THESIS

EFFECTS OF ANTIMICROBIAL TREATMENTS ON FOOD SAFETY, QUALITY AND SHELF-LIFE OF BEEF

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ABSTRACT

EFFECTS OF ANTIMICROBIAL TREATMENTS ON FOOD SAFETY, QUALITY AND SHELF-LIFE OF BEEF

Three separate studies were conducted to evaluate effects of antimicrobial treatments on food safety, quality and shelf-life of beef. The first study explored efficacy of an antimicrobial intervention against inoculated pathogens on beef cheek meat and tongues during refrigerated and frozen storage, whereas the second study focused on the effect of an antimicrobial intervention on product flavor volatile formation in these products. The third study evaluated effects of commonly used antimicrobial intervention combinations on the flavor profile of ground beef.

Beef variety meats, including cheek meat and tongues, carry a higher level of microbiological contamination than whole muscle cuts. Presence of pathogens on these edible variety meats could pose a domestic and international food safety concern. The objective of the first study was to evaluate antimicrobial effects of peroxyacetic acid (PAA) acidified with a sulfuric acid and sodium sulfate blend (aPAA) against inoculated populations of Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella enterica* on beef cheek meat and tongues during refrigerated (4°C) and frozen (-20°C) storage. Prerigor cheek meat and tongue samples were inoculated (5 to 6 log CFU/cm^2) with a 14-strain mixture of rifampicin-resistant (100 µg/ml) STEC (two strains each of serogroups O26, O45, O103, O111, O121, O145, and O157) or a 6-serotype strain mixture of *Salmonella enterica* (serotypes Agona, Anatum, Saintpaul,

Reading, Newport and Typhimurium DT104 var. Copenhagen).After inoculation, cheek meat samples had pathogen counts of 6.0 log CFU/cm^2 and tongues had 5.6 log CFU/cm2. Inoculated samples were left untreated (control) or were immersed for 10 s in water or a solution of aPAA. The aPAA treatment was comprised of 400 ppm PAA and was acidified to a pH of 1.2 with a commercial blend of sulfuric acid and sodium sulfate. Untreated and treated cheek meat and tongue samples were individually packaged (cheek meat: aerobically; tongues: vacuum-packaged) and were stored at 4°C for up to 60 (cheek meat) or 90 (tongues) days, or at -20°C for up to 150 days (both product types). Samples were analyzed for inoculated pathogen populations and aerobic plate counts on the day of inoculation and application of immersion treatments (day-0) and throughout storage at 4°C or -20°C. Treatment of samples with aPAA reduced ($P < 0.05$) initial (day-0) inoculated pathogen counts on cheek meat samples by 1.0 (STEC) and 1.1 to 1.2 (*Salmonella*) log CFU/cm^2. Initial (day-0) reductions ($P < 0.05$) obtained for aPAA-treated tongues, when compared to the untreated controls, were 1.6 (STEC) and 1.8 (*Salmonella*) log CFU/cm^2. Overall, initial pathogen reductions of 0.4 to 0.5 (cheek meat) and 0.3 to 0.5 (tongues) log CFU/cm^2 were obtained with the water treatment, irrespective of inoculum type. Pathogen (STEC and *Salmonella*) counts did not increase during storage of cheek meat and tongues at 4°C; this was expected since the minimum temperature for growth for both pathogens is >4°C. In fact, regardless of immersion treatment, STEC and *Salmonella* populations generally decreased during storage at 4°C. Pathogen counts of aPAA-treated cheek meat samples analyzed on day-60 were 0.9 (STEC) and 2.2 (*Salmonella*) log CFU/cm^2, respectively, lower than counts of corresponding day-0 samples. For aPAA-treated tongues stored at 4°C, STEC and *Salmonella* populations on samples stored for 90 days were 0.5 ($P \geq 0.05$) and 2.3 ($P < 0.05$) log CFU/cm^2, respectively, lower than corresponding pathogen populations recovered on day-0 samples.

Pathogen counts of aPAA-treated cheek meat and tongue samples decreased during storage at -20°C for 150 days. Specifically, counts of cheek meat samples on day-150 were 1.1 (STEC) and 1.5 (*Salmonella*) log CFU/cm^2 lower ($P < 0.05$) than day-0 counts. Tongues held at -20°C for 150 days had STEC and *Salmonella* counts that were 1.6 and > 3.3 log CFU/cm^2, respectively, lower than the counts obtained on day-0.

Variety meats contribute considerable value to the beef industry, and many edible by-products are almost exclusively sold in the export market (Schaefer & Arp, 2017). Due to international demand, there is a growing need for high quality, desirable and safe variety meats. The objective of the second study was to evaluate effects of aPAA on quality attributes (aroma, lipid oxidation, and chemical profile) of beef cheek meat and tongues during refrigerated and frozen storage. Sensory aroma evaluations, lipid oxidation and Rapid Evaporative Ionization Mass Spectrometry (REIMS) analyses were performed on beef cheek meat and tongue samples that were treated with aPAA or were left untreated (control), and subsequently stored at 4°C or -20°C for up to 90 or 150 days, respectively. Identifying and validating antimicrobial interventions that will maintain flavor and shelf life that meets the expectations of the export markets to which the products are shipped is of great importance to the industry. Trained panelists evaluated odor attributes (i.e., overall off-odor, sour and rancid) at multiple time points during storage. On the last day of aerobic storage at 4°C (day-60), untreated cheek meat samples were rated greater ($P < 0.05$) for overall off-odor and rancid attributes than aPAA-treated samples. No differences ($P \geq 0.05$) in overall off-odor were obtained between untreated and aPAA-treated cheek meat samples stored for 150 days at -20°C. Similarly, no differences ($P \geq 0.05$) in any of the odor attributes evaluated were found between untreated and aPAA-treated vacuum-packaged tongue samples stored at 4°C for up to 90 days, or at -20°C for up to 150 days.

Thiobarbituric acid reactive substances (TBARS) were used to evaluate lipid oxidation. No differences ($P \geq 0.05$) in lipid oxidation were observed between untreated and treated cheek meat samples on day-0 of storage at 4°C. However, on every subsequent analysis day during the aerobic storage period at 4°C, cheek meat samples treated with aPAA had higher ($P < 0.05$) TBARS values than those of the control samples. Results of assessment using REIMS suggested differences in individual samples analyzed and potential to classify samples based on metabolomic characteristics.

Multiple hurdles technology has proven multiple intervention steps are more effective any single intervention; therefore, sequential decontamination steps are utilized (Bacon et al., 2000; Graves-Delmore et al., 1998). The objective of the third study was to characterize flavor profiles of beef treated with a combination of common pre-chilling and post-chilling antimicrobial interventions. Beef briskets collected from the harvest floor were utilized as the beef source. Briskets were not chilled until initial treatments were applied. Following collection, briskets were treated in a factorial arrangement of interventions commonly applied pre and post-chilling. Whole beef briskets were treated pre-chilling, to emulate the impact of harvest floor interventions. Briskets were spray treated (15 s, 15 psi) with lactic acid (4.5%; LA), peroxyacetic acid acidified with sulfuric acid and sodium sulfate blend (pH 1.2; aPAA) or left untreated (CONTROL). Briskets were chilled at 2°C for 24 h then divided into four equal sections, and randomly assigned to one of each of the four post-chilling treatments. Post-chilling treatments were also spray treated (15s, 15 psi) with lactic acid (4.5%; LA), a commercial blend of lactic and citric acid (LAC), peroxyacetic acid acidified with sulfiuric acid and sodium sulfate blend (pH 1.2; aPAA) or left untreated (CONTROL). Samples were then stored at 2°C for 48 to 72 h prior to processing. Each sample was ground twice and formed into 28 g patties and stored at -

20°C until analysis. At the time of sensory analysis, patties were cooked to 71°C. A subset of samples (N = 72; n = 6) were analyzed for fatty acid composition and organic volatile compounds.

Each sample was analyzed by a trained sensory panel to evaluate flavor attributes. Trained taste panelist ratings for sour and chemical were rated highest ($P < 0.01$) for the LA pre-chilling treatment, compared to the CONTROL and aPAA. Ratings for the browned attribute were greater ($P < 0.05$) for samples subjected to aPAA than CONTROL or LA. No differences ($P \geq 0.05$) were found for beef flavor ID, roasted, metallic, fat-like, sour, rancid, warmed over, or liver-like ratings due to pre-chilling treatments. Post-chilling treatments did not create any significant ($P \geq 0.05$) flavor attribute differences.

Fatty acid analysis showed very minimal differences due to use of any chemical interventions. Only C10:0 was affected by treatment; LA treatment post-chilling produced greater ($P < 0.05$) concentrations of C10:0 than LAC or CONTROL samples. When volatile acid compounds were assessed, relative abundance of pentanal was greater ($P < 0.05$) in LA-treated post-chilling intervention samples than in CONTROL, LAC, or aPAA samples. Similarly, relative abundance of hexanoic acid and pentanol were greater ($P < 0.05$) in samples treated with LA post-chilling than for samples from CONTROL or LAC treated samples. Overall, LA-treated samples resulted in various slight off-flavor attributes, with increased sour and chemical attributes. However, in general, minimal differences were associated with use of chemical antimicrobial interventions.

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CHAPTER I

Review of Literature

Escherichia coli

Escherichia coli (*E. coli*) is a predominant species of naturally occurring facultative anaerobes found in the gastrointestinal tract of animals and humans (Feng et al., 2011). *E. coli* is transmitted to humans through contaminated food, water, or direct contact with infected animals or people (Mead & Griffin, 1998). Most *E. coli* bacteria are harmless, however, some are pathogenic and can cause foodborne illness (CDC, 2018). Infection symptoms include diarrhea, and cramps, and in some extreme cases, infection can lead to hemolytic uremic syndrome (HUS). Diarrheagenic *E. coli* strains, commonly referred to as pathogenic strains, are classified into six pathotypes including; enterohemorrhagic, enterotoxigenic, enteropathogenic, enteroaggregative, enteroinvasive and diffusely adherent *E. coli* (CDC, 2018). Enterohemorrhagic *E. coli* (EHEC), also termed verocytotoxin-producing or Shiga toxin-producing *E. coli* (STEC), have been recognized as the primary cause of hemorrhagic colitis which can progress to the potentially fatal HUS (Feng et al., 2011).

According to U.S. Food and Drug Administration's "Bad Bug Book" (2012), STEC serotypes are rod shaped, gram-negative bacteria that are named according to their somatic (O) and flagella (H) antigens (FDA, 2012; Gould, 2012). Over 250 different O serogroups of *E. coli* have been found to produce Shiga toxins, and more than 100 serotypes of STEC's have been associated with human illnesses (Hughes et al., 2006). *E. coli* O157:H7, a relatively prevalent serogroup, is the most commonly reported type causing EHEC infections (Brooks et al., 2005; FDA, 2012).

Many of the pathogens of greatest concern were not recognized as causes of foodborne illness until just a few decades ago (Mead et al., 1999). *E. coli* O157:H7 was first acknowledged as a pathogen in 1982, following an investigation of an outbreak of hemorrhagic colitis (Rangel et al., 2005). Subsequently, Karmali et al. (1983) reported an association between *E. coli* and HUS. In 1993, a devastating *E. coli* O157:H7 outbreak resulted in over 700 illnesses and 4 deaths, due to improper cooking of contaminated ground beef (Bell et al., 1994). This latter event catalyzed reform in food safety and began an era of intensified efforts to reduce risk of this pathogen in the food supply (Wheeler et al., 2014). Thereafter, screening processes were improved and clinical laboratories started examining more stool specimens for *E. coli* O157:H7 (Boyce et al., 1995). Major restructuring within the industry came from a zero-tolerance policy in 1996, when the United States Department of Agriculture – Food Safety and Inspection Services (USDA-FSIS) declared *E. coli* O157:H7 an adulterant in ground beef (FSIS, 1996a) . This evaluation of the food safety system led to mandated Hazard Analysis Critical Control Point (HACCP) implementation for the meat industry.

Brooks et al. (2005) examined 940 non-O157 STEC isolates and identified the most common serotypes. Six STEC serogroups, (O26, O111, O103, O121, O45 and O145) were found to collectively account for a majority (71%) of the non-O157 STEC isolates and are referred to in the United States as the “Big 6”. These non-O157 STEC serogroups are estimated to cause over 110,000 illnesses annually (Scallan et al., 2011). In 2011, the Big 6 were declared adulterants in raw ground beef and other non-intact beef products (FSIS, 2011b). Routine testing was implemented on June 4, 2012 for sampling raw beef trimmings (FSIS, 2012). The Centers for Disease Control and Prevention (CDC) has reported nine outbreaks due to non-O157 STEC

strains since 2010. The foods implicated in these outbreaks included lettuce, clover sprouts, flour, ground beef, and other causes (CDC, 2018).

Salmonella

Salmonella are a rod-shaped, gram-negative bacteria known to cause illness for over 100 years (FSIS, 2011a). *Salmonella* are facultative bacteria belonging to the *Enterobacteriaceae* family (Montville et al., 2012). A majority of the *Salmonella* that infect humans and animals are of the *Salmonella enterica* species (Coburn et al., 2007). Within *Salmonella enterica*, there are six subspecies, including enterica, salamae, arizonae, diarizonae, houtenae, and indica (Fierer & Guiney, 2001). These subspecies are further divided into over 2,500 serotypes that are classified based on flagellar, carbohydrate and lipopolysaccharide structures (Coburn et al., 2007). *Salmonella* has a short lag phase and can grow very rapidly (Tajkarimi, 2007). The temperature range for growth is between 5-45°C, but optimal growth occurs at 35-37°C. Additionally, these bacteria are able to grow at pH levels ranging from 4.5 to 9.5 (Montville et al., 2012). *Salmonella* can be found in a variety of foods including fruits, vegetables, spices, eggs, chicken, red meat, and even processed foods (CDC, 2019). Some reported outbreaks in the United States in 2018 were linked to cake mix, cereal, raw clover sprouts, melons, dried coconut, ground beef, chicken, and pet guinea pigs.

Salmonellosis is an infection caused by *Salmonella* bacteria, and patients develop diarrhea, fever and cramps within 8 to 72 hours after infection, with the illness lasting 4 to 7 days (FSIS, 2011a). The Centers for Disease Control and Prevention (CDC; 2019) estimated that over 1.2 million illnesses are caused by *Salmonella* annually in the United States (U.S.). Nontyphoidal *Salmonella* spp. is the leading cause of both hospitalizations and deaths; 23,000 hospitalizations and 450 deaths each year (Scallan et al., 2011). For every reported case of *Salmonella* that is

confirmed by a laboratory test, there are an estimated 30 additional people with the illness that were not reported (CDC, 2019). *Salmonella* is also a worldwide concern, estimated to cause as many as 1.3 billion illnesses annually (Coburn et al., 2007). Although anyone is susceptible to salmonellosis, those with a weak immune system, such as the elderly, young children, or those with other illnesses are at a higher risk (FDA, 2012).

Beef Carcass Contamination

Skeletal muscle from animals has historically been considered sterile prior to slaughter (Huffman, 2002). Grau (1986) describes the carcass as being, "a source of edible tissue sandwiched between two regions that are heavily contaminated with microorganisms". Carcasses become contaminated from the hide, fecal material, and paunch contents from the animal itself, in addition to cross-contamination during the slaughter process from tools, equipment, employees and other contacts (Huffman, 2002; Lahr, 1996). Pathogens such as STEC, *Salmonella* spp. and *Campylobacter* spp. are naturally found in the digestive system of cattle and are shed in their feces (Chapman et al., 1997; Fedorka-Cray et al., 1998; Reid et al., 2002; Wesley et al., 2000). Barkocy-Gallagher et al. (2003) reported *Salmonella* prevalence in fed beef cattle feces at time of slaughter ranging from 2% - 9%, and non-O157 STEC from 14% - 27%. Cattle being transported encounter several potential sources of contamination. Hide contamination transpires from direct and indirect fecal contamination in cattle production, in addition to lairage environments (Arthur et al., 2010). High prevalence rates of *E. coli* O157:H7 on hides has been shown to increase from 50% on cattle hides at the feedlot to 94% on hides removed on the harvest floor (Arthur et al., 2007). Furthermore, only 29% of the *E. coli* O157:H7 isolates recovered at the harvest facility matched those recovered from the feedlot samples, indicating that the majority were likely introduced during transportation and lairage.

Transfer of pathogens occurs in lairage from animal-to-animal, animal-to-environment, and environment-to-animal routes (Small et al., 2002). Despite washing of lairage areas, Small et al. (2002) demonstrated that *E. coli* O157:H7 was harbored from one day to the next. Furthermore, Barkocy-Gallagher et al. (2001) showed that 66% of isolates recovered post-harvest matched those of the pre-harvest isolates within groups of cattle, indicating carcass contamination occurring from animals within the same lot. There are many opportunities for transferring pathogens among animals in the same lot, in addition to cross-contamination across lots in later stages of processing.

High incidence rates of *E. coli* O157 on cattle hides makes potential carcass contamination a main concern during hide removal (Elder et al., 2000; Madden et al., 2004; Nou et al., 2003). The brisket region has been found to possess high contamination rates (Bell, 1997). McEvoy et al. (2000) reported higher contamination levels on the brisket than the hock, cranial back, bung and inside round, which are all believed to be areas prone to contamination. Brisket contamination is predominantly due to the initial cut of the de-hiding process as the contaminated knife contacts the surface, in conjunction with other potential contact with contaminated surfaces during harvest (Reid et al., 2002).

