 0.38$^{bc}$ | 0.08$^{b}$ | 0.04$^{a}$ | 1.92$^{a}$ |
| Hour 48 | 0.40$^{bc}$ | 0.77$^{b}$ | 0.05$^{a}$ | 0.43$^{a}$ |
| Hour 60 | 0.97$^{ab}$ | 0.32$^{b}$ | 1.17$^{a}$ | 0.86$^{a}$ |
| Hour 72 | 1.74$^{a}$ | 4.17$^{a}$ | 2.00$^{a}$ | - |
| SEM[3] | 0.67 | 0.67 | 0.81 | 1.22 |

BBFT 2

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 0.00$^{b}$ | 0.86$^{a}$ | 0.25$^{b}$ | 0.77$^{a}$ |
| Hour 12 | 0.00$^{b}$ | 0.51$^{a}$ | 1.72$^{b}$ | 0.64$^{a}$ |
| Hour 24 | 0.00$^{b}$ | 0.00$^{a}$ | 6.68$^{a}$ | 0.00$^{a}$ |
| Hour 36 | 0.00$^{b}$ | 0.00$^{a}$ | 0.82$^{b}$ | 0.48$^{a}$ |
| Hour 48 | 0.67$^{ab}$ | 0.00$^{a}$ | 0.00$^{b*}$ | 0.72$^{a}$ |
| Hour 60 | 1.77$^{a}$ | 1.24$^{a}$ | 0.03$^{b}$ | 0.94$^{a}$ |
| Hour 72 | 1.87$^{a}$ | 0.57$^{a}$ | 0.32$^{b}$ | - |
| SEM[3] | 0.43 | 0.57 | 0.78 | 0.52 |

[1]LSmeans analyses were performed within each treatment (Control, BBFT 1, or BBFT 2), within each dark storage time (7, 14, 18, or 21 days), between display times.
[2]Following 21 days dark storage, after exceeding pre-determined spoilage microorganism thresholds at 0 hours display time, the last sampling time was revised to 60 hours to monitor lean color changes at a closer interval.
[3]Pooled standard error (largest) of the mean within treatment, within pull time.
[a-c] LSmeans with different superscripts within treatment, within column are different ($P < 0.05$).
[*] Indicates negative LSmeans; reported as 0.00%.

**Table 3.6.** Least squares means[1] of trained panelist-determined redness intensity values (0-100%; pale - intense) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C).

Control

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 85.98$^{ab}$ | 85.64$^{a}$ | 74.95$^{a}$ | 74.23$^{ab}$ |
| Hour 12 | 88.70$^{a}$ | 85.80$^{a}$ | 64.38$^{b}$ | 80.57$^{a}$ |
| Hour 24 | 86.53$^{ab}$ | 85.64$^{a}$ | 70.92$^{ab}$ | 67.76$^{c}$ |
| Hour 36 | 86.00$^{ab}$ | 84.04$^{ab}$ | 69.02$^{ab}$ | 67.97$^{c}$ |
| Hour 48 | 82.10$^{b}$ | 78.77$^{bc}$ | 65.91$^{ab}$ | 71.80$^{bc}$ |
| Hour 60 | 84.90$^{ab}$ | 76.94$^{c}$ | 69.30$^{ab}$ | 59.90$^{d}$ |
| Hour 72 | 71.10$^{c}$ | 59.37$^{d}$ | 67.64$^{ab}$ | - |
| SEM[3] | 2.10 | 2.00 | 3.64 | 3.29 |