Contamination rates of *Salmonella* and STEC in cattle feces, on cattle hides and carcasses are variable throughout the seasons and have been reported to be highest in the summer months and lowest throughout the winter (Barkocy-Gallagher et al., 2003). Implementing good management practices (GMP) for pre-harvest preventative strategies involved with transportation, feed and water, and live animal treatments can assist in reducing pathogenic *E. coli* shedding (Callaway, 2010; Loneragan & Brashears, 2005). Although successful, contamination can still occur later in production. The slaughter process also should minimize

bacterial contamination of the carcass from the hide and gastrointestinal tract and remove any contamination which may have occurred (Buege & Ingham, 2003). Wheeler et al. (2014) listed three issues in the production process that ultimately impact risk of contamination: "1) level of pathogens contaminating the 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". Therefore, a whole systematic approach is required to control pathogen contamination on beef carcasses.

Multiple Hurdles for Pathogen Control

Multiple hurdles technology is a method used to control risk of pathogens in the meat industry throughout several processing stages. This system involves multiple sequential treatments, which together, are more effective than any single process (Bacon et al., 2000; Graves-Delmore et al., 1998; Kang et al., 2001). This method was implemented to meet microbiological performance criteria by linking traditional "hurdle technologies" with HACCP concepts (Leistner, 1992). Multiple interventions assist in managing food safety critical control points in HACCP plans by preventing, eliminating or reducing safety hazards (Leistner & Gould, 2012). Chemical interventions are applied at multiple points, including but not limited to, hide washing, pre-evisceration, final rail washing and pre chilling, as well as employing physical methods of steam vacuuming and knife trimming (BIFSCO, 2016).

Arthur et al. (2004) tested prevalence of *E. coli* O157 at various stages of processing to assess the effectiveness of interventions. Across plants sampled, *E. coli* O157 was present on 76% of cattle hides at time of slaughter and dropped to 15% and 4% after pre-evisceration and post-evisceration, respectively. Moreover, prevalence after the final carcass wash dropped to 0.3% and was not detectable after carcasses were chilled. Another study recovered *E. coli* O157

from 26.2%, 13.0%, 43.4%, 18.3%, and 1.9% of fecal samples, hides, pre-evisceration carcasses, post-evisceration carcasses, and carcasses post-intervention, respectively (Elder et al., 2000). Consecutive decontamination methods are advantageous to meet performance standards or zero tolerance policies to ensure safety and quality of meat products.

Chemical Antimicrobial Interventions

Antimicrobials are chemicals present or added to food that kill or retard growth of microorganisms (Concia et al., 2007; Davidson et al., 2013). Many antimicrobial solutions are utilized in multiple hurdle systems including acetic acid, acidified sodium chlorite, citric acid, hypobromous acid, lactic acid, peroxyacetic acid, and other organic acid blends (BIFSCO, 2016; FSIS, 2019). Microorganisms from the hide, gastrointestinal track or environment which contaminate the surface of the carcasses, are initially quite vulnerable to chemical decontamination (Acuff, 2005). However, effectiveness of antimicrobials depends on the concentration and temperature of the acid, contact time, application pressure, tissue type, surface composition, and the sensitivity of the organisms (Hardin et al., 1995). Although extensive research has been conducted on decontamination efficacy based on various factors, more recent research has been focused on product quality implications.

Trimming Interventions

Converting beef carcasses to ground beef introduces many potential sources for contamination. Bacteria present on the outer surface of intact meat is distributed throughout the entire product and surface area is increased during grinding (Ayres, 1955; Emswiler et al., 1976). Additional contamination can occur due to further processing, contact with various equipment, personnel handling of product by plant personnel and other environmental conditions (Eisel et

al., 1997). Moreover, ground beef is a combination of multiple loads, including an assortment of varying hygienic statuses and locations including foreign markets (Bosilevac et al., 2007).

Salmonella can be harbored in lymph nodes (LN) of healthy feedlot and cull cattle (Arthur et al., 2008; Brichta-Harhay et al., 2012; Gragg et al., 2013; Webb et al., 2017). Koohmaraie et al. (2012) stated that 18% of lymph nodes contained *Salmonella*. Since complete removal of is not practically possible, these PLN may contribute to *Salmonella* contamination of ground beef products (Vipham et al., 2015). Although FSIS regulation allows 5 out of 53 samples to test positive for *Salmonella* when regulatory performance standard samples are tested, some specific programs such as the Agricultural Marketing Service's school lunch program have a zero tolerance standard (Ollinger & Bovay, 2017).

Despite effective carcass surface interventions, contamination may be inevitable when producing ground beef. Therefore, antimicrobials such as lactic acid, acidified sodium chlorite, peroxyacetic acid, sodium metasilicate, potassium lactate, and cetylpyridinium chloride have been evaluated for their decontamination efficacy on ground beef (Bosilevac et al., 2004; Ellebracht et al., 1999; Ellebracht et al., 2005; Pohlman et al., 2002; Ransom et al., 2003; Stivarius et al., 2002). In a multiple hurdles system, ground beef will have been exposed to antimicrobial interventions at several steps of production in order to meet the performance standards or zero tolerance policy.

Hot Water and Steam

Hot water has frequently been used as a pre-evisceration, post-evisceration, carcass wash or trimming intervention, and is a key step of many multiple hurdle systems (Greig et al., 2012). Several studies have shown effectiveness of hot water as a decontamination method (Baird et al., 2006; Castillo et al., 1998; Ellebracht et al., 1999; Gorman et al., 1995; Hardin et al., 1995;

Kalchayanand et al., 2012; Smith & Graham, 1978; Smith & Kavey, 1990). Hot water has even been indicated to be more effective than lactic acid on post-evisceration beef carcasses (Bosilevac et al., 2006). Gill et al. (1999) reported hot water treatments can effectively reduce microbial contamination without causing unacceptable damage to the appearance of the carcass products.

In initial attempts to meet the zero tolerance for fecal contamination, there was substantial carcass weight loss due to excessive trimming (Koohmaraie et al., 2005). In 1996, USDA-FSIS approved use of steam vacuuming as an alternative to knife trimming for visual contamination less than one inch in size (FSIS, 1996b). Although these steam vacuum techniques are effective for spot treatment, they are not efficient for an entire carcass (Dorsa et al., 1997).

Steam pasteurization is a process to apply steam to the carcass surface, and has been shown to be very effective in reducing contamination on pre-rigor beef carcasses (Nutsch et al., 1998). Minihan et al. (2003) reported significant reductions in *Enterobacteriaceae* from steam pasteurization, however, not complete decontamination. Use of steam pasteurization is therefore suggested to serve as an aid in processing. Advantages of steam pasteurization include the ability to uniformly cover surfaces without creating waste water treatment concerns since no chemicals are used and the systems can operate fully automated (Phebus et al., 1997).

Organic Acids

Many organic acids are approved as a safe and suitable antimicrobial to be used as an aqueous solution for pre-chilled and post-chilled carcasses, subprimals, trimmings and variety meats (FSIS, 2013). Organic acids are considered weak acids, as they do not fully dissociate in water, but can depending on the pH (Mani-López et al., 2012). Weak acids have a more powerful

antimicrobial activity at lower pH levels (Axelsson, 2004). Temperature and pH of the solution, greatly impact the acid's ability to inactivate pathogens (Conner & Kotrola, 1995). Antimicrobial activity of organic acids are improved when the pH is equal to or below the acid dissociation constant of the acid (pK_a; Mani-López et al., 2012). The pK_a is the pH at which half of the total acid is undissociated. An organic acid in the undissociated state is readily soluble in cell membranes allowing for antimicrobial activity (Baird-Parker, 1980). A near-neutral pH environment inside the cell causes the acid to dissociate into a free proton and acid anion, acidifying the cell interior (Davidson et al., 2013). Lipophilic organic acids interfere with the permeability of the cell membrane, leading to acidification of the cell, which is believed to be the main cause of inhibition and death of microorganisms (Baird-Parker, 1980). Microbial factors influencing antimicrobial activity include the bacteria's inherent resistance, initial levels, growth rate, cell structure, life cycle state and ability to form biofilms (Davidson et al., 2013).

Lactic Acid

Lactic acid is the most common organic acid applied in the meat industry due to effectiveness and cost advantages (Wheeler et al., 2014). Typically, lactic acid is used as a decontamination intervention for beef carcasses post-evisceration (Bosilevac et al., 2006). The antibacterial activity of lactic acid is largely due to its ability to penetrate the cell membrane, reducing the intracellular pH and disrupting the transmembrane proton force (Alakomi et al., 2000). Lactic acid is generally recognized as safe (GRAS) and can be applied at concentrations of up to 5% solution on carcasses before fabrication (FSIS, 2013). A 4% solution of lactic acid has been shown to be an effective decontaminant for chilled beef (Gill & Badoni, 2004). This application after chilling is beneficial for reducing levels of pathogens in ground beef (Castillo,

Lucia, Mercado, & Acuff, 2001; Castillo, Lucia, Mercado, Roberson, et al., 2001). Lactic acid also is approved for use on beef heads and tongues with a 2.0 to 2.8% solution (FSIS, 2013).

Peroxyacetic Acid (PAA)

Peroxyacetic acid or peracetic acid (PAA) is the peroxide of acetic acid (Kitis, 2004). Use of PAA can be effective in an aqueous solution or as an aerosol or vapor for sterilization (Baldry, 1983; Kraemer & Johnstone, 1955). Peroxyacetic acid is a strong oxidant and disinfectant with the chemical formula $CH_3COO\text{-}OH$ (Kitis, 2004). Although PAA is a more potent antimicrobial agent than hydrogen peroxide, but is considerably less stable (Santoro et al., 2007). Solutions of PAA are a colorless, clear liquid with a strong pungent acetic acid odor (Kitis, 2004). The chemical is approved as an antimicrobial agent on various products including meat or poultry carcasses, parts, trim and organs (FSIS, 2013). Peroxyacetic acid has a wide spectrum of antimicrobial activity due to its bactericidal, fungicidal, sporicidal effectiveness, and has been used as a wastewater disinfectant (Kitis, 2004). Unlike other chemical water disinfectants, PAA does not form hazardous byproducts (Santoro et al., 2007).

Beefxide®

Beefxide® (Bx) is a commercial antimicrobial compound comprised of a blend of lactic and citric acid. Treatment of beef tips with 2.5% Bx reduced *E. coli* O157:H7 and *Salmonella* contamination levels by 1.4 and 1.1 log CFU/100 cm^2, respectively (Laury et al., 2009). A 2.5% concentration of Bx was later compared to a 2.9% concentration of lactic acid on further processed beef products by Hendricks et al. (2014). This study concluded that both antimicrobial agents were similar in their efficacy as antimicrobial interventions. Tansawat et al. (2013) also concluded that generic *E. coli* and coliform counts were reduced by Bx. Consumer acceptability

traits were also evaluated and ground beef treated with Bx resulted in a darker, less brown color, as well as lower lipid oxidation levels compared to the control samples.

Beef Variety Meats

In the United States, the term 'offal' refers to edible and inedible harvest organ meats and includes components of the live animal that are not part of the carcass final product. Variety meats are the wholesale edible by-products that are segregated, chilled, processed, and inspected according to federal guidelines (Ockerman & Hansen, 1988). Edible by-products contribute a significant portion of the U.S. export market, averaging 23-25% of the volume, and 14-19% of the value of beef exports (Marti et al., 2011). In 2018, the U.S. exported over 310,000 metric tons of variety meats alone, totaling over $890 million (USMEF, 2018). Up to 60% of variety meats across species are exported, however some products such as beef tongues and livers are almost exclusively sold in export markets (Schaefer et al., 2017). Although not often desired in the United States, variety meats play an important role in the diets of many people in other countries, as they provide a more affordable, nutrient dense protein product that is accessible. According to USDA's extra labeling claims, tongues have been found to be an excellent source of protein, zinc, riboflavin (B_2), niacin(B_3), and vitamin B_{12}, as well as a good source of iron, phosphorus, and vitamin B_6 (Kesterson et al., 2018).

Furthermore, the United States Meat Export Federation (USMEF) conveyed how variety meats have served as a first point of entry for U.S. beef into developing markets (Igoe, 2013). Opening access to other countries benefits the entire industry for additional trade opportunities. Due to the international demand for these variety meats, there is an increasing need to supply a highly desirable and safe product.

Beef Cheek Meat

Cheek meat from bovine heads may be sold without further processing, or can be incorporated into other products including, but not limited to, ground beef (Schmidt et al., 2014). Beef cheek meat can be used in meat food products and identified as "beef" unless otherwise restricted in specific product regulatory standards. For example, cheek meat is limited to 25% in ground beef, and if the cheek meat exceeds 2%, its presence must be declared on the label (USDA, 2005).

Beef head and cheek meat in the U.S. is customarily sold to Mexican markets. These products are typically transported via refrigerated truck, usually frozen, and traditionally in polyethylene lined boxes (Smith et al., 1983). Beef cheek meat was traditionally consumed in ethic markets as barbacoa. The earliest record of the word barbacoa dates back to the 16th century where it was used to describe a process of cooking (Fernandez, 1526). Traditional preparation of 'babacoe de cabeza' (barbecued beef heads) included cooking whole bovine heads in a pit of hot coals (Montano, 1992). Although authentic barbacoa is less common in current times, the style is often mimicked (Montano, 1992; Valdez, 2016).

Beef Tongues

Tracing back to the pioneer days, tongues were deemed a delicacy due to being tender and rich with luscious flavors (Ashbrook, 1955). In present times, the domestic market does not value variety meats; hence, the majority are exported. Over 90% of beef tongues produced in the U.S. are exported to northern Asia and Mexico (Igoe, 2013). Japan is the dominant market for U.S. beef tongues, where they were valued at $286 million in 2016 (Schaefer et al., 2017). Tongues can be sold fresh, frozen, pickled, smoked, canned or in comminuted products (Pearson & Dutson, 2013). Typical preparation is long moist-heat cookery. In Japan, the tongue is thinly

sliced and popular in yakiniku barbeque. Some cultures have color preferences for tongues, but color generally is not considered to be an indication of quality; merely a result of breed influence of the animal (McLagan, 2011).

When tongues are collected on the harvest floor, the esophagus, pharynx, the great cornu bone and related cartilage are removed. Later the tip of the epiglottis, larynx, trachea, and salivary glands are removed (Pearson et al., 2013). Additionally, palatine and lingual tonsils must be removed as they are identified as specified risk materials (SRM) since they are high risk for prion contamination (FSIS, 2008). Lingual tonsils are located at the base of the tongue under the skin, just behind the last papilla, and a transverse cut should be made caudal to the last vallate papillae to ensure complete removal (FSIS, 2017).

Salvia and mucus from the mouth can be unrecognized sources of microbial contamination (Gill & Jones, 1998). Therefore, tongues are scalded before processing in order to remove the mucous membrane (Gonulalan et al., 2004). Additionally, tongues are skinned before consumption. Other countries have a grading system for tongues and the Canadian beef quality audit reported 65% qualifying for #1 (no defects), 30% as #2 (minor surface defects) and 5% being condemned (Van Donkersgoed et al., 2001). Earlier National Beef Quality Audits in the United States reported tongue condemnation rates of 7% in 2000, and 9.7% in 2005 (Garcia et al., 2008; McKenna et al., 2002). Tongues condemned in 2000 were associated with hair sores (34.8%), cactus tongue (17.6%), abscesses (14.75) and miscellaneous reasons (34.5%; McKenna et al., 2002).

Variety Meat Microbiological Quality

Edible variety meats, including head/cheek meat and tongues, carry a higher level of microbiological contamination than whole muscle cuts (Kalchayanand et al., 2008). Mouths of

cattle harbor large numbers of bacteria (Gill, McGinnis, et al., 1999; Grau, 1986). Moreover, higher contamination levels could be due to the removal of heads before carcasses reach multiple hurdles intervention systems during harvest and/or poor hygienic and chilling conditions of these products (Gill, McGinnis, et al., 1999; Kalchayanand et al., 2008). The head is more exposed to contamination, since the head is the lowest portion of the hanging carcass while processing, (Schmidt et al., 2014). In early stages of dressing, the animal's head is at particular risk due to contamination dripping down, unclean water splashing up, and increased contact with surfaces (Etcheverría et al., 2010). A majority of facilities remove the head after exsanguination and hide removal, and it is suspended on a conveyor. Subsequently, the tongue is removed and hung with the head and washed to remove blood and ingesta before inspection (DeOtte et al., 2010). Some facilities also pre-wash the head with water in addition to using a mechanical wash cabinet (Galloway et al., 2013).

High microbial loads, and pathogen contamination of edible variety meats have been reported. Carney et al. (2006) reported a 3.0% prevalence rate of *Escherichia coli* O157 on beef head meat, and concentrations of *Enterobacteriaceae* from 0.7 to 3.0 log CFU/g. Meyer et al. (2010) isolated *Salmonella* from 2.2% of beef tongues. Also, beef tongues were found to have the highest contamination rate among five variety meats tested (tongues, livers, hearts, kidneys, and lungs) Gill et al. (1999) reported total aerobic populations of 4.5 log CFU/cm^2 on tongues in the heads of carcasses, while washing resulted in reductions of 1 to 2 log CFU/cm^2. Aerobic plate counts from tongues after washing and chilling have been reported to be 2.6 to 4.5 and up to 5.2 log CFU/g (Delmore et al., 1999; Rothenberg et al., 1982). Previously, the challenge of variety meat quality was believed to be due to poor handling and improper temperature management rather than inherent characteristics of the meat (Delmore et al., 1999). Vanderzant

et al. (1985) suggested that proper handling, packaging, and storage conditions would be the most advantageous means for protecting exported variety meats. As technology advanced, additional interventions including antimicrobial treatments have been utilized to better control the microbiological quality of variety meats.