BBFT 1

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 85.51$^{ab}$ | 78.37$^{b}$ | 76.20$^{a}$ | 84.17$^{a}$ |
| Hour 12 | 87.06$^{ab}$ | 83.17$^{ab}$ | 67.20$^{a}$ | 75.53$^{b}$ |
| Hour 24 | 85.85$^{ab}$ | 86.60$^{a}$ | 68.80$^{a}$ | 75.27$^{b}$ |
| Hour 36 | 84.48$^{ab}$ | 89.40$^{a}$ | 68.27$^{a}$ | 59.96$^{c}$ |
| Hour 48 | 89.01$^{a}$ | 77.43$^{b}$ | 68.55$^{a}$ | 51.30$^{c}$ |
| Hour 60 | 86.88$^{ab}$ | 86.20$^{a}$ | 74.03$^{a}$ | 48.35$^{d}$ |
| Hour 72 | 83.18$^{b}$ | 56.20$^{c}$ | 54.80$^{b}$ | - |
| SEM[3] | 1.77 | 2.46 | 3.54 | 3.75 |

BBFT 2

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 86.03$^{ab}$ | 87.31$^{a}$ | 78.00$^{a}$ | 79.17$^{a}$ |
| Hour 12 | 86.87$^{ab}$ | 83.21$^{abc}$ | 72.23$^{abc}$ | 82.17$^{a}$ |
| Hour 24 | 84.93$^{ab}$ | 86.15$^{ab}$ | 68.77$^{bc}$ | 62.48$^{bc}$ |
| Hour 36 | 89.63$^{a}$ | 77.49$^{cd}$ | 76.05$^{ab}$ | 67.10$^{bc}$ |
| Hour 48 | 83.30$^{b}$ | 75.42$^{d}$ | 73.73$^{abc}$ | 55.70$^{c}$ |
| Hour 60 | 82.13$^{b}$ | 80.37$^{bcd}$ | 66.52$^{c}$ | 59.15$^{bc}$ |
| Hour 72 | 83.10$^{b}$ | 77.98$^{cd}$ | 55.20$^{d}$ | - |
| SEM[3] | 2.14 | 2.25 | 3.43 | 3.33 |

[1]LSmeans analyses were performed within each treatment (Control, BBFT 1, or BBFT 2), within each dark storage time (7, 14, 18, or 21 days), between display times.

[2]Following 21 days dark storage, after exceeding pre-determined spoilage microorganism thresholds at 0 hours display time, the last sampling time was revised to 60 hours to monitor lean color changes at a closer interval.

[3]Pooled standard error (largest) of the mean within treatment, within pull time.

[a-d] LSmeans with different superscripts within treatment, within column are different ($P < 0.05$).

**Table 3.7.** Least squares means[1] of L* (lightness) values of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C).

Control

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | $56.89^{bc}$ | $58.86^{b}$ | $51.63^{c}$ | $64.48^{a}$ |
| Hour 12 | $54.60^{c}$ | $55.34^{b}$ | $55.97^{bc}$ | $52.75^{b}$ |
| Hour 24 | $58.99^{ab}$ | $56.15^{b}$ | $57.26^{ab}$ | $56.72^{b}$ |
| Hour 36 | $56.47^{c}$ | $56.72^{b}$ | $57.58^{ab}$ | $57.74^{b}$ |
| Hour 48 | $55.75^{c}$ | $58.35^{b}$ | $61.46^{a}$ | $58.27^{b}$ |
| Hour 60 | $56.51^{c}$ | $57.97^{b}$ | $59.22^{ab}$ | $58.78^{ab}$ |
| Hour 72 | $60.32^{a}$ | $76.40^{a}$ | $58.33^{ab}$ | - |
| SEM[3] | 0.79 | 2.22 | 1.74 | 2.01 |

BBFT 1

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | $51.09^{bc}$ | $50.58^{b}$ | $46.18^{c}$ | $56.99^{ab}$ |
| Hour 12 | $48.45^{c}$ | $52.61^{b}$ | $50.57^{bc}$ | $58.04^{a}$ |
| Hour 24 | $52.49^{b}$ | $53.50^{b}$ | $52.73^{ab}$ | $52.32^{abc}$ |
| Hour 36 | $49.69^{bc}$ | $50.43^{b}$ | $54.13^{ab}$ | $50.81^{c}$ |
| Hour 48 | $49.10^{c}$ | $50.67^{b}$ | $54.33^{ab}$ | $50.51^{c}$ |
| Hour 60 | $50.05^{bc}$ | $50.13^{b}$ | $56.91^{a}$ | $51.69^{bc}$ |
| Hour 72 | $55.92^{a}$ | $72.35^{a}$ | $56.78^{a}$ | - |
| SEM[3] | 1.11 | 0.96 | 1.69 | 1.20 |

BBFT 2

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | $50.85^{ab}$ | $53.81^{b}$ | $49.04^{c}$ | $60.74^{a}$ |
| Hour 12 | $43.65^{b}$ | $52.40^{bc}$ | $52.98^{bc}$ | $56.30^{b}$ |
| Hour 24 | $53.22^{a}$ | $50.18^{cd}$ | $52.98^{bc}$ | $52.85^{bc}$ |
| Hour 36 | $49.96^{ab}$ | $49.60^{d}$ | $56.60^{ab}$ | $53.33^{bc}$ |
| Hour 48 | $49.00^{ab}$ | $53.38^{b}$ | $53.89^{ab}$ | $53.22^{bc}$ |
| Hour 60 | $49.84^{ab}$ | $53.22^{b}$ | $58.33^{a}$ | $51.32^{c}$ |
| Hour 72 | $53.28^{a}$ | $76.26^{a}$ | $56.08^{ab}$ | - |
| SEM[3] | 2.88 | 0.92 | 1.56 | 3.33 |

[1]LSmeans analyses were performed within each treatment (Control, BBFT 1, or BBFT 2), within each dark storage time (7, 14, 18, or 21 days), between display times.

[2]Following 21 days dark storage, after exceeding pre-determined spoilage microorganism thresholds at 0 hours display time, the last sampling time was revised to 60 hours to monitor lean color changes at a closer interval.

[3]Pooled standard error of the mean within treatment, within pull time.

[a-d] LSmeans with different superscripts within treatment, within column are different ($P < 0.05$).

**Table 3.8.** Least squares means[1] of a* (redness) values of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C).

Control

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | $24.20^{ab}$ | $24.54^{a}$ | $27.04^{a}$ | $28.39^{a}$ |
| Hour 12 | $25.36^{a}$ | $22.45^{ab}$ | $22.86^{bc}$ | $24.89^{b}$ |
| Hour 24 | $23.99^{ab}$ | $23.92^{a}$ | $20.43^{c}$ | $26.14^{b}$ |
| Hour 36 | $24.36^{ab}$ | $20.69^{bc}$ | $23.97^{ab}$ | $23.00^{c}$ |
| Hour 48 | $23.06^{bc}$ | $20.00^{c}$ | $23.05^{bc}$ | $22.91^{c}$ |
| Hour 60 | $20.52^{d}$ | $20.48^{bc}$ | $20.39^{c}$ | $21.41^{c}$ |
| Hour 72 | $21.35^{cd}$ | $20.33^{bc}$ | $16.88^{d}$ | - |
| SEM[3] | 0.72 | 0.82 | 1.12 | 0.63 |

BBFT 1

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | $26.43^{b}$ | $28.87^{a}$ | $29.98^{a}$ | $30.34^{a}$ |
| Hour 12 | $28.47^{a}$ | $24.40^{b}$ | $25.92^{b}$ | $28.76^{a}$ |
| Hour 24 | $26.38^{b}$ | $29.86^{a}$ | $23.02^{c}$ | $28.93^{a}$ |
| Hour 36 | $28.33^{a}$ | $23.73^{bc}$ | $25.99^{b}$ | $22.84^{b}$ |
| Hour 48 | $25.27^{b}$ | $22.94^{c}$ | $25.22^{b}$ | $23.35^{b}$ |
| Hour 60 | $22.81^{c}$ | $22.53^{c}$ | $23.64^{c}$ | $23.95^{b}$ |
| Hour 72 | $22.94^{c}$ | $22.82^{c}$ | $19.09^{d}$ | - |
| SEM[3] | 0.49 | 0.47 | 0.49 | 1.20 |