Although there have been many studies addressing decontamination of beef carcasses, fewer have been focused on variety meats. Delmore et al. (2000) evaluated hot water, acetic acid, lactic acid or trisodium phosphate as intervention treatments on beef variety meats. Reductions in aerobic bacteria were reported across all variety meats, but no differences were found from any treatment in reducing the *E. coli* counts on cheek meat or tongues. Lack of effectiveness was believed to be due to the physical structure of the meat and low initial counts. Hot water and 2.0% lactic acid spray treatments have been demonstrated as effective decontamination washes for the reduction of *E. coli* O157:H7 on bovine cheek and head meat (Kalchayanand et al., 2008). However, the oral cavity, which has been shown to have *E. coli* O157:H7 present, may be unaffected by spray treatments on the external beef head surface (Schmidt et al., 2014). Consequently, immersing beef cheek meat in various antimicrobial solutions (AFTEC, Beefxide, hypobromous acid, lactic acid, levunlinic acid, and sodium dodecyl sulfate) and hot water was found to be the most effective in reducing STECs and *Salmonella* (Schmidt et al., 2014).

Presence of pathogens on edible variety meats could pose a domestic and international food safety concern if the products are not adequately cooked before consumption. Additionally, bacterial spoilage of chilled products can affect the quality attributes of exported variety meats. The perishability of these products, from both a quality and microbiological standpoint, present a challenge for international trade.

Variety Meat Packaging

Historically, exported variety meats have encountered problems when arriving at destination, including failing to meet quality standards, deterioration of product during transit, and unsuitable chilling procedures. Reports from the 1970's described edible offal products with a very short shelf-life of only 2-3 days, due to poor handling, chilling and packaging (Patterson, 1971; Patterson & Gibbs, 1979). Therefore, many variety meats were frozen to extend their shelf-life, which lowers the product's value (Sheridan & Lynch, 1988). Berry et al. (1982) evaluated the quality of frozen beef tongues before and following transcontinental and transoceanic shipments, (being in transit for 7- 9 days) and no differences were detected in off-odor scores or bacterial counts.

Variety meat shelf-life was extended by 1-2 weeks under vacuum packaged conditions, with significantly lower counts than when packaged in uncovered or polyvinyl chloride film-wrapped products (Rothenberg et al., 1982). Another concern of variety meat in the export market is the chilling process prior to packaging. Vanderzant et al. (1985) showed aerobic plate counts were much higher from tongues that were not pre-chilled prior to packaging. Variety meats stored in aerobic packaging at refrigerated temperatures have been shown to have dominant presence of pseudomonads (Stanbridge & Davies, 1998).

Meat Quality

Beef Flavor

Beef palatability is dependent upon the acceptance of tenderness, flavor, juiciness (O'Quinn et al., 2018). Traditionally, tenderness has been considered the most important palatability factor, and consumers have displayed a willingness to pay premiums on guaranteed tender steaks (Boleman et al., 1997; Miller et al., 2001; Shackelford et al., 2001). The 2015

National Beef Tenderness Survey showed majority of steaks at the retail level from the rib and loin would be classified as “very tender” or “tender” (Martinez et al., 2017). Therefore, when tenderness is at an acceptable level, the focus turns to flavor. More recent studies have shown flavor to be the largest factor influencing eating satisfaction of beef (Corbin et al., 2015; Goodson et al., 2002; Killinger et al., 2004; Lucherk et al., 2016; O’Quinn et al., 2012). Feuz et al. (2004) showed not only tenderness, but flavor, marbling, aging, and origin influenced consumers’ willingness to pay. Flavor has surpassed the ranking of tenderness for the perceived importance for retailers, as they strive to meet consumer demands. (Igo et al., 2013). Consumers’ eating satisfaction is the top priority, and flavor plays a critical role in the eating experience.

Meat flavor is a complex system, encompassing a combination of taste compounds received on the tongue, as well as volatile compounds traveling through nasal pathways (Aaslyng & Meinert, 2017). Flavor includes aromatics, basic tastes, feeling factors and aftertastes (Adikari et al., 2011). Beef flavor is not a single attribute, although it was originally defined as one single measurement (AMSA, 1978). Due to the lack of adequate descriptors, studies began developing their own independent methods for quantifying flavor until standards were later updated (AMSA, 1995). For example, Johnson and Civille (1986) developed a system for quantifying warmed-over flavor, using descriptions including beef-lean, beef-fat, browned, serum/bloody, grainy/cowy, oxidized/rancid/painty, fishy and cardboard. Then Lynch et al. (1986) generated the factors of beefiness, fat, freshness, stale/off, bloody/serumy, metallic/sharp, dairy/milky, sweet, sour, bitter, salty, oily, metallic and astringency/drying. Multiple studies followed with variability in terminology to describe flavors present in beef (Campbell et al., 2001; Luchsinger et al., 1997; Maughan et al., 2012; Miller et al., 1996; Yancey et al., 2005). Therefore, a universal lexicon for beef flavor was developed (Adikari et al., 2011). Thirty-eight aroma and

flavor characteristics in beef were identified and defined, which built the foundation for trained sensory analysis.

Inherent flavor of beef is influenced by lipid content, feeding/diet, oxidation, myoglobin, and pH (Calkins & Hodgen, 2007). Vast research has been conducted on the fundamentals of beef flavor, however, little focus has been towards how conventional beef processing practices influences flavor. Standard production practices in the beef harvest process, such as use of antimicrobial interventions for controlling pathogen contamination, could have an impact on the quality of the meat. Various studies have evaluated the influence on color of various antimicrobials on ground beef, with lack of major differences in the resulting meat color (Pohlman et al., 2002; Stivarius, Pohlman et al. 2002a; Stivarius et al. 2002b). Jimenez-Villarreal et al. (2003) concluded utilizing chlorine dioxide, cetylpyridinium chloride, lactic acid or trisodium phosphate had little to no effect on ground beef color. Additional quality analysis was conducted, and antimicrobial treatments showed no negative impacts on beef patty quality evaluated by instrumental color, lipid oxidation, cooking characteristics and sensory odor or taste attributes. Interventions applied to hot carcasses can result in discoloration of fat or tissue surfaces, however, discoloration is minimized at low concentrations (Acuff, 2005).

Pohlman et al. (2009) examined the effect of PAA and other single antimicrobial interventions on the microbial, instrumental color and odor characteristics of beef. No differences were found in instrumental color measurements or odor analysis. Quilo et al. (2009) studied the sensory properties of trimmings tumbled with a single antimicrobial (potassium lactate, sodium metasilicate, peroxyacetic acid, or acidified sodium chlorite). No differences in sensory odor, sensory taste, lipid oxidation, instrumental color, shear characteristics or cooking characteristics were found. Harris et al. (2012) reported that untrained panelists could not detect

differences between control and antimicrobial spray (acetic acid, lactic acid, acidified sodium chlorite or water)-treated ground beef in a triangle test. Eastwood et al. (2018) examined effects of multiple antimicrobial treatments (hot water, lactic acid, acidified sodium chlorite, and Beefxide®) on the quality of ground beef. No trends in color or consumer likability were found due to any single or combined antimicrobial treatment, and it was concluded those food safety interventions have minimal negative impacts on ground beef quality.

Aromas

Odors transpire when volatile compounds bind to receptors in the olfactory bulb behind the nasal cavity, simulating a response (Brewer, 2007). Over a thousand compounds have been identified in the volatile constituents of meat products (Shahidi, 1994). However, only a small fraction have been reported to possess meaty aroma characteristics (Shahidi, 1989). Lipids in meat and water soluble compounds are important precursors to the flavor of cooked meat (Khan et al., 2015).

Fatty acids that do not contain any double bonds are classified as saturated. By contrast, monounsaturated fatty acids have one double bond, and polyunsaturated fats contain multiple double bonds. Beef fat is comprised of predominantly monounsaturated and saturated fatty acids with oleic (C18:1), palmitic (C18:0), and stearic (C16:0) acid being most abundant (Valsta et al., 2005). Unsaturated fatty acids oxidize more readily than saturated fats, which can lead to rancid off flavors (Mottram, 1998). Food quality can be equated with consumer acceptance, and consumer's sense of smell is the ultimate discriminator of food's flavor and quality (Lawless, 1991).

Thiobarbituric Acid Reactive Substances (TBARS)

Lipid oxidation is one of the main factors restricting the quality of meat products as it leads to discoloration, development of off flavors and off odors, drip losses and production of

potential toxins (Morrissey et al., 1998). Lipid oxidation is a deterioration reaction which intensifies through the storage period of meat products. Oxidation results in rancid off-flavors and becomes unacceptable to consumers as the products are held for extended periods of time (Gray et al., 1996).

Thiobarbituric acid reactive substances (TBARS) measure presence of oxidized lipids. Malondialdehyde (MDA) is one of the most abundant aldehydes produced during oxidation, and hence the most commonly used indicator of oxidation (Reitznerová et al., 2017). In 1944, Kohn and Liversedge first used the 2-thibarbituric acid (TBA) reaction as a measure of lipid oxidation. Patton and Kurtz (1951) then reported MDA as being responsible for the pink complex formed with TBA. A distillation method was developed later, which measures samples on a spectrophotometer for analysis of MDA (mg/kg of samples; Tarladgis et al., 1960).

Rapid Evaporative Ionization Mass Spectrometry

Rapid Evaporative Ionization Mass Spectrometry (REIMS) is a relatively new technology that has demonstrated applications in human health, and more recently in food science. Traditional mass spectrometry is utilized for food analysis, but extensive sample preparation and analysis time is required. Conversely, ambient mass spectrometry requires no sample preparation and has very fast sampling times. Over thirty different techniques of ambient mass spectrometry, including REIMS, have been used to analyze food including meat, fish, dairy products, oils, nuts, fruits and vegetables (Black et al., 2016).

REIMS has applications in human medicine, for real time characterization of cancerous tissues in surgery and other prognostic and diagnostic practices (Schäfer et al., 2009; Vaqas et al., 2016). Analysis of the aerosols released during electrosurgical dissection is commonly referred to as the intelligent knife (“iKnife”; Balog et al., 2013). In food science, REIMS

provides a successful test for identification of animal tissues from anatomical origin, species and breed (Balog et al., 2016). Other practical functions have included detecting fish fraud, identifying pork carcasses with boar taint, or if swine were fed ractopamine (Black et al., 2017; Guitton et al., 2018; Verplanken et al., 2017). Additionally, REIMS has exhibited benefits as an authenticity application, able to accurately identify various offals within minced beef (Black et al., 2019).

CHAPTER II

Effect of Antimicrobial Treatment of Beef Cheek Meat and Tongues on Pathogen Survival/Death During Refrigerated and Frozen Storage

Summary

Beef variety meats, including cheek meat and tongues, carry a higher level of microbiological contamination than whole muscle cuts (Kalchayanand et al., 2008). Presence of pathogens on these edible variety meats could pose a domestic and international food safety concern. The objective of this study was to evaluate antimicrobial effects of peroxyacetic acid (PAA) acidified with a sulfuric acid and sodium sulfate blend (aPAA) against inoculated populations of Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella enterica* on beef cheek meat and tongues during refrigerated (4°C) and frozen (-20°C) storage. Prerigor cheek meat and tongue samples were inoculated (5 to 6 log CFU/cm^2) with a 14-strain mixture of rifampicin-resistant (100 µg/ml) STEC (including two strains each of serogroups O26, O45, O103, O111, O121, O145, and O157) or a 6-serotype strain mixture of *Salmonella enterica* (serotypes Agona, Anatum, Saintpaul, Reading, Newport and Typhimurium DT104 var. Copenhagen). Inoculated samples were left untreated (control) or were immersed for 10 s in water or a solution of aPAA. The aPAA treatment was comprised of 400 ppm PAA and was acidified to a pH of 1.2 with a commercial blend of sulfuric acid and sodium sulfate. Untreated and treated cheek meat and tongue samples were individually packaged (cheek meat: aerobically; tongues: vacuum-packaged) and were stored at 4°C for up to 60 (cheek meat) or 90 (tongues)

days, or at -20°C for up to 150 days (both product types). Samples were analyzed for inoculated pathogen populations and aerobic plate counts on the day of inoculation and application of immersion treatments (day-0) and throughout storage at 4°C or -20°C. After inoculation, cheek meat samples had pathogen counts of 6.0 log CFU/cm^2 and tongues had 5.6 log CFU/cm2. Treatment of samples with aPAA reduced ($P < 0.05$) initial (day-0) inoculated pathogen counts on cheek meat samples by 1.0 (STEC) and 1.1 to 1.2 (*Salmonella*) log CFU/cm^2. Initial (day-0) reductions ($P < 0.05$) obtained for aPAA-treated tongues, when compared to the untreated controls, were 1.6 (STEC) and 1.8 (*Salmonella*) log CFU/cm^2. Overall, initial pathogen reductions of 0.4 to 0.5 (cheek meat) and 0.3 to 0.5 (tongues) log CFU/cm^2 were obtained with the water treatment, irrespective of inoculum type. Pathogen (STEC and *Salmonella*) counts did not increase during storage of cheek meat and tongues at 4°C; this was expected since the minimum temperature for growth for both pathogens is > 4°C. In fact, regardless of immersion treatment, STEC and *Salmonella* populations generally decreased during storage at 4°C. Pathogen counts of aPAA-treated cheek meat samples analyzed on day-60 were 0.9 (STEC) and 2.2 (*Salmonella*) log CFU/cm^2, respectively, lower than counts of corresponding day-0 samples. For aPAA-treated tongues stored at 4°C, STEC and *Salmonella* populations on samples stored for 90 days were 0.5 ($P \geq 0.05$) and 2.3 ($P < 0.05$) log CFU/cm^2, respectively, lower than corresponding pathogen populations recovered on day-0 samples. Pathogen counts of aPAA-treated cheek meat and tongue samples decreased during storage at -20°C for 150 days. Specifically, counts of cheek meat samples on day-150 were 1.1 (STEC) and 1.5 (*Salmonella*) log CFU/cm^2 lower ($P < 0.05$) than day-0 counts. Tongues held at -20°C for 150 days had STEC and *Salmonella* counts that were 1.6 and > 3.3 log CFU/cm^2, respectively, lower than the counts obtained on day-0.

Introduction

Beef variety meats, including cheek meat and tongues, carry a higher level of microbiological contamination than whole muscle cuts (Kalchayanand et al., 2008). Presence of pathogens on these edible variety meats could pose a domestic and international food safety concern if the products are not adequately cooked before consumption. *Escherichia coli* O157 on beef head meat has been reported having a 3.0% prevalence rate, and concentrations of *Enterobacteriaceae* counts ranging from 0.7 to 3.0 log CFU/g (Carney et al., 2006). Additionally, *Salmonella* has been present on 2.2% of beef tongues, and tongues were reported of having the highest contamination rate of variety meats tested (Meyer et al., 2010).

Antimicrobial intervention systems are a common practice within the U.S. beef industry to control pathogen contamination of beef carcasses, cuts, and trimmings (Bacon et al., 2000). The antimicrobials predominately used within the domestic beef industry are lactic acid and peroxyacetic acid and are traditionally applied via a spray cabinet. This has been an effective intervention practice for the entire carcass (Wheeler et al., 2014). However, heads are typically removed before the final carcass wash (Kalchayanand et al., 2008). Therefore, head/cheek meat and tongues are typically more heavily contaminated than whole carcasses and subsequent muscle cuts. Many beef processing facilities currently do not use, or use with limited metrics of viability, antimicrobial interventions for cheek meat or other offal items. Identifying an effective intervention system specifically for beef variety meats could be beneficial for the industry. The objective of this study was to evaluate antimicrobial effects of peroxyacetic acid (PAA) acidified with a sulfuric acid and sodium sulfate blend (aPAA) against inoculated populations of Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella enterica* on beef cheek meat and tongues during refrigerated (4°C) and frozen (-20°C) storage.

Materials and Methods

Bacterial strains and preparation of inocula

Two inoculum mixtures were utilized in this study: (i) a 14-strain rifampicin-resistant (100 μg/ml) STEC mixture, and, (ii) a 6-strain mixture of hydrogen sulfide-producing *Salmonella enterica* serotypes (Table 2.1). Specifically, the STEC inoculum included two strains of *E. coli* serotype O157:H7, and two strains each of the "Big-Six" non-O157 STEC serogroups (i.e., O26, O45, O103, O111, O121, and O145). Included in the *Salmonella* inoculum was one strain each of serotypes Agona, Anatum, Saintpaul, Reading, Newport and Typhimurium DT104 var. Copenhagen. Working cultures of the STEC and *Salmonella* strains were maintained at 4°C on plates of MacConkey sorbitol agar (Difco, Becton Dickinson and Co. [BD], Sparks, MD) supplemented with rifampicin (SMAC+rif; 100 μg/ml; Sigma-Aldrich, St. Louis, MO) and xylose lysine deoxycholate agar (XLD; Acumedia-Neogen, Lansing, MI), respectively. The rifampicin resistance phenotype of the STEC strains and the hydrogen sulfide-producing ability of the *Salmonella* strains (indicated by the formation of black-centered colonies on XLD agar) was used to selectively enumerate and differentiate the inoculum populations from beef product-associated microflora on untreated and treated beef cheek meat and tongue samples.

The inoculum preparation procedure was initiated three days prior to the start of each inoculation trial. Specifically, a single colony from the SMAC+rif or XLD plate of each strain was separately inoculated into 10 ml of tryptic soy broth (TSB; Difco, BD) supplemented with rifampicin (100 μg/ml; TSB+rif) for the rifampicin-resistant STEC strains, or TSB (without rifampicin) for the *Salmonella* strains. Inoculated broths were incubated at 35°C for 24 ± 2 h. Broth cultures were then subcultured once by transferring a 0.1-ml aliquot of the first broth culture into 10 ml of fresh TSB+rif or TSB. After incubation (35°C, 22 h), cultures of the 14

STEC strains, or six *Salmonella* serotypes strains, were combined, and cells were harvested by centrifugation (6,000 × g, 15 min, 4°C; Sorvall Legend X1R centrifuge, Thermo Scientific, Waltham, MA). The resulting pellets were washed with 10 ml of phosphate-buffered saline (PBS, pH 7.4; Sigma-Aldrich), recentrifuged, and resuspended in 140 ml (STEC) or 60 ml (*Salmonella*) of PBS. The concentration of the resulting cell suspensions was 8 to 9 log CFU/ml.

Inoculation of prerigor beef cheek meat and tongues

Two repetitions (trials), initiated on two separate days, were performed for each product (cheek meat and tongues), inoculum (STEC and *Salmonella*) and storage temperature (4°C and -20°C) combination. On each trial day, prerigor beef cheek meat and/or whole beef tongues, with tonsils removed, were collected at a commercial processing facility in Colorado. Tissues were transferred hot in an insulated container to the Center for Meat Safety & Quality (Department of Animal Sciences, Colorado State University, Fort Collins, CO) within 1 h of collection.

Cheek meat was cut into 5 × 10 cm pieces that were placed on trays lined with alcohol-sterilized aluminum foil and inoculated under a biological safety cabinet (Figure 2.1). A 100 µl-aliquot of the prepared STEC or *Salmonella* inoculum was deposited on one side of each sample and spread across the entire surface with a sterile L-shaped disposable spreader. Samples were held for 10 min at room temperature (25 ± 3°C) to allow for bacterial cell attachment and were then flipped over, using flame-sterilized forceps, and the second side was inoculated in the same manner as the first.

Tongues were sectioned into thirds, resulting in approximately 16 × 8 cm-sized portions of the external surface area of the tongue. Tongue portions were placed on foil-lined trays and sterile cotton swabs, moistened with edible carcass ink, were used to mark two separate 6 × 4 cm areas on the surface of each tongue portion (Figure 2. 2). The carcass ink was left to dry for at

least 5 min before depositing a 25 µl aliquot of the STEC or *Salmonella* inoculum within each marked area. After evenly spreading the inoculum within each 24-cm^2 area, samples were left undisturbed for 20 min to allow for bacterial cell attachment.

The target inoculation level for both tissues was approximately 5 to 6 log CFU/cm^2. Following inoculation, cheek meat and tongue samples were either subjected to one of the immersion treatments outlined below, or, in the case of the untreated controls, were immediately packaged.

Antimicrobial treatment of beef cheek meat and tongue samples

Within each inoculum type (i.e., STEC and *Salmonella*), cheek meat and tongue samples were assigned to one of three immersion treatments: an untreated control, water (at room temperature, 25 ± 3°C), or a solution of aPAA. The aPAA was comprised of 400 ppm PAA (Kroff, Pittsburg, PA) that was acidified to a pH of 1.2 with a commercial blend of sulfuric acid and sodium sulfate (Centron; Zoetis, Parsippany, NJ). For the water and aPAA treatments, batches of nine pieces at a time of inoculated cheek meat were placed into a 92 oz Whirl-Pak bag (Nasco, Modesto, CA), then 1.5 L of water or aPAA was immediately added to the bag, fully immersing all nine samples. Samples in the bag were gently agitated for 10 s after which, the solution was poured off, and samples were placed in a sterile strainer, for 5 min, to allow excess liquid to drain. Sterile forceps were then used to place cheek meat samples into individual 24-oz Whirl-Pak bags for subsequent storage or microbial analysis (day-0 samples). The same treatment procedure was followed for tongues; however, 2 L of treatment solution was used to ensure all samples were fully immersed. After draining for 5 min, tongues were placed in 6 x 8 in vacuum bags (3-mil. standard barrier; Clarity Vacuum Pouches; Kansas City, MO) for subsequent storage or

microbial analysis (day-0 sample). Vacuum bags were sealed using a vacuum packager (Koch Equipment, Model No. UV 225, Kansas City, MO).

Storage of beef cheek meat and tongue samples

Samples were randomly assigned to a storage temperature, that being refrigerated (chilled) at 4°C, or frozen at -20°C, creating an equal representation of samples per product type, inoculum type, and immersion treatment (Table 2.2). Cheek meat samples, individually packaged in Whirl-Pak bags (aerobic environment), were stored at 4°C for up to 60 days, and at -20°C for up to 150 days. Individual vacuum-packaged tongues were stored at 4°C for up to 90 days and at -20°C for up to 150 days. The analysis of cheek meat samples stored at 4°C was concluded at day-60 because of the presence of high levels of spoilage flora (9.1 to 9.7 log CFU/cm^2) on these samples. Therefore, the cheek meat refrigerated storage period was shorter than the 90-day period for the tongue samples.

Microbiological analyses

Cheek meat and tongue samples were analyzed for aerobic plate counts and inoculated bacterial populations on the day of inoculation and application of immersion treatments (day-0) and on at least six subsequent time points during storage (Table 2.3). Samples stored at -20°C were removed from the freezer and placed at 4°C for approximately 20 h before microbial analysis to allow samples to thaw. On each sampling day (Table 2.3), cheek meat samples were aseptically transferred to a filtered 24-oz Whirl-Pak filter bag, and 145 ml of Dey-Engley (D/E) neutralizing broth (Difco, BD) was added. For tongue samples, the external surface of the two 24 cm^2 marked areas was excised (Figure 2. 2), using sterile scalpels, and placed together into a 24-oz Whirl-Pak filter bag. A 35 ml volume of D/E neutralizing broth was added to the tongue samples.

All samples were mechanically agitated (Masticator, IUL Industries, Barcelona, Spain) for 2 min and then serially diluted (10-fold) in 0.1% buffered peptone water (BPW; Difco). Appropriate dilutions were surface plated (0.1 or 1 ml) in duplicate on: (i) tryptic soy agar (TSA; Acumedia-Neogen) to determine aerobic plate counts; (ii) SMAC+rif for inoculated rifampicin-resistant STEC populations; or (iii) XLD agar for inoculated *Salmonella* populations. Colonies were enumerated following incubation of plates at 35°C for 24 h (SMAC+rif and XLD) or 25°C for 72 h (TSA). Uninoculated pre-rigor cheek meat and tongue samples were also analyzed on day-0 of each trial (three samples per trial) for levels of the natural microflora (on TSA), and for any naturally present rifampicin-resistant (on SMAC+rif) or hydrogen sulfide-producing populations (on XLD agar).

Statistical analysis

Two repetitions (trials) were performed for each product (cheek meat or tongues), inoculum type (STEC or *Salmonella*), immersion treatment (untreated, water, or aPAA) and storage temperature (4°C or -20°C) combination, and within each repetition, three samples were analyzed on each sampling day (n = 6). The study was designed as a randomized complete block with trial day as the block. Bacterial counts were log transformed and expressed as least-squares means (log CFU/cm^2). Data (aerobic plate counts or pathogen counts) for each product, inoculum type, and storage temperature combination (e.g., cheek meat inoculated with STEC and stored at 4°C) were analyzed separately using the mixed procedure in SAS (version 9.4, SAS Institute, Cary, NC). The main effects of treatment and storage day were evaluated, as well as the interaction. All least-squares means were separated using a significance level of $\alpha = 0.05$.

Results and Discussion

Microbial populations of uninoculated samples.

Microbial analysis of uninoculated and untreated beef cheek meat and tongue samples, on day-0 of each trial, indicated absence (< 0.2 log CFU/cm^2) of naturally occurring rifampicin-resistant (on SMAC+rif) and hydrogen sulfide-producing (on XLD agar) microflora. Therefore, colony counts recovered with SMAC+rif and XLD agar from both untreated and treated inoculated samples (Tables 2.4 to 2.12) were those of the inoculated pathogen inocula (STEC or *Salmonella*). Levels of the natural microflora, as recovered on TSA, associated with the uninoculated cheek meat and tongue samples were 3.6 ± 0.6 log CFU/cm^2 and 4.1 ± 0.4 log CFU/cm^2, respectively (data not shown in tables).

Beef cheek meat inoculated with STEC

Results for the STEC-inoculated cheek meat samples stored at 4°C or -20°C are shown in Tables 2.5 and 2.6. Initial (day-0) STEC and aerobic plate counts of 6.0 and 6.1 log CFU/cm^2, respectively, were obtained for the untreated inoculated (control) cheek meat samples (Tables 2.5 and 2.6). Following immersion treatment (10 s) with water or aPAA, STEC counts were reduced by 0.4 and 1.0 log CFU/cm^2, respectively (Tables 2.5 and 2.6). Similarly, initial aerobic plate counts were reduced by 0.4 (water) and 0.9 (aPAA) log CFU/cm^2 (Tables 2.5 and 2.6). Bacterial count reductions obtained with the water treatment reflect the physical removal of cells during the immersion treatment, whereas reductions obtained with the aPAA treatment are due to a combination of the killing effect from the active ingredients of the antimicrobial solution as well as the physical removal of cells.

As expected, pathogen counts did not increase during aerobic storage of cheek meat samples at 4°C for 60 days (Table 2.5). This was expected since the minimum growth temperature for pathogenic *E. coli* is 6.5°C (FDA, 2016). In fact, STEC populations for all treatments decreased over the 60-day storage period. Untreated, and water or aPAA-treated

samples analyzed on day-60 had STEC counts that were 1.6, 1.3 and 0.9 log CFU/cm^2, respectively, lower than counts of corresponding day-0 samples (Table 2.5). By contrast, aerobic plate counts for all treatments increased ($P < 0.05$) during storage at 4°C due to the growth of spoilage microflora. Aerobic plate counts of samples treated with aPAA were lower ($P < 0.05$) than those of the control and water-treated samples from day-10 to day-45 of storage. By day-60, however, aerobic plate counts for all treatments ranged from 9.1 to 9.7 log CFU/cm^2 (Table 2.5).

Bacterial counts (STEC and aerobic plate counts) of untreated and treated cheek meat samples stored for 150 days at -20°C were lower ($P < 0.05$) or similar ($P \geq 0.05$) to those obtained for day-0 samples (Table 2.6). Specifically, , pathogen counts of the control, water-treated and aPAA-treated samples were 0.5, 0.4 and 1.1 log CFU/cm^2, respectively, lower ($P < 0.05$) than the corresponding day-0 counts (i.e., 6.0, 5.6 and 5.0 log CFU/cm^2, respectively). Therefore, aPAA treatment of cheek meat samples did not only reduce initial STEC counts by 1.0 log CFU/cm^2 on the day of treatment (day-0), but also resulted in an additional reduction of 1.1 log CFU/cm^2 by the end of the 150-day frozen storage period (Table 2.6).

Beef cheek meat inoculated with Salmonella

Results for the *Salmonella*-inoculated cheek meat samples stored at 4°C are shown in Table 2.7. Initial (day-0) bacterial counts of inoculated untreated (control) cheek meat samples were 6.0 (*Salmonella*) and 6.2 (aerobic plate counts) log CFU/cm^2 (Table 2.7). Treatment with aPAA reduced ($P < 0.05$) the pathogen and aerobic plate counts by 1.1 and 1.0 log CFU/cm^2, respectively, while the water treatment lowered initial bacterial counts by 0.5 (*Salmonella*; $P \geq 0.05$) and 0.6 (aerobic plate counts; $P < 0.05$) log CFU/cm^2 (Table 2.7).

Irrespective of immersion treatment, *Salmonella* counts did not increase during the 60-d refrigerated (4°C) aerobic storage of cheek meat samples. This was expected since the minimum

growth temperature for *Salmonella* is 5.2°C (FDA, 2016). Pathogen populations for all treatments actually decreased ($P < 0.05$) during storage (Table 2.7). More specifically, *Salmonella* counts of the untreated, and water or aPAA-treated samples analyzed on day-60 were 2.4, 1.8 and 2.2 log CFU/cm^2, respectively, lower ($P < 0.05$) than counts of corresponding day-0 samples (Table 2.7). Cheek meat samples treated with aPAA on day-60 had pathogen counts 0.9 log CFU/cm^2 lower than the untreated samples (Table 2. 7). On the other hand, aerobic plate counts of cheek meat samples increased during storage at 4°C, regardless of treatment, due to growth of spoilage microbial populations (recovered with TSA; Table 2.7). At all sampling points apart from day-0, however, aerobic plate counts of the aPAA-treated samples were lower ($P < 0.05$) than those of the control and water-treated products.

Results for the *Salmonella*-inoculated cheek meat samples stored at -20°C are shown in Table 2.8. Day-0 *Salmonella* and aerobic plate counts of the untreated samples, and subsequent reductions obtained following treatment with water or aPAA, were similar to those obtained for samples destined for storage at 4°C (Tables 2.7 and 2.8). Specifically, initial *Salmonella* counts of 6.0 log CFU/cm^2 were reduced ($P < 0.05$) by 0.4 (water) and 1.2 (aPAA) log CFU/cm^2, and, initial aerobic plate counts of 6.1 log CFU/cm^2 were reduced ($P < 0.05$) by 0.4 (water) and 1.0 (aPAA) log CFU/cm^2 (Table 2.8). Additional reductions of bacterial populations (*Salmonella* and aerobic plate counts) were obtained during frozen storage of untreated and treated cheek meat samples (Table 2.8). Specifically, with regard to the aPAA treatment, day-15 through day-150 samples had *Salmonella* counts that were 1.0 to 1.5 log CFU/cm^2 lower ($P < 0.05$) than the day-0 counts (Table 2.8). After 150 days, aPAA-treated cheek meat samples had pathogen counts 2.0 and 1.6 log CFU/cm^2 lower than the untreated and water-treated samples respectively (Table 2.8).

Beef tongues inoculated with STEC

Results for the STEC-inoculated tongues stored at 4°C or -20°C are shown in Tables 2.9 and 2.10, respectively. Initial (day-0) bacterial counts, as recovered with SMAC+rif and TSA, were 5.6 and 5.9 log CFU/cm^2, respectively, for untreated tongue samples (Tables 2.9 and 2.10). Treatment with aPAA for 10 s reduced the pathogen counts by 1.6 log CFU/cm^2, and aerobic plate counts by 1.0 log CFU/cm^2 (Tables 2.9 and 2.10). Counts of water-treated tongues were similar to those of the untreated control samples, regardless of recovery medium (Tables 2.9 and 2.10).

Pathogen counts of vacuum-packaged untreated and water-treated tongues decreased during storage at 4°C, and by day-90, STEC counts were 1.9 and 1.7 log CFU/cm^2, respectively, lower ($P < 0.05$) than the counts obtained on day-0 for these treatments (Table 2.9). For the aPAA-treated samples, STEC counts for tongues stored for 90 days were similar ($P \geq 0.05$) to those obtained for the day-0 samples. STEC counts of the aPAA treatment were lower ($P < 0.05$) than those of the control through day-60 of refrigerated storage (Table 2.9). Aerobic plate counts of vacuum-packed tongue samples increased during storage at 4°C due to the growth of spoilage populations. Regardless of immersion treatment, counts for samples stored for 90 days were 0.7 to 1.0 log CFU/cm^2 higher than counts obtained for day-0 tongue samples (Table 2.9). Aerobic plate counts of aPAA-treated tongues remained lower than the aerobic plate counts of the control and water-treated samples throughout the 90-day storage period (Table 2.9).

Vacuum-packaged tongue samples stored at -20°C for 150 days had bacterial counts (STEC and aerobic plate counts) that were lower than the counts obtained for day-0 samples (Table 2.10). For the aPAA-treated samples, STEC and aerobic plate counts on day-150 of storage were 1.6 and 1.2 log CFU/cm^2, respectively, lower than the corresponding day-0 counts

(Table 2.10). For the control and water-treated tongues, bacterial counts at the end of the frozen storage period were 0.6 (SMAC+rif) and 0.5 (TSA) log CFU/cm^2 lower than the corresponding counts on day-0 (Table 2.10). After 150 days, aPAA-treated tongue samples had pathogen counts 2.6 log CFU/cm^2 lower than the untreated samples (Table 2.10).

Beef tongues inoculated with Salmonella

Results for the *Salmonella*-inoculated tongues stored at 4°C or -20°C are shown in Tables 2.11 and 2.12, respectively. Untreated control tongue samples inoculated with *Salmonella* had initial pathogen counts of 5.6 log CFU/cm^2 and treatment with water or aPAA reduced the inoculated populations by 0.5 and 1.8 log CFU/cm^2, respectively (Tables 2.11 and 2.12). Aerobic plate counts of untreated tongues were 6.0 log CFU/cm^2, and the water and aPAA treatments reduced these counts by 0.3 ($P \geq 0.05$) and 1.5 ($P < 0.05$) log CFU/cm^2, respectively (Tables 2.11 and 2.12).

Salmonella counts of vacuum-packaged tongues, regardless of treatment, decreased during storage at 4°C (Table 2.11). More specifically, pathogen counts of untreated, water-treated and aPAA-treated samples on day-90 of storage were 1.8, 2.2, and 2.3 log CFU/cm^2, respectively, lower than the counts obtained on day-0 (Table 2.11). After 90 days, aPAA-treated tongue samples had pathogen counts 2.3 and 1.4 log CFU/cm^2 lower than the untreated and water-treated samples respectively (Table 2.11). Aerobic plate counts of untreated and water-treated tongues increased ($P < 0.05$) by 0.9 and 1.3 log CFU/cm^2, respectively, during storage at 4°C due to the growth of spoilage populations. However, the aerobic plate counts of aPAA-treated tongues remained largely unchanged ($P \geq 0.05$) during the 90-day storage time. Aerobic plate counts of aPAA-treated tongues were lower (by 1.2 to 2.8 log CFU/cm^2; $P < 0.05$) than the

aerobic plate counts of the control and water-treated samples throughout the 90-day storage period (Table 2.11).