BBFT 2

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | $27.25^{ab}$ | $27.74^{a}$ | $29.73^{a}$ | $29.47^{a}$ |
| Hour 12 | $28.23^{a}$ | $27.27^{a}$ | $24.54^{ab}$ | $26.76^{b}$ |
| Hour 24 | $26.93^{b}$ | $24.83^{b}$ | $28.05^{a}$ | $27.10^{ab}$ |
| Hour 36 | $26.60^{b}$ | $21.73^{c}$ | $24.50^{ab}$ | $23.37^{c}$ |
| Hour 48 | $25.99^{b}$ | $22.26^{c}$ | $24.72^{ab}$ | $21.54^{c}$ |
| Hour 60 | $23.22^{c}$ | $22.55^{c}$ | $23.29^{ab}$ | $21.11^{c}$ |
| Hour 72 | $23.96^{c}$ | $22.44^{c}$ | $19.53^{b}$ | - |
| SEM[3] | 0.46 | 0.61 | 2.33 | 0.82 |

[1]LSmeans analyses were performed within each treatment (Control, BBFT 1, or BBFT 2), within each dark storage time (7, 14, 18, or 21 days), between display times.
[2]Following 21 days dark storage, after exceeding pre-determined spoilage microorganism thresholds at 0 hours display time, the last sampling time was revised to 60 hours to monitor lean color changes at a closer interval.
[3]Pooled standard error of the mean within treatment, within pull time.
[a-d] LSmeans with different superscripts within treatment, within column are different ($P < 0.05$).

**Table 3.9.** Least squares means[1] of b* (yellowness) values of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C).

Control

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 21.44[bc] | 21.64[b] | 24.00[a] | 23.84[a] |
| Hour 12 | 22.89[a] | 19.42[c] | 20.12[bc] | 21.61[b] |
| Hour 24 | 21.44[bc] | 18.42[c] | 18.26[cd] | 23.22[a] |
| Hour 36 | 22.78[ab] | 18.15[c] | 22.27[ab] | 20.48[bc] |
| Hour 48 | 21.85[abc] | 19.01[c] | 22.34[a] | 20.22[c] |
| Hour 60 | 19.01[d] | 19.17[c] | 19.26[cd] | 19.32[c] |
| Hour 72 | 20.81[c] | 25.04a | 17.27[d] | - |
| SEM[3] | 0.48 | 0.50 | 0.75 | 0.49 |

BBFT 1

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 21.44[ab] | 23.45[b] | 25.76[a] | 24.53[ab] |
| Hour 12 | 24.38[ab] | 19.75[cd] | 21.45[cd] | 23.25[b] |
| Hour 24 | 27.90[a] | 19.06[cd] | 18.89[e] | 25.12[a] |
| Hour 36 | 25.64[ab] | 18.80[d] | 22.89[b] | 19.04[c] |
| Hour 48 | 22.99[ab] | 19.72[cd] | 22.68[bc] | 19.65[c] |
| Hour 60 | 19.89[b] | 20.09[c] | 20.99[d] | 19.33[c] |
| Hour 72 | 20.81[b] | 27.54[a] | 17.64[e] | - |
| SEM[3] | 2.25 | 0.42 | 0.46 | 0.49 |

BBFT 2

| | Days Dark Storage | | | |
|---|---|---|---|---|
| Display Time | 7 | 14 | 18 | 21 |
| Hour 0 | 22.25[bc] | 22.49[b] | 25.54[a] | 24.25[a] |
| Hour 12 | 24.30[a] | 20.47[c] | 20.40[d] | 22.23[b] |
| Hour 24 | 22.47[b] | 18.29[d] | 18.90[e] | 23.80[ab] |
| Hour 36 | 23.85[a] | 17.70[d] | 21.82[bc] | 20.17[c] |
| Hour 48 | 23.54[a] | 19.09[cd] | 22.91[b] | 18.82[c] |
| Hour 60 | 19.54[d] | 20.16[c] | 21.29[cd] | 19.93[c] |
| Hour 72 | 21.20[c] | 26.00[a] | 17.88[e] | - |
| SEM[3] | 0.36 | 0.50 | 0.41 | 0.65 |

[1]LSmeans analyses were performed within each treatment (Control, BBFT 1, or BBFT 2), within each dark storage time (7, 14, 18, or 21 days), between display times.