Salmonella and aerobic plate counts of vacuum-packaged tongue samples, irrespective of treatment, stored at -20°C for 150 days were lower than the counts obtained for day-0 samples (Table 2.12). For the control and water-treated tongues, pathogen counts on day-150 were 0.7 and 0.8 log CFU/cm^2, respectively, lower ($P < 0.05$) than the corresponding day-0 counts (Table 2.12). For the aPAA-treated samples, counts on day-150 of storage were > 3.3 (*Salmonella*) and 2.9 (aerobic plate counts) log CFU/cm^2, respectively, lower than the corresponding day-0 counts (Table 2.12). After 150 days, aPAA-treated tongue samples had pathogen counts < 4.4 and < 3.8 log CFU/cm^2 lower than the untreated and water-treated samples respectively (Table 2.12).

Numerically greater reductions were observed for counts of *Salmonella* compared to STEC for initial reductions and throughout storage. *Salmonella* has been shown to be more acid sensitive than *E. coli* (Gorden & Small, 1993). Acid resistance of *E. coli* is speculated to be an adaptation to become successfully established as normal microflora in the digestive system of mammals (Gorden et al., 1993). Additionally, aPAA-treated tongues resulted in greater initial reductions than the aPAA-treated cheek meat when compared to the untreated samples. It has been demonstrated treating cheek meat present challenges due the physical structure that can prevent exposure to interventions (Delmore et al., 2000). Additionally, the limitations of spray-applied head wash interventions are not affective for treating cheek meat, showing additional measures are required for beef cheek meat (Kalchayanand et al., 2008; Schmidt et al., 2014). This study determined aPAA not only resulted in initial reductions, but continued reductions throughout storage, showing the efficacy of aPAA.

Conclusions

Application of the 10 s aPAA immersion treatment reduced (day-0) initial inoculated pathogen counts on cheek meat by 1.0 (STEC) and 1.1 to 1.2 (*Salmonella*) log CFU/cm^2. Pathogen counts did not increase during aerobic storage of cheek meat samples at 4°C. This finding was expected because the minimum temperature for growth of pathogenic *E. coli* is 6.5°C, and 5.2°C for *Salmonella*. STEC and *Salmonella* populations, for all cheek meat treatments, decreased during the 60-day storage period at 4°C. Pathogen counts of the aPAA-treated cheek meat samples analyzed on day-60 were 0.9 (STEC) and 2.2 (*Salmonella*) log CFU/cm^2, respectively, lower ($P < 0.05$) than counts of corresponding day-0 samples. Pathogen and aerobic plate counts of untreated and treated cheek meat samples stored for 150 days at -20°C were lower ($P < 0.05$) than those obtained for day-0 samples.

Immediate (day-0) reductions of 1.6 (STEC) and 1.8 (*Salmonella*) log CFU/cm^2 were obtained following treatment (10 s) of tongues with aPAA. As noted for the cheek meat samples, STEC and *Salmonella* counts for all treatments decreased during the 90-day storage period at 4°C. Pathogen counts of aPAA-treated tongues stored for 90 days at 4°C were 0.5 (STEC) and 2.3 (*Salmonella*) log CFU/cm^2 lower than corresponding day-0 counts. For the aPAA-treated tongues stored at -20°C, pathogen counts on day-150 of storage were 1.6 log (STEC) and > 3.3 log (*Salmonella*) CFU/cm^2 lower than the corresponding day-0 counts.

TABLE 2. 1. Bacterial strains used.

Inoculum	Serotype or serogroup	Strain ID	Origin	Source
Shiga toxin-producing *E. coli*	O157:H7	ATCC 43895[a]	Raw hamburger	ATCC[a]
	O157:H7	C1-072	Bovine feces	Carlson et al. (2009)
	O26:H11	hSTEC_03	Human	USMARC[b]
	O26:H2	93.0494	Human	ERC[c]
	O45	99E_2750	Human	USMARC
	O45	O45-2	Human	USMARC
	O103	MDR0089	Beef	USMARC
	O103:H2	90.1764	Cow	ERC
	O111:H8	hSTEC_08	Human	USMARC
	O111	4.0522	Cow	ERC
	O121	10896	Human	USMARC
	O121	imp_450	Beef	USMARC
	O145:NM	hSTEC_22	Human	USMARC
	O145	MAY109	Beef	USMARC
Salmonella	Agona	Nonfed plant 1	Cattle hides	Bacon et al. (2000)
	Anatum	Fed plant 3	Cattle hides	Bacon et al. (2000)
	Saintpaul	Fed plant 2	Cattle hides	Bacon et al. (2000)
	Reading	Fed plant 1	Cattle hides	Bacon et al. (2000)
	Newport	FSL S5-436	Bovine	Cornell University[d]
	Typhimurium DT104 var. Copenhagen	Fed plant 2	Cattle hides	Bacon et al. (2000)

[a]ATCC, American Type Culture Collection, Manassas, VA

[b]Dr. Tommy Wheeler, U.S. Meat Animal Research Center (USMARC), U.S. Department of Agriculture, Agricultural Research Service, Clay Center, NE

[c]Dr. Chitrita DebRoy, *E. coli* Reference Center (ERC), Pennsylvania State University, University Park

[d]Dr. Martin Wiedmann, Department of Food Science, Cornell University, Ithaca, NY.

TABLE 2. 2. Product types, inoculum types, storage temperatures, immersion treatments, and number of samples.

Product Type	Inoculum	Storage Temperature	Treatment	Number of Samples
Cheek meat	STEC[1]	4°C	Untreated	42
			Water	42
			aPAA	42
		-20°C	Untreated	42
			Water	42
			aPAA	42
	Salmonella[2]	4°C	Untreated	42
			Water	42
			aPAA	42
		-20°C	Untreated	42
			Water	42
			aPAA	42
Tongues	STEC	4°C	Untreated	48
			Water	48
			aPAA	48
		-20°C	Untreated	42
			Water	42
			aPAA	42
	Salmonella	4°C	Untreated	48
			Water	48
			aPAA	48
		-20°C	Untreated	42
			Water	42
			aPAA	42

aPAA: Peroxyacetic acid acidified with a sulfuric acid and sodium sulfate blend
[1]14-strain mixture of Shiga toxin-producing *Escherichia coli*
[2]6-serotype strain mixture of *Salmonella enterica*

TABLE 2. 3. Sampling days for microbiological analysis of untreated and treated inoculated cheek meat and tongue samples stored at 4°C or -20°C.

Product Type	Storage Temperature	Sampling Days
Cheek Meat	4°C	0, 5, 10, 15, 30, 45, 60
	-20°C	0, 15, 30, 60, 90, 120, 150
Tongues	4°C	0, 5, 10, 15, 30, 45, 60, 90
	-20°C	0, 15, 30, 60, 90, 120, 150

TABLE 2. 4. Microbiological analysis *P*-values for main effects of treatment, and storage day, as well as the interaction.

Product Type	Inoculum	Storage Temperature	Culture Medium	Treatment × Storage Day Interaction *P*-value	Treatment Main Effect *P*-value	Storage Day Main Effect *P*-value
Cheek meat	STEC	4°C	SMAC+rif	0.0542	< 0.0001	< 0.0001
			TSA	< 0.0001	< 0.0001	< 0.0001
		-20°C	SMAC+rif	< 0.0001	< 0.0001	< 0.0001
			TSA	0.0271	< 0.0001	< 0.0001
	Salmonella	4°C	XLD	0.1296	< 0.0001	< 0.0001
			TSA	0.0004	< 0.0001	< 0.0001
		-20°C	XLD	< 0.0001	< 0.0001	< 0.0001
			TSA	< 0.0001	< 0.0001	< 0.0001
Tongues	STEC	4°C	SMAC+rif	0.0003	< 0.0001	< 0.0001
			TSA	0.9421	0.0135	< 0.0001
		-20°C	SMAC+rif	0.0921	< 0.0001	< 0.0001
			TSA	0.1557	< 0.0001	< 0.0001
	Salmonella	4°C	XLD	0.6581	< 0.0001	< 0.0001
			TSA	0.0158	< 0.0001	< 0.0001
		-20°C	XLD	< 0.0001	< 0.0001	< 0.0001
			TSA	0.0957	< 0.0001	< 0.0001

STEC: Shiga toxin-producing *Escherichia coli*; SMAC+rif: MacConkey sorbitol agar; TSA: tryptic soy agar; XLD: xylose lysine deoxycholate agar

TABLE 2. 5. Adjusted least-squares means estimates of rifampicin-resistant Shiga toxin-producing *Escherichia coli* (**STEC**) counts (SMAC+rif) and aerobic plate counts (TSA) for inoculated (14-strain STEC mixture; 5-6 log CFU/cm^2) prerigor beef **cheek meat** that was left untreated (control) or treated by immersing (10 s) in water or peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, and aerobically stored at **4°C** for up to 60 days.

		Mean Counts (log CFU/cm^2) ± Standard Deviation						
		Storage Day						
Culture Medium	Treatment	0	5	10	15	30	45	60
SMAC+rif[*]	Control	6.0 ± 0.0	5.4 ± 0.2	5.5 ± 0.2	5.3 ± 0.0	5.2 ± 0.1	5.0 ± 0.3	4.4 ± 0.6
	Water	5.6 ± 0.1	5.3 ± 0.1	5.1 ± 0.2	5.1 ± 0.1	4.8 ± 0.1	4.5 ± 0.2	4.3 ± 0.4
	aPAA	5.0 ± 0.2	4.7 ± 0.1	4.6 ± 0.2	4.5 ± 0.1	4.4 ± 0.2	4.0 ± 0.5	4.1 ± 0.4
TSA	Control	6.1 ± 0.0^{fg}	5.9 ± 0.0^{fg}	5.9 ± 0.1^{fg}	7.0 ± 0.7^{d}	8.9 ± 0.2^{b}	9.3 ± 0.2^{ab}	9.7 ± 0.1^{a}
	Water	5.7 ± 0.1^{fgh}	5.5 ± 0.2^{ghi}	5.8 ± 0.5^{fgh}	6.8 ± 0.6^{de}	8.9 ± 0.2^{b}	9.1 ± 0.2^{ab}	9.4 ± 0.3^{ab}
	aPAA	5.2 ± 0.1^{hij}	4.9 ± 0.3^{ghi}	5.1 ± 0.1^{ij}	5.0 ± 0.3^{ij}	6.3 ± 1.6^{ef}	7.6 ± 1.4^{c}	9.1 ± 0.3^{b}

SMAC+rif: MacConkey sorbitol agar supplemented with rifampicin (100 μg/ml); TSA: tryptic soy agar

[*] Per Table 2. 4, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed

[a-j] Means without a common superscript letter differ ($P < 0.05$)

TABLE 2. 6. Adjusted least-squares means estimates of rifampicin-resistant Shiga toxin-producing *Escherichia coli* (**STEC**) counts (SMAC+rif) and aerobic plate counts (TSA) for inoculated (14-strain STEC mixture; 5-6 log CFU/cm^2) prerigor beef **cheek meat** that was left untreated (control) or treated by immersing (10 s) in water or peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, and aerobically stored at **-20°C** for up to 150 days.

		Mean Counts (log CFU/cm^2) ± Standard Deviation						
		Storage Day						
Culture Medium	Treatment	0	15	30	60	90	120	150
SMAC+rif	Control	6.0 ± 0.0^{a}	5.7 ± 0.1^{bcd}	5.8 ± 0.1^{bc}	5.6 ± 0.1^{bcd}	5.8 ± 0.1^{b}	5.6 ± 0.1^{cd}	5.5 ± 0.1^{de}
	Water	5.6 ± 0.1^{bcd}	5.5 ± 0.1^{ed}	5.5 ± 0.2^{ed}	5.3 ± 0.2^{f}	5.4 ± 0.2^{ef}	5.4 ± 0.1^{ef}	5.2 ± 0.2^{f}
	aPAA	5.0 ± 0.2^{g}	4.2 ± 0.1^{h}	4.1 ± 0.2^{hi}	4.2 ± 0.3^{h}	4.2 ± 0.1^{h}	4.1 ± 0.2^{h}	3.9 ± 0.2^{i}
TSA	Control	6.1 ± 0.0^{a}	6.0 ± 0.1^{ab}	6.0 ± 0.1^{ab}	5.8 ± 0.2^{bcde}	5.9 ± 0.1^{bcde}	6.0 ± 0.1^{abc}	5.9 ± 0.1^{bcd}
	Water	5.7 ± 0.1^{defg}	5.8 ± 0.1^{cdef}	5.8 ± 0.2^{cdef}	5.6 ± 0.1^{fgh}	5.4 ± 0.2^{h}	5.6 ± 0.0^{efgh}	5.5 ± 0.2^{gh}
	aPAA	5.2 ± 0.1^{i}	4.8 ± 0.2^{jk}	4.8 ± 0.3^{j}	4.7 ± 0.2^{jk}	4.4 ± 0.4^{l}	4.6 ± 0.2^{kl}	4.6 ± 0.3^{jkl}

SMAC+rif: MacConkey sorbitol agar supplemented with rifampicin (100 µg/ml); TSA: tryptic soy agar
[a-l] Means within each culture medium without a common superscript letter differ ($P < 0.05$).

TABLE 2. 7. Adjusted least-squares means estimates of ***Salmonella*** counts (XLD) and aerobic plate counts (TSA) for inoculated (5-6 log CFU/cm^2; 6-*Salmonella* serotype strain mixture) prerigor beef **cheek meat** that was left untreated (control) or treated by immersing (10 s) in water or peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, and aerobically stored at **4°C** for up to 60 days.

		Mean Counts (log CFU/cm^2) ± Standard Deviation						
		Storage Day						
Culture Medium	Treatment	0	5	10	15	30	45	60
XLD[*]	Control	6.0 ± 0.0	5.6 ± 0.1	5.2 ± 0.2	5.1 ± 0.2	4.8 ± 0.2	4.5 ± 0.2	3.6 ± 0.5
	Water	5.5 ± 0.1	5.2 ± 0.1	4.5 ± 0.5	4.7 ± 0.1	4.5 ± 0.2	4.0 ± 0.3	3.7 ± 0.3
	aPAA	4.9 ± 0.2	4.4 ± 0.2	3.3 ± 1.2	4.1 ± 0.3	3.8 ± 0.3	3.8 ± 0.3	2.7 ± 1.3
TSA	Control	6.2 ± 0.5^{f}	5.9 ± 0.1^{fg}	6.1 ± 0.3^{fg}	7.1 ± 0.7^{e}	8.8 ± 0.2^{c}	9.5 ± 0.2^{abc}	9.8 ± 0.1^{ab}
	Water	5.6 ± 0.0^{gh}	5.6 ± 0.0^{gh}	5.8 ± 0.5^{fg}	7.1 ± 0.5^{e}	9.0 ± 0.2^{bc}	9.3 ± 0.2^{abc}	9.4 ± 0.2^{ab}
	aPAA	5.2 ± 0.3^{hi}	4.9 ± 0.1^{i}	4.9 ± 0.2^{i}	4.9 ± 0.3^{i}	6.9 ± 1.3^{e}	7.8 ± 1.0^{d}	8.8 ± 1.3^{c}

XLD: xylose lysine deoxycholate agar; TSA: tryptic soy agar.

[*] Per Table 2.4, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed.

[a-j] Means within each culture medium without a common superscript letter differ ($P < 0.05$).

TABLE 2. 8. Adjusted least-squares means estimates of ***Salmonella*** counts (XLD) and aerobic plate counts (TSA) for inoculated (5-6 log CFU/cm^2; 6-*Salmonella* serotype strain mixture) prerigor beef **cheek meat** that was left untreated (control) or treated by immersing (10 s) in water or peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, and aerobically stored at **-20°C** for up to 150 days.

		Mean Counts (log CFU/cm^2) ± Standard Deviation						
		Storage Day						
Culture Medium	Treatment	0	15	30	60	90	120	150
XLD	Control	6.0 ± 0.1^{a}	5.6 ± 0.1^{b}	5.5 ± 0.1^{b}	5.5 ± 0.1^{bc}	5.4 ± 0.1^{bc}	5.3 ± 0.1^{cd}	5.3 ± 0.1^{cde}
	Water	5.6 ± 0.1^{b}	5.2 ± 0.1^{def}	5.0 ± 0.1^{efg}	5.0 ± 0.1^{fg}	5.1 ± 0.1^{efg}	5.0 ± 0.1^{gh}	4.9 ± 0.0^{gh}
	aPAA	4.8 ± 0.2^{h}	3.8 ± 0.3^{i}	3.7 ± 0.2^{ij}	3.3 ± 0.3^{l}	3.5 ± 0.2^{jk}	3.5 ± 0.3^{kl}	3.3 ± 0.1^{l}
TSA	Control	6.1 ± 0.1^{a}	5.8 ± 0.1^{b}	5.9 ± 0.0^{b}	5.9 ± 0.1^{ab}	5.7 ± 0.2^{b}	5.8 ± 0.1^{b}	5.8 ± 0.0^{b}
	Water	5.7 ± 0.0^{b}	5.4 ± 0.2^{c}	5.4 ± 0.1^{c}	5.4 ± 0.1^{c}	5.3 ± 0.2^{cd}	5.3 ± 0.1^{cd}	5.2 ± 0.1^{cd}
	aPAA	5.1 ± 0.1^{d}	4.5 ± 0.3^{e}	4.5 ± 0.4^{e}	4.0 ± 0.2^{fg}	3.9 ± 0.5^{g}	4.2 ± 0.3^{f}	4.1 ± 0.1^{fg}

XLD: xylose lysine deoxycholate agar; TSA: tryptic soy agar.

[a-l] Means within each culture medium without a common superscript letter differ ($P < 0.05$).

TABLE 2. 9 Adjusted least-squares means estimates of rifampicin-resistant Shiga toxin-producing *Escherichia coli* (**STEC**) counts (SMAC+rif) and aerobic plate counts (TSA) for inoculated (14-strain STEC mixture; 5-6 log CFU/cm^2) prerigor beef **tongues** that were left untreated (control) or treated by immersing (10 s) in water or peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, vacuum sealed and stored at **4°C** for up to 90 days.

		Mean Counts (log CFU/cm^2) ± Standard Deviation							
		Storage Day							
Culture Medium	Treatment	0	5	10	15	30	45	60	90
SMAC+rif	Control	5.6 ± 0.2^{a}	5.1 ± 0.2^{bc}	4.9 ± 0.2^{cd}	4.6 ± 0.1^{de}	4.3 ± 0.3^{ef}	4.1 ± 0.4^{fg}	3.9 ± 0.4^{ghi}	3.7 ± 0.4^{hij}
	Water	5.3 ± 0.3^{ab}	4.8 ± 0.3^{cd}	4.6 ± 0.3^{de}	4.4 ± 0.3^{ef}	4.1 ± 0.2^{fg}	3.9 ± 0.5^{ghi}	3.7 ± 0.4^{hij}	3.6 ± 0.3^{hijk}
	aPAA	4.0 ± 0.4^{fgh}	3.6 ± 0.6^{hijk}	3.5 ± 0.2^{ijk}	3.3 ± 0.7^{jk}	3.4 ± 0.3^{jk}	3.5 ± 0.2^{ijk}	3.2 ± 0.3^{k}	3.5 ± 0.4^{hijk}
TSA*	Control	5.9 ± 0.2	5.6 ± 0.2	5.5 ± 0.3	5.3 ± 0.3	6.0 ± 0.2	6.4 ± 0.5	6.8 ± 0.3	6.6 ± 0.5
	Water	5.8 ± 0.2	5.9 ± 0.3	5.8 ± 0.3	5.4 ± 0.3	6.1 ± 0.6	6.5 ± 0.4	7.0 ± 0.4	6.8 ± 0.2
	aPAA	4.9 ± 0.5	4.3 ± 0.9	4.1 ± 0.3	4.0 ± 0.6	4.2 ± 0.7	4.9 ± 0.6	6.0 ± 1.5	5.7 ± 1.2

SMAC+rif: MacConkey sorbitol agar supplemented with rifampicin (100 μg/ml); TSA: tryptic soy agar

* Per Table 2.4, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed

[a-k] Means without a common superscript letter differ ($P < 0.05$).

TABLE 2. 10. Adjusted least-squares means estimates of rifampicin-resistant Shiga toxin-producing *Escherichia coli* (**STEC**) counts (SMAC+rif) and aerobic plate counts (TSA) for inoculated (14-strain STEC mixture; 5-6 log CFU/cm^2) prerigor beef **tongues** that were left untreated (control) or treated by immersing (10 s) in water or peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, vacuum sealed and stored at **-20°C** for up to 150 days.

		Mean Counts (log CFU/cm^2) ± Standard Deviation						
		Storage Day						
Culture Medium	Treatment	0	15	30	60	90	120	150
SMAC+rif[*]	Control	5.6 ± 0.2	5.1 ± 0.3	5.1 ± 0.2	5.0 ± 0.2	5.1 ± 0.2	4.8 ± 0.3	5.0 ± 0.2
	Water	5.3 ± 0.3	4.8 ± 0.3	4.7 ± 0.2	4.7 ± 0.3	4.8 ± 0.2	4.5 ± 0.2	4.7 ± 0.2
	aPAA	4.0 ± 0.4	2.6 ± 0.8	2.3 ± 0.5	2.5 ± 0.5	2.5 ± 0.9	1.9 ± 0.6	2.4 ± 1.0
TSA[#]	Control	5.9 ± 0.2	5.5 ± 0.3	5.5 ± 0.2	5.5 ± 0.1	5.6 ± 0.2	5.2 ± 0.3	5.4 ± 0.2
	Water	5.8 ± 0.2	5.6 ± 0.3	5.5 ± 0.4	5.3 ± 0.5	5.5 ± 0.4	5.3 ± 0.3	5.3 ± 0.4
	aPAA	4.9 ± 0.5	3.6 ± 0.7	3.5 ± 0.7	3.5 ± 0.7	3.5 ± 0.8	3.3 ± 0.9	3.7 ± 0.8

SMAC+rif: MacConkey sorbitol agar supplemented with rifampicin (100 μg/ml); TSA: tryptic soy agar

[*,#] Per Table 2.4, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed

TABLE 2. 11. Adjusted least-squares means estimates of ***Salmonella*** counts (XLD) and aerobic plate counts (TSA) for inoculated (5-6 log CFU/cm^2; 6-*Salmonella* serotype strain mixture) prerigor beef **tongues** that were left untreated (control) or treated by immersing (10 s) in water or peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, vacuum sealed and stored at **4°C** for up to 90 days.

		Mean Counts (log CFU/cm^2) ± Standard Deviation							
		Storage Day							
Culture Medium	Treatment	0	5	10	15	30	45	60	90
XLD[*]	Control	5.6 ± 0.2	5.3 ± 0.3	5.2 ± 0.2	5.2 ± 0.2	4.6 ± 0.3	4.2 ± 0.4	3.8 ± 0.5	3.8 ± 0.4
	Water	5.1 ± 0.3	4.5 ± 0.4	4.6 ± 0.3	4.6 ± 0.3	3.7 ± 0.4	3.4 ± 0.3	3.3 ± 0.3	2.9 ± 0.3
	aPAA	3.8 ± 0.5	2.9 ± 0.7	3.1 ± 0.3	3.1 ± 0.3	2.4 ± 0.7	2.0 ± 1.0	2.4 ± 0.9	1.5 ± 0.9
TSA	Control	6.0 ± 0.3^{cd}	5.9 ± 0.5^{cde}	5.8 ± 0.4^{cde}	5.5 ± 0.4^{de}	6.0 ± 0.5^{cde}	6.3 ± 0.2^{bc}	6.8 ± 0.3^{ab}	6.9 ± 0.3 ^{ab}
	Water	5.7 ± 0.2^{cde}	5.6 ± 0.3^{de}	5.8 ± 0.2^{cde}	5.4 ± 0.4^{e}	6.3 ± 0.6^{bc}	7.0 ± 0.4^{a}	7.1 ± 0.3^{a}	7.0 ± 0.2^{a}
	aPAA	4.5 ± 0.4^{f}	4.1 ± 0.8^{fgh}	4.1 ± 0.6^{fgh}	3.7 ± 0.6^{gh}	3.7 ± 0.8^{h}	4.2 ± 1.5^{fgh}	4.7 ± 0.8^{f}	4.3 ± 0.6^{gf}

XLD: xylose lysine deoxycholate agar; TSA: tryptic soy agar

[*] Per Table 2.4, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed

[a-h] Means without a common superscript letter differ ($P < 0.05$).

TABLE 2. 12. Adjusted least-squares means estimates of ***Salmonella*** counts (XLD) and aerobic plate counts (TSA) for inoculated (5-6 log CFU/cm^2; 6-*Salmonella* serotype strain mixture) prerigor beef **tongues** that were left untreated (control) or treated by immersing (10 s) in water or peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, vacuum sealed and stored at **-20°C** for up to 150 days.

		Mean Counts (log CFU/cm^2) ± Standard Deviation						
		Storage Day						
Culture Medium	Treatment	0	15	30	60	90	120	150
XLD	Control	5.6 ± 0.2^{a}	5.0 ± 0.3^{b}	5.1 ± 0.1^{b}	5.0 ± 0.1^{b}	5.1 ± 0.2^{b}	4.9 ± 0.2^{bc}	4.9 ± 0.2^{bcd}
	Water	5.1 ± 0.3^{b}	4.6 ± 0.2^{bcde}	4.5 ± 0.3^{cde}	4.5 ± 0.3^{cde}	4.4 ± 0.2^{de}	4.5 ± 0.1^{cde}	4.3 ± 0.2^{e}
	aPAA	3.8 ± 0.5^{f}	1.4 ± 0.8^{gh}	1.7 ± 0.5^{g}	1.6 ± 0.5^{gh}	1.3 ± 0.8^{gh}	1.2 ± 0.7^{h}	$< 0.5 \pm 0.4^{i}$
TSA[*]	Control	6.0 ± 0.3	5.6 ± 0.3	5.6 ± 0.2	5.5 ± 0.2	5.7 ± 0.4	5.5 ± 0.2	5.4 ± 0.3
	Water	5.7 ± 0.2	5.5 ± 0.3	5.5 ± 0.2	5.3 ± 0.3	5.3 ± 0.4	5.4 ± 0.2	4.9 ± 0.3
	aPAA	4.5 ± 0.4	3.4 ± 1.0	3.3 ± 0.4	3.6 ± 0.7	3.2 ± 0.8	3.6 ± 0.5	2.9 ± 0.2

XLD: xylose lysine deoxycholate agar; TSA: tryptic soy agar

Means with a less than symbol (<) indicate that at least one sample had a count that was below the detection limit (< 0.2 log CFU/cm^2)

[*] Per Table 2.4, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed

[a-i] Means without a common superscript letter differ ($P < 0.05$).

FIGURE 2. 1. Cheek meat samples cut into 5 × 10 cm pieces on trays lined with alcohol-sterilized aluminum foil being inoculated under a biological safety cabinet.

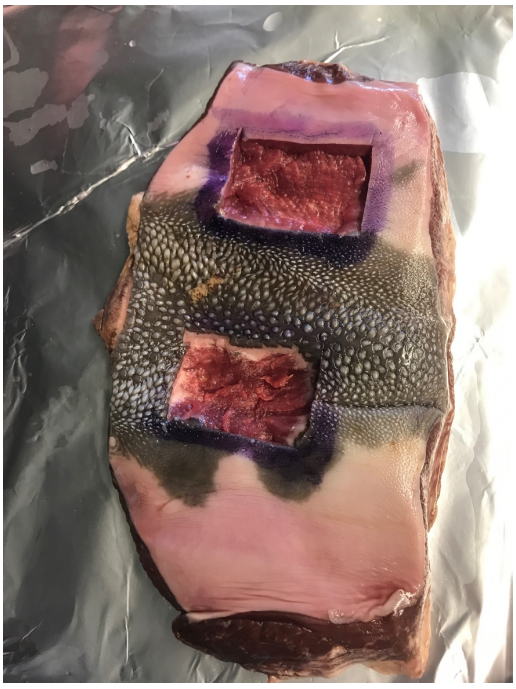

FIGURE 2. 2. Beef tongue sample showing two 6 × 4 cm marked, using edible carcass ink, areas on the surface. The surface of the tongue sample, within the marked areas, was excised for microbiological analysis.

CHAPTER III

Effect of Antimicrobial Treatment of Beef Cheek Meat and Tongues on Quality During Refrigerated and Frozen Storage

Summary

Variety meats contribute considerable value to the beef industry, and many edible by-products are almost exclusively sold in the export market (Schaefer et al., 2017). Due to international demand, there is a growing need for high quality, desirable and safe variety meats. The objective of this study was to evaluate effects of aPAA on quality attributes (aroma, lipid oxidation, and chemical profile) of beef cheek meat and tongues during refrigerated and frozen storage. Sensory evaluations, lipid oxidation and Rapid Evaporative Ionization Mass Spectrometry (REIMS) analyses were performed on beef cheek meat and tongue samples that were treated with aPAA or were left untreated (control), and subsequently stored at either 4°C or -20°C. Trained panelists evaluated odor attributes (i.e., overall off odor, sour and rancid) at multiple time points during storage. On the last day (day 60) of aerobic storage (4°C), untreated cheek meat samples were rated greater ($P < 0.05$) than aPAA-treated samples for overall off-odor and rancid attributes. No differences ($P \geq 0.05$) in overall off-odor were observed between untreated and aPAA-treated cheek meat samples stored for 150 days at -20°C. Similarly, no differences ($P \geq 0.05$) in any of the odor attributes evaluated were found between untreated and aPAA-treated vacuum-packaged tongue samples stored at 4°C for up to 90 days, or -20°C for up to 150 days. Thiobarbituric acid reactive substances (TBARS) were used to evaluate lipid oxidation. No differences ($P \geq 0.05$) in lipid oxidation were observed between untreated and

treated cheek meat samples on day 0 of storage at 4°C. However, on every subsequent analysis day during the aerobic storage period at 4°C, cheek meat samples treated with aPAA had higher ($P < 0.05$) TBARS values than those of the control samples. REIMS results provided visual differences of individual samples analyzed, showing the potential to classify samples based on metabolomic characteristics.

Introduction

Variety meats contribute considerable value to beef industry, and many edible by-products are almost exclusively sold in the export market (Schaefer et al., 2017). Although variety meats are not a commonly consumed product in the United States, they play an important role in the diets of many people in other countries as an accessible, affordable protein source, and often provide a staple in cultural cuisines. However, products like beef tongues are actually viewed as a delicacy and bring a premium. Customers in Japan are willing to pay significantly more than domestic consumers for beef tongues (Obara, 2010). In 2016, Japan imported 19,000 metric tons of U.S. beef tongues valued at $286 million (Schaefer et al., 2017). Overall, edible by-products contribute a significant portion of the U.S. export market averaging 23-25% of the volume and 14-19% of the value of beef exports (Marti et al., 2011). In 2018, the U.S. exported over 310,000 metric tons of variety meats, totaling over $890 million (USMEF, 2018). Increased demand in the export market has driven a higher value to every individual fed beef animal sold in the United States. Therefore, due to international demand, there is a growing need for high quality, desirable and safe variety meats.

Beef cheek meat is typically sold intact to Mexico and Latin America, but has become more prevalent elsewhere, as barbacoa street tacos are growing in popularity. Traditionally,

cheek meat has been transported frozen in polyethylene lined boxes. Tongues however are predominantly packaged in vacuum packaging, and chilled or frozen for transoceanic shipments.

Bovine head/cheek meat and tongues carry a higher level of microbiological contamination than whole muscle cuts (Kalchayanand et al., 2008). The anatomical location, harvest processes, and handling conditions have been shown to contribute to higher contamination rates of head/cheek meat (Gill, McGinnis, et al., 1999). This creates a food safety concern for pathogens, as well as a quality concern with spoilage bacteria.

To ensure food safety and quality attributes, antimicrobial treatments can be utilized. The use of organic acids (lactic and acetic), hot water and trisodium phosphate are a few treatments that can be used to decontaminate beef variety meats (Delmore et al., 2000). Kalchayanand et al. (2008) determined hot water, lactic acid (2%), and FreshFx could be used as decontamination washes for the reduction of *E. coli* O157:H7 on beef cheek meat. An antimicrobial can decrease spoilage and maintain quality to protect the export marketability.

Materials and Methods

Sample collection, antimicrobial treatment and packaging.

Two repetitions (trials), initiated on two separate days, were performed for each product (cheek meat and tongues), treatment (untreated control and aPAA) and storage temperature (4°C and -20°C) combination. On each trial day, prerigor beef cheek meat and whole beef tongues, with tonsils removed, were collected at a commercial processing facility in Colorado. Tissues were transported hot in an insulated container to the Center for Meat Safety & Quality (Department of Animal Sciences, Colorado State University, Fort Collins, CO) within 1 h of collection. Pre-rigor cheek meat was cut into 5 × 10 cm pieces. Tongues were sectioned into

thirds, resulting in approximately 16 × 8 cm-sized portions of the external surface area of the tongue.

Cheek meat and tongue samples were assigned to one of two treatments: an untreated control, or a solution of aPAA. The aPAA was comprised of 400 ppm PAA (Kroff, Pittsburg, PA) that was acidified to a pH of 1.2 with a commercial blend of sulfuric acid and sodium sulfate (Centron; Zoetis, Parsippany, NJ). For the aPAA treatments, batches of nine pieces at a time of cheek meat were placed into a 92-oz Whirl-Pak bag (Nasco, Modesto, CA), then 1.5 L of aPAA was immediately added to the bag, fully immersing all nine samples. Samples in the bag were gently agitated for 10 s after which, the solution was poured off, and samples were placed in a sterile strainer for 5 min to allow excess liquid to drain. Sterile forceps were used to place cheek meat samples into individual 24-oz Whirl-Pak bags for subsequent storage or microbial analysis (day-0 samples). The same treatment procedure was followed for tongues; however, 2 L of treatment solution was used to ensure all samples were fully immersed. After draining for 5 min, tongues were placed in individual 6 x 8 in vacuum bags (3-mil. standard barrier; Clarity Vacuum Pouches; Kansas City, MO)and were sealed using a vacuum packager (Koch Equipment, Model No. UV 225, Kansas City, MO).

Storage of beef cheek meat and tongue samples

Samples were randomly assigned to a storage temperature (chilled at 4°C, or frozen at -20°C), creating an equal representation of samples per product type and immersion treatment (Table 3.1). On predetermined storage days (Table 3.2), samples were prepared for analysis of aroma, lipid oxidation and volatiles as described below on each of three samples per treatment in each of two trials. Samples stored at -20°C were removed from the freezer and placed at 4°C for approximately 20 h prior to sensory aroma analysis to allow samples to thaw.

Odor analysis

Cheek meat and tongue samples were analyzed for odor attributes on the day of application of immersion treatments and packaging of samples (day-0), and on at least four subsequent time points during storage (Table 3.2). For the odor analysis, 10 g of surface tissue was excised from multiple locations of the cheek meat or tongue sample was placed in a 100 ml glass test tube, and capped. At the time of evaluation, panelists were instructed to vortex the sample for 10 s and quickly open the lid and smell the sample. Six panelists who were trained to assess and rate off odors evaluated 12 samples (three samples/product type/treatment) during each panel. Each sample was evaluated for overall off-odor intensity, sour, and rancid attributes using an unstructured scale, anchored at both ends (0 = absence or low intensity, 100 = extreme intensity). Panelist ratings were captured using an electronic ballot produced by an online survey software (Qualtrics, Provo, UT) and a single average for each sample was obtained.

Lipid oxidation

Lipid oxidation was determined by quantifying the malondialdehyde (MDA) concentration. A 5 g portion was collected from multiple locations across the surface of each sample, and stored at -80°C, until analysis. At the time of analysis, the 5 g sample was mixed with trichloroacetic acid (Sigma-Aldrich, Louis, MO), homogenized in a blender (Laboratory blender 7012G, Warning Commercial, Torrington, CT) for 1 min, and filtered using Whatman No. 1 filter paper (Whatman, Clifton, NJ). Next, duplicate samples comprised of 1 ml of filtrate mixed with 1 ml of thiobarbituric acid (20 mM; MP Biomedicals, Solon, OH) were incubated at 25°C for 20 h. The absorbance of samples at 532 nm (Model UV-1800 spectrophotometer, Shimadzu, Canbyl, OR) was reported as thiobarbituric acid reactive substances (TBARS) values in ppm.

Rapid Evaporative Ionization Mass Spectrometry (REIMS)

Volatile compounds were evaluated using the Rapid Evaporative Ionization Mass Spectrometry (REIMS) technology. After being excised for odor and TBARS, samples were held at -80°C until REIMS analysis. Before analysis, samples were thawed at 4°C for approximately 16 h. Samples were analyzed using a Synapt G2 Si Q-ToF fitted with a REIMS ionization source coupled with a monopolar electrosurgical hand piece ("iKnife", Waters Corporation, Milford, MA) powered by an Erbotom ICC 300 electrosurgical generator (Erbe Elektromedizin GmbH, Tubingen, Germany) using the "dry cut" mode at a power of 40W. A continual flow (200 µl/min) of 2 ng/ml leucine-enkephalin was introduced directly to the REIMS source during sampling. The cone voltage was set to 40 V and the heater bias to 80 V. At least five "burns" were collected for each sample, with each burn lasting approximately 1 s. Spectra were collected in negative mode ionization from 100-1,000 m/z. Preprocessing was performed using the Abstract Model Builder (AMX) software (Beta version 1.0.1581.0, Waters Corporation) and included lock mass correction (leucine-enkephalin), background subtraction using standard Masslynx preprocessing algorithms, and normalization to total ion current. Peak binning was performed at intervals of 0.5 m/z resulting in a total of 1,800 bins. The bins from the five burns were summed to create a single value for each sample, resulting in a final data matrix containing 1,800 variables (m/z bins) per observation.

Statistical analysis

Two repetitions (trials) were performed for each product (cheek meat or tongues), immersion treatment (untreated or aPAA) and storage temperature (4°C or -20°C) combination, and, within each repetition, three samples were analyzed for aroma, lipid oxidation (TBARS) and REIMS on each sampling day (n = 6). The study was designed as a randomized complete block

with trial day as the block. Data (odor panel attributes or TBARS values) for each product and storage temperature combination were analyzed separately using the mixed procedure in SAS (version 9.4, SAS Institute, Cary, NC). The main effects of immersion treatment and storage day were evaluated, as well as the interaction. All least-squares means were separated using a pairwise comparisons test at a significance level of $\alpha = 0.05$.

Results and Discussion

Odor analysis

Odor panels were used to quantify aromatic attributes of beef cheek meat and tongues over an extended refrigerated or frozen storage period (Tables 3.3 to 3.7). No differences ($P \geq 0.05$) in overall off odor were obtained between untreated and aPAA-treated cheek meat stored aerobically for up to 45 days at 4°C (Table 3.4). By day-60, however, untreated samples were rated higher ($P < 0.05$) than aPAA-treated samples for overall off-odor. Similarly, for the rancid attribute evaluated, no differences ($P \geq 0.05$) were observed between the two treatments for the first 30 days, then, at day-45 and day-60, untreated cheek meat samples were rated higher ($P < 0.05$) than the samples treated with aPAA (Table 3.4).

For cheek meat stored for up to 150 days at -20°C, no differences ($P \geq 0.05$) in overall off-odor were found between untreated and aPAA-treated samples (Table 3.5). Sour attributes were similar between treatments, and rancid ratings tended to be numerically higher for aPAA-treated samples (Table 3.5). No differences ($P \geq 0.05$) in any of the odor attributes evaluated were observed between untreated and aPAA-treated vacuum-packaged tongue samples stored at 4°C for 90 days (Table 3.6), or -20°C for 150 days (Table 3.7). Ratings for each of the attributes (overall off-odor, sour, rancid) were relatively similar between the two treatments throughout each of the storage periods.

Although ratings for odor attributes of cheek meat throughout the 60 days of 4°C storage remained similar, by day-60, untreated control samples were rated higher ($P < 0.05$) for overall off-odor than aPAA-treated samples. Ratings for rancid attributes in untreated samples also were rated greater ($P < 0.05$) by the end of the storage period. No differences ($P \geq 0.05$) in overall off-odor were found between untreated and aPAA-treated cheek meat samples stored for 150 days at -20°C. For vacuum-packaged tongues, ratings for overall off-odor were numerically higher for untreated samples compared to aPAA-treated tongues. Ratings for sour, and rancid were similar between both treatments for tongues stored at 4°C or -20°C.

Thiobarbituric Acid Reactive Substances

Thiobarbituric Acid Reactive Substances (TBARS) were used to evaluate lipid oxidation of untreated and aPAA-treated cheek meat and tongues across an extended refrigerated and frozen storage period. No differences ($P \geq 0.05$) in lipid oxidation were observed between untreated and treated cheek meat samples on day-0 of storage at 4°C (Table 3.8). However, on every subsequent analysis day during the aerobic storage period at 4°C, cheek meat samples treated with aPAA had higher ($P < 0.05$) mean TBARS values than those of the control samples (Table 3.8). Similarly, for cheek meat samples stored at -20°C, TBARS values of the aPAA-treated samples were higher ($P < 0.05$) than those of the control samples on all analysis days except for day-0 and day-120 (Table 3.9). Statistical analysis of the TBARS values for tongue samples stored at 4°C or -20°C indicated that there were no differences ($P \geq 0.05$) between the untreated and aPAA-treated samples (Tables 3.8 and 3.9).

Lipid oxidation results showed more differences in cheek meat than tongues, as expected, since cheek meat was stored aerobically whereas tongues were vacuum packaged. No differences ($P \geq 0.05$) in lipid oxidation were observed between untreated and treated cheek meat samples on

day-0 of storage at 4°C. However, on every subsequent analysis day during the aerobic storage period at 4°C, cheek meat samples treated with aPAA had greater ($P < 0.05$) TBARS values than those of the control samples. Similar results were obtained for cheek meat stored at -20°C. Regardless of storage temperature, no differences ($P \geq 0.05$) were found between the untreated and aPAA-treated tongue samples. The higher lipid oxidation in samples treated with aPAA was expected, as the antimicrobial is a prooxidant.

Rapid Evaporative Ionization Mass Spectrometry (REIMS)

Rapid Evaporative Ionization Mass Spectrometry (REIMS) analysis of the cheek meat and tongue samples in this study resulted in a data matrix of metabolomic fingerprints that included 1,800 m/z bins per sample. Individual peaks from each sample's burns were binned in intervals of 0.5 m/z, starting with 100.25 and ending with 999.75, for a total of 1,800 variables. Data binning is a technique used to account for minor errors during data collection. The AMX software condensed the 1,800 peaks into principle components, to maximize separation within data sets. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to visually plot samples to show differences in the molecular profile of samples (Figures 3.1 to 3.6). To utilize molecular profiles generated by REIMS, or any of the ambient ionization techniques, as a means to classify samples, machine learning algorithms are necessary to generate a predictive model. Machine learning is described as "The process of rapidly finding and characterizing patterns in complex data" (Gredell et al., 2019). The model for this study is under development and will be a continued effort to validate the prediction modeling system.

The OPLS-DA plots are shown in Figures 3.1 to 3.6. Cheek meat stored at 4°C had the clearest clusters grouped by storage day (Figure 3.1). Larger differences across samples create better opportunities to see distinctions between the variable of interest. Cheek meat stored at -

20°C also had distinguishable separation of storage day groupings (Figure 3.2). When cheek meat samples from across all storage days were classified based on treatment, separation between control and aPAA-treated samples was observed (Figure 3.3). Tongue samples did not have as many clear visual differences for storage day (Figures 3.4 and 3.5). Additionally, Figure 3.6 shows samples plainly divided based on tissue type, where REIMS could clearly classify based on tissue tyoe.. Misclassification rates are shown in Table 3.10, where 100% of tongue samples were correctly identified in their respective classification category after prediction. The ability to classify offals with REIMS has also been shown by Black et al. (2016). Future applications of REIMS could include identifying offals or tissue types within ground meat products.

Ambient mass spectrometry is a relatively new approach that enables ionization of molecules under ambient conditions with no sample preparation and very fast sampling times. This study showed the prospective accuracy to classify samples into like-groups. The potential of this technology is to use the metabolomic fingerprint of individual samples to predict various quality characteristics, such as odor, or lipid oxidation very quickly. However, this requires the usage of unique algorithms, and various mathematical approaches. The data collected will be further analyzed, as models are custom built. However, this study shows differences can still be observed, showing the potential to classify samples based on metabolomic characteristics.

TABLE 3. 1. Product types, storage temperatures, immersion treatments, and number of samples.

Product Type	Storage Temperature	Treatment	Number of Samples
Cheek meat	4°C	Untreated	30
		aPAA	30
	-20°C	Untreated	48
		aPAA	48
Tongues	4°C	Untreated	42
		aPAA	42
	-20°C	Untreated	48
		aPAA	48

aPAA: Peroxyacetic acid (400ppm) acidified (pH 1.2) with a sulfuric acid and sodium sulfate blend

TABLE 3. 2. Sampling days for aroma, lipid oxidation and volatiles analyses of untreated and treated cheek meat and tongue samples stored at 4°C or -20°C.

Product Type	Storage Temperature	Sampling Days
Cheek Meat	4°C	0, 15, 30, 45, 60[1]
	-20°C	0, 30, 60, 90, 105, 120, 135, 150
Tongues	4°C	0, 15, 30, 45, 60, 80, 90
	-20°C	0, 30, 60, 90, 105, 120, 135, 150

[1]Analyses of cheek meat samples stored at 4°C were concluded at day-60 due to significant product spoilage

TABLE 3. 3. Aroma analysis *P*-values for the main effects of treatment, and storage day, as well as the interaction.

Product Type	Storage Temperature	Odor Attribute	Treatment × Storage Day Interaction *P*-value	Treatment Main Effect *P*-value	Storage Day Main Effect *P*-value
Cheek meat	4°C	Overall Off-Odor	0.0027	0.0035	0.0093
		Sour	0.5280	0.2090	0.0236
		Rancid	0.0025	0.0854	0.0091
	-20°C	Overall Off-Odor	0.0055	< 0.0001	< 0.0001
		Sour	0.4038	0.0665	0.0019
		Rancid	0.1767	< 0.0001	< 0.0001
Tongues	4°C	Overall Off-Odor	0.9876	0.1465	0.3607
		Sour	0.9928	0.0343	0.2553
		Rancid	0.9947	0.4943	0.4652
	-20°C	Overall Off-Odor	0.0633	0.0193	0.0430
		Sour	0.1123	0.0260	0.0847
		Rancid	0.1244	0.0254	0.0516

TABLE 3. 4. Least-squares means of trained panel odor attributes[1] for prerigor beef **cheek meat** that was left untreated (control) or treated by immersing (10 s) in peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, and aerobically stored at **4°C** for up to 60 days.

		Storage Day						
Odor Attribute	Treatment	0	15	30	45	60	SEM[2]	*P* - Value
Overall Off-Odor	Control	4.90^{e}	15.99^{de}	46.55^{bc}	49.23^{bc}	80.05^{a}	3.35	< 0.01
	aPAA	6.13^{e}	24.91^{cde}	32.87^{bcd}	35.30^{bcd}	57.17^{b}		
Sour[*]	Control	2.40	5.28	7.61	15.21	17.99	2.63	0.53
	aPAA	1.33	4.35	14.04	16.22	27.13		
Rancid	Control	1.03^{e}	6.65^{de}	15.05^{bcd}	26.14^{b}	40.06^{a}	4.29	< 0.01
	aPAA	1.62^{e}	13.64^{cd}	17.83^{bc}	13.18^{d}	24.55^{bc}		

[1]Attributes were scored using a 100-point numerical scale: 0 = none and 100 = extremely intense

[2] Standard error (largest) of the least squares means

[*] Per Table 3.3, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed

[a-e] Means within an odor attribute without a common superscript letter differ ($P < 0.05$)

TABLE 3. 5. Least-squares means of trained panel odor attributes[1] for prerigor beef **cheek meat** that was left untreated (control) or treated by immersing (10 s) in peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, aerobically stored at **-20°C** for up to 150 days.

		Storage Day									
Odor Attribute	Treatment	0	30	60	90	105	120	135	150	SEM[2]	*P* - Value
Overall Off-Odor	Control	4.98^{e}	24.92^{cde}	29.55^{bcd}	18.72^{de}	17.26^{de}	36.33^{abcd}	45.50^{ab}	40.67^{abc}	10.11	0.01
	aPAA	6.19^{e}	30.97^{bcd}	31.13^{bcd}	35.21^{abcd}	22.37^{de}	38.22^{abcd}	53.27^{a}	45.18^{ab}		
Sour[*]	Control	1.18	10.06	10.52	5.64	5.35	17.22	16.30	17.05	3.74	0.40
	aPAA	2.26	13.56	12.57	12.64	5.46	15.44	17.91	17.17		
Rancid[#]	Control	0.60	10.72	5.87	8.17	5.66	14.89	23.95	8.29	7.28	0.18
	aPAA	1.18	15.08	11.74	15.82	8.82	25.44	34.09	13.76		

[1]Attributes were scored using a 100-point numerical scale: 0 = none and 100 = extremely intense

[2] Standard error (largest) of the least squares means

[*, #] Per Table 3.3, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed

[a-e] Means within an odor attribute without a common superscript letter differ ($P < 0.05$)

TABLE 3. 6. Least-squares means of trained panel odor attributes[1] for prerigor beef **tongue**s that were left untreated (control) or treated by immersing (10 s) in peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, vacuum sealed and stored at **4°C** for up to 90 days.

		Storage Day									
Odor Attribute	Treatment	0	15	30	45	60	70	80	90	SEM[2]	*P* - Value
Overall Off-Odor[*]	Control	7.08	23.11	33.79	27.42	41.10	36.78	36.36	40.45	10.60	0.99
	aPAA	6.93	22.18	32.47	26.82	36.72	33.39	34.06	38.81		
Sour[#]	Control	4.29	10.84	11.56	10.12	21.78	18.58	17.42	17.49	5.05	0.99
	aPAA	3.56	9.66	10.73	7.13	18.72	15.39	14.50	15.47		
Rancid[+]	Control	1.55	7.69	6.69	10.94	8.24	10.50	13.69	17.51	5.71	0.99
	aPAA	1.47	6.83	7.72	11.40	6.72	9.36	12.22	15.67		

[1]Attributes were scored using a 100-point numerical scale: 0 = none and 100 = extremely intense

[2] Standard error (largest) of the least squares means

[*, #, +] Per Table 3.3, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed

TABLE 3. 7. Least-squares means of trained panel odor attributes[1] for prerigor beef **tongue**s that were left untreated (control) or treated by immersing (10 s) in peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Untreated and treated samples were individually packaged, vacuum sealed and stored at **-20°C** for up to 150 days.

		Storage Day									
Odor Attribute	Treatment	0	30	60	90	105	120	135	150	SEM[2]	*P* - Value
Overall Off-Odor[*]	Control	7.08	30.36	25.61	30.08	28.30	50.61	41.31	39.93	9.67	0.06
	aPAA	6.98	31.28	30.22	30.61	31.96	47.63	53.45	42.22		
Sour[#]	Control	4.26	14.93	9.53	16.10	7.93	29.73	15.93	18.03	5.55	0.11
	aPAA	3.46	17.65	14.83	14.64	14.13	30.77	18.41	17.49		
Rancid[+]	Control	1.51	6.77	6.46	10.05	7.75	19.66	17.45	8.09	4.96	0.12
	aPAA	1.48	7.53	6.34	12.27	8.83	18.54	24.52	11.35		

[1]Attributes were scored using a 100-point numerical scale: 0 = none and 100 = extremely intense

[2] Standard error (largest) of the least squares means

[*, #, +] Per Table 3.3, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed

TABLE 3. 8. **TBARS**[1] values (ppm) of beef cheek meat and tongues that were left untreated (control) or were treated by immersing (10 s) in peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Samples were individually packaged aerobically (cheek meat) or vacuum sealed (tongues) and stored at 4°C for 60 (cheek meat) or 90 (tongues) days.

		Storage Day								
Product Type	Treatment	0	15	30	45	60	80	90	SEM[2]	*P* - Value
Cheek Meat	Control	0.10[d]	0.28[d]	0.96[d]	0.77[d]	0.93[d]			0.37	< 0.01
	aPAA	0.14[d]	1.98[c]	2.23[bc]	2.94[ab]	3.30[a]				
Tongues[*]	Control	0.13	0.10	0.12	0.14	0.50	0.17	0.31	0.12	0.38
	aPAA	0.10	0.08	0.49	0.12	0.46	0.17	0.21		

[1] Thiobarbituric acid reactive substances (TBARS)

[2] Standard error (largest) of the least squares means

[*] Per Table 3.3, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed

[a-e] Means within each product type without a common superscript letter differ ($P < 0.05$)

TABLE 3. 9. **TBARS**[1] values (ppm) of beef cheek meat and tongues that were left untreated (control) or were treated by immersing (10 s) in peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA). Samples were individually packaged aerobically (cheek meat) or vacuum sealed (tongues), and stored at **-20°C** for up to 150 days.

		Storage Day									
Product Type	Treatment	0	30	60	90	105	120	135	150	SEM[2]	*P* - Value
Cheek Meat	Control	0.11^{e}	0.09^{e}	0.13^{e}	0.22^{e}	0.15^{e}	0.32^{cd}	0.16^{de}	0.24^{de}	0.11	< 0.01
	aPAA	0.14^{e}	0.54^{ab}	0.63^{ab}	0.46^{bc}	0.75^{a}	0.43^{cd}	0.63^{ab}	0.52^{bc}		
Tongues[*]	Control	0.13	0.11	0.11	0.09	0.08	0.10	0.09	0.12	0.04	0.34
	aPAA	0.10	0.13	0.24	0.11	0.13	0.17	0.12	0.14		

[1] Thiobarbituric acid reactive substances (TBARS)

[2] Standard error (largest) of the least squares means

[*] Per Table 3.3, the immersion treatment × storage day interaction was not significant ($P \geq 0.05$), therefore, mean separation was not performed

[a-e] Means within each product type without a common superscript letter differ ($P < 0.05$)

TABLE 3. 10 Misclassification matrix[1] of tissue type predicted[2] by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of raw beef cheek meat or tongues collected using rapid evaporative ionization mass spectrometry (REIMS).

	Predicted Class		
Reference Class	Tongue	Cheek Meat	Correct
Tongue	157	0	100%
Cheek Meat	1	105	99.06%

[1]Number of samples falling into each respective classification category after prediction.
[2]Models were built using 80% of the original data and tested using the remaining 20%.

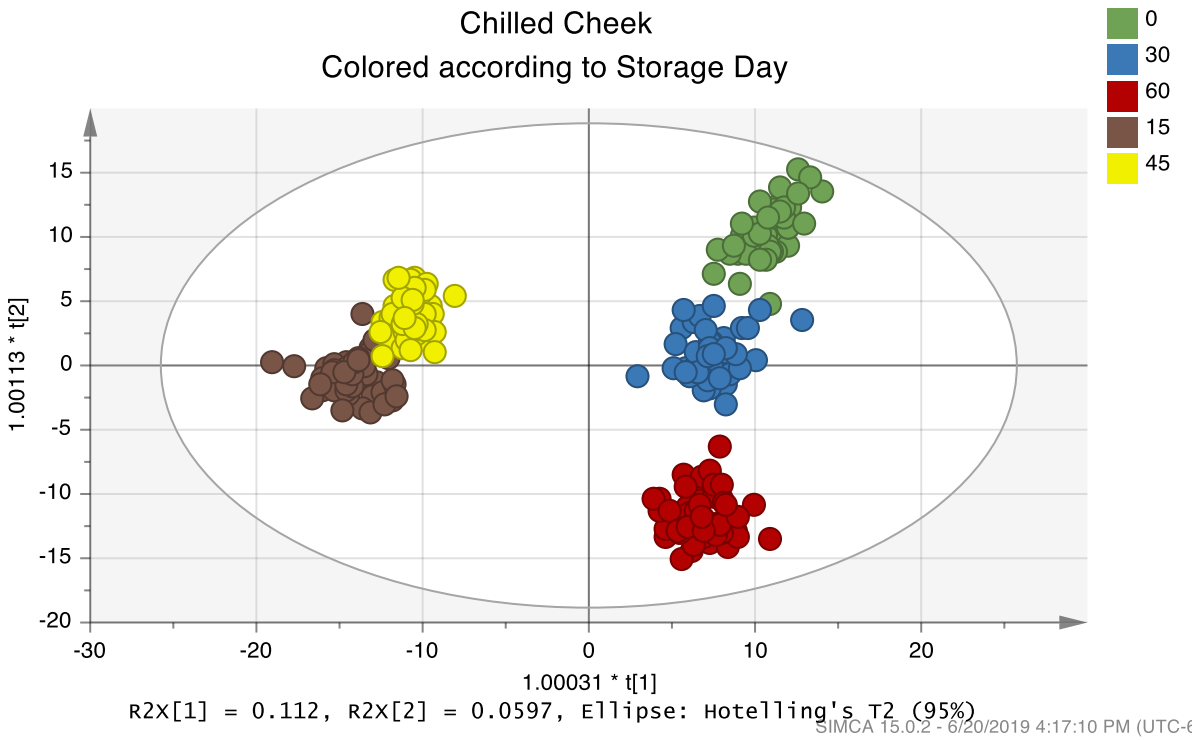

FIGURE 3. 1. Orthogonal partial least squares discriminant plot of refrigerated **(4°C) cheek meat**, classifying samples based on storage day, across both treatments (i.e., untreated control, and treated with peroxyacetic acid [400 ppm] acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend).

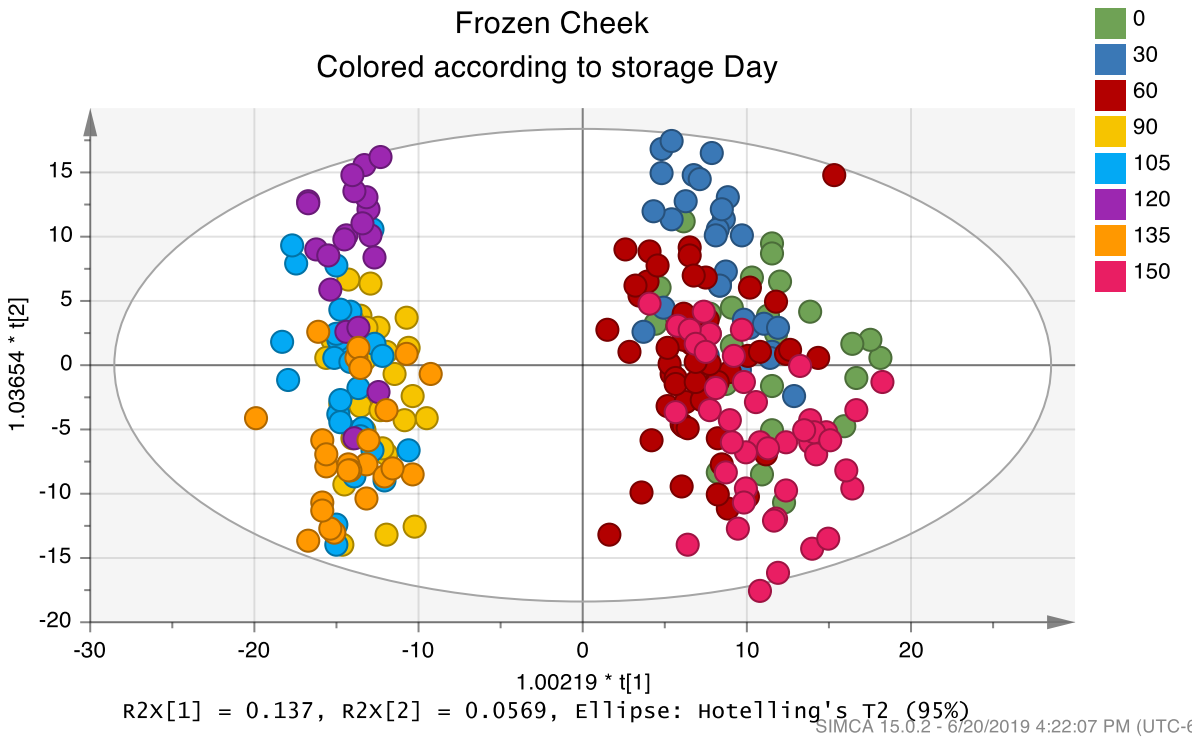

FIGURE 3. 2. Orthogonal partial least squares discriminant plot of frozen **(-20°C) cheek meat**, classifying samples based on storage day, across both treatments (i.e., untreated control, and treated with peroxyacetic acid [400 ppm] acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend).

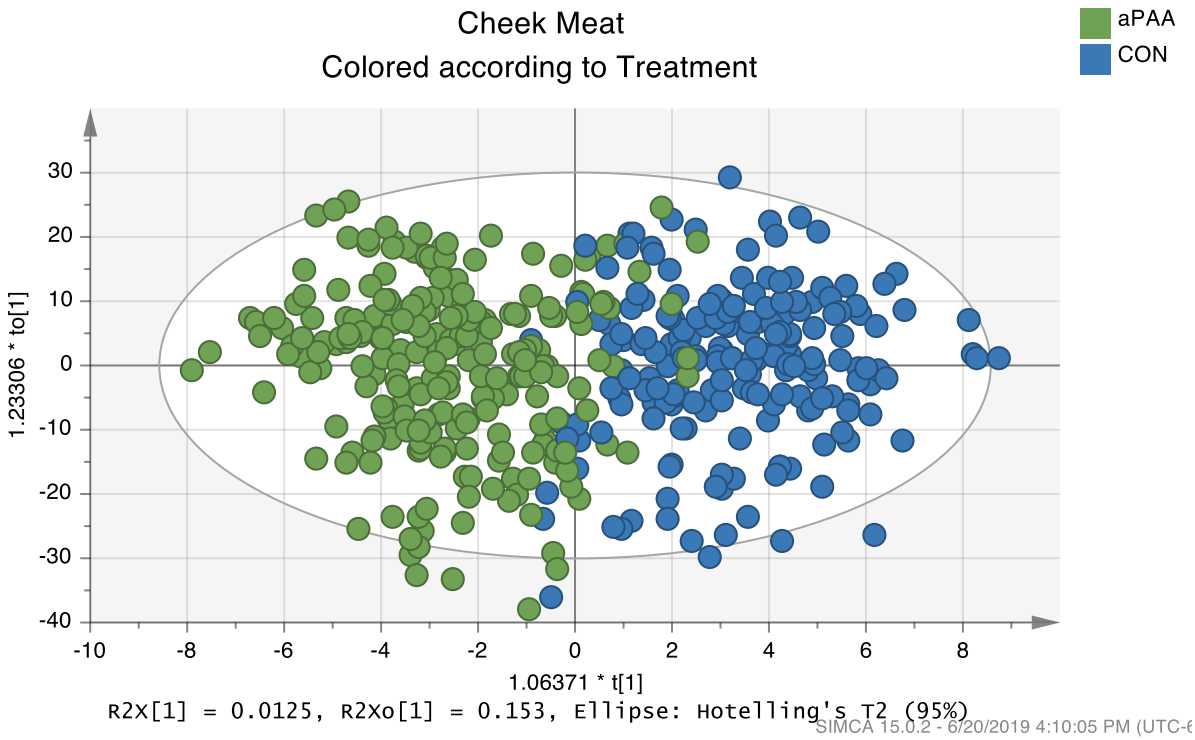

FIGURE 3. 3. Orthogonal partial least squares discriminant plot of all cheek meat samples, classifying based on treatment (i.e., untreated control [CON], and treated with peroxyacetic acid [400 ppm] acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend [aPAA]), across all storage days.

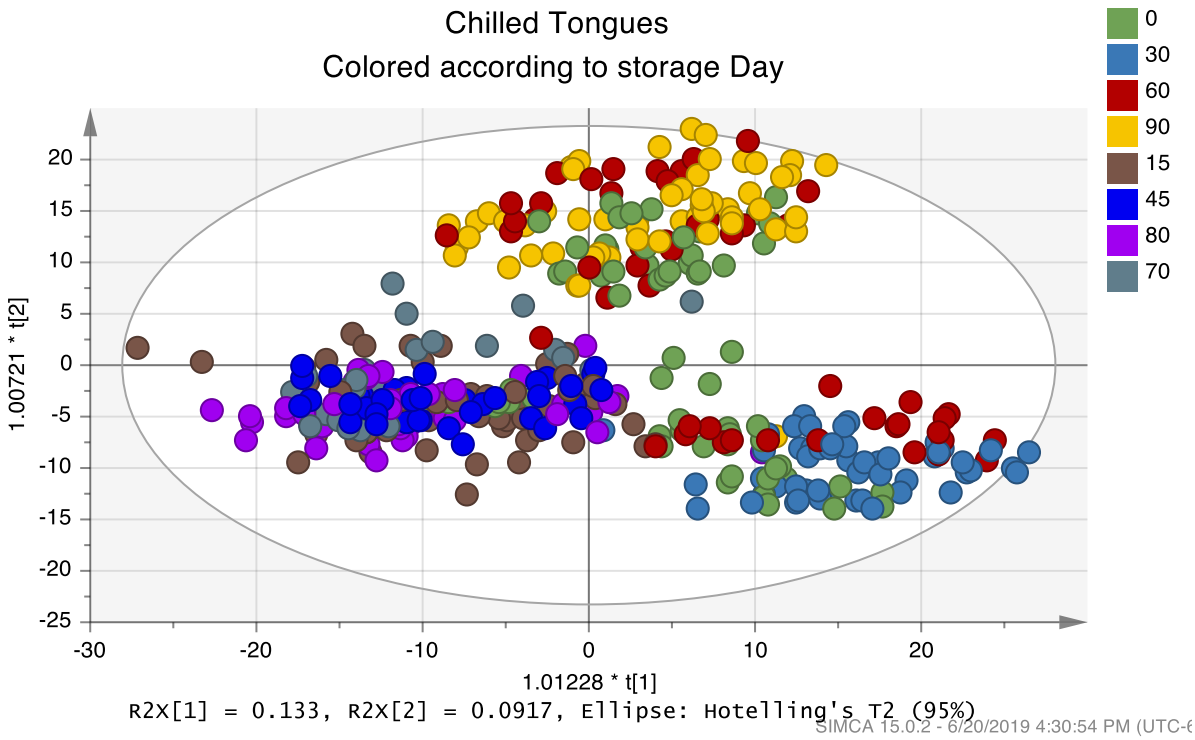

FIGURE 3. 4. Orthogonal partial least squares discriminant plot of refrigerated **(4°C) tongues**, classifying samples based on storage day, across both treatments (i.e., untreated control, and treated with peroxyacetic acid [400 ppm] acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend).

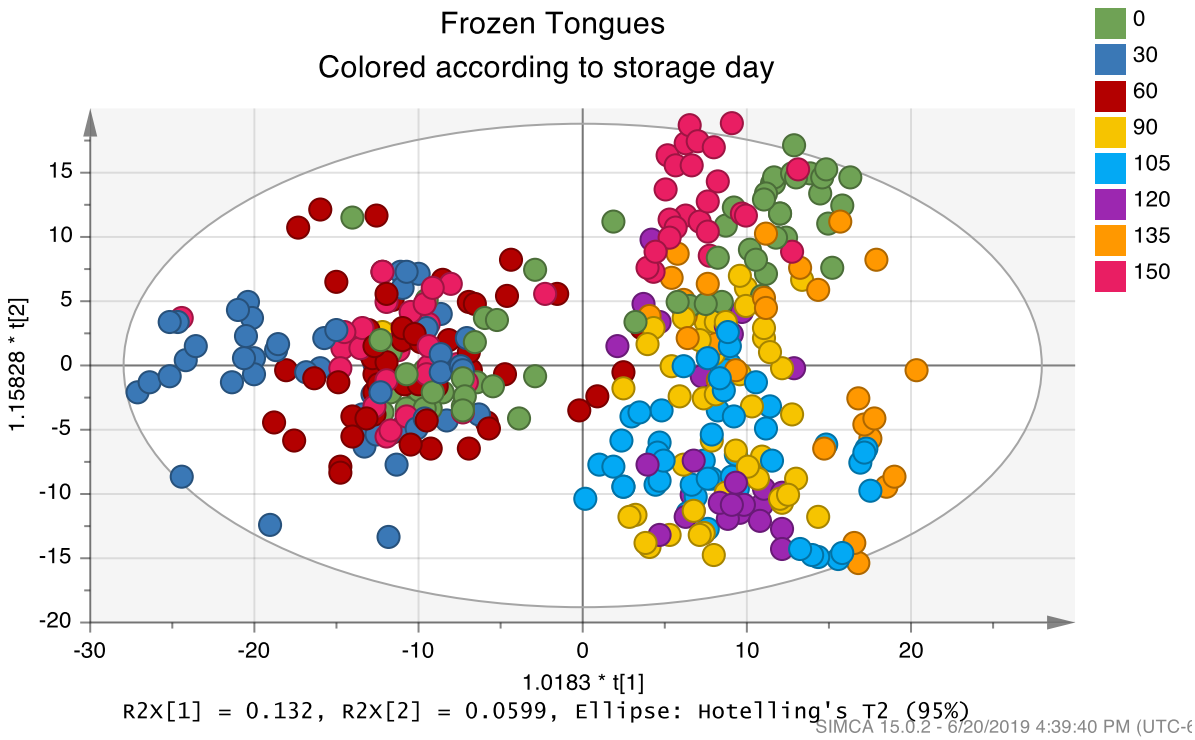

FIGURE 3. 5. Orthogonal partial least squares discriminant plot of frozen **(-20°C) tongues**, classifying samples based on storage day (0, 30, 60, 90, 105, 120, 135 or 150 days), across both treatments (i.e., untreated control, and treated with peroxyacetic acid [400 ppm] acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend).