[2]Following 21 days dark storage, after exceeding pre-determined spoilage microorganism thresholds at 0 hours display time, the last sampling time was revised to 60 hours to monitor lean color changes at a closer interval.

[3] Pooled standard error of the mean within treatment, within pull time.

[a-e] LSmeans with different superscripts within treatment, within column are different ($P < 0.05$).

**Table 3.10.** Least squares means[1] of 2-thiobarbituric acid reactive substances analysis (TBAR; mgMDA/kg) of ground beef samples at 0, 12, 24, 36 48, 60, and 72 hours[2] retail case display (4°C) following 7, 14, 18, or 24 days vacuum-packaged dark storage (4°C).

Control

| Display Time | Days Dark Storage 7 | 14 | 18 | 21 |
|---|---|---|---|---|
| Hour 0 | 1.51$^{ab}$ | 1.38$^{b}$ | 0.70$^{c}$ | 1.72$^{a}$ |
| Hour 24 | 1.25$^{b}$ | 1.39$^{b}$ | 1.96$^{b}$ | 1.66$^{a}$ |
| Hour 48 | 1.80$^{ab}$ | 1.54$^{b}$ | 1.72$^{b}$ | 1.96$^{a}$ |
| Hour 60 | - | - | - | 2.25$^{a}$ |
| Hour 72 | 2.16$^{a}$ | 2.02$^{a}$ | 2.27$^{a}$ | - |
| SEM[3] | 0.20 | 0.06 | 0.07 | 0.25 |

BBFT 1

| Display Time | Days Dark Storage 7 | 14 | 18 | 21 |
|---|---|---|---|---|
| Hour 0 | 1.68$^{a}$ | 1.64$^{b}$ | 1.84$^{b}$ | 2.45$^{a}$ |
| Hour 24 | 1.91$^{a}$ | 1.69$^{b}$ | 2.57$^{a}$ | 2.63$^{a}$ |
| Hour 48 | 1.94$^{a}$ | 2.51$^{a}$ | 2.81$^{a}$ | 2.69$^{a}$ |
| Hour 60 | - | - | - | 2.78$^{a}$ |
| Hour 72 | 2.03$^{a}$ | 2.67$^{a}$ | 2.81$^{a}$ | - |
| SEM[3] | 0.13 | 0.21 | 0.13 | 0.11 |

BBFT 2

| Display Time | Days Dark Storage 7 | 14 | 18 | 21 |
|---|---|---|---|---|
| Hour 0 | 1.33$^{a}$ | 1.88$^{b}$ | 1.54$^{b}$ | 2.49$^{a}$ |
| Hour 24 | 2.36$^{a}$ | 2.31$^{ab}$ | 2.93$^{b}$ | 2.67$^{a}$ |
| Hour 48 | 1.83$^{a}$ | 3.05$^{a}$ | 2.81$^{a}$ | 2.71$^{a}$ |
| Hour 60 | - | - | - | 2.71$^{a}$ |
| Hour 72 | 2.03$^{a}$ | 1.96$^{b}$ | 2.87$^{a}$ | - |
| SEM[3] | 0.28 | 0.23 | 0.13 | 0.11 |

[1]LSmeans analyses were performed within each treatment (Control, BBFT 1, or BBFT 2), within each dark storage time (7, 14, 18, or 21 days), between display times.

[2]Following 21 days dark storage, after exceeding pre-determined spoilage microorganism thresholds at 0 hours display time, the last sampling time was revised to 60 hours to monitor TBAR changes at a closer interval.

[3]Pooled standard error of the mean within treatment, within pull time.

[a-c] LSmeans with different superscripts within treatment, within column are different ($P < 0.05$).

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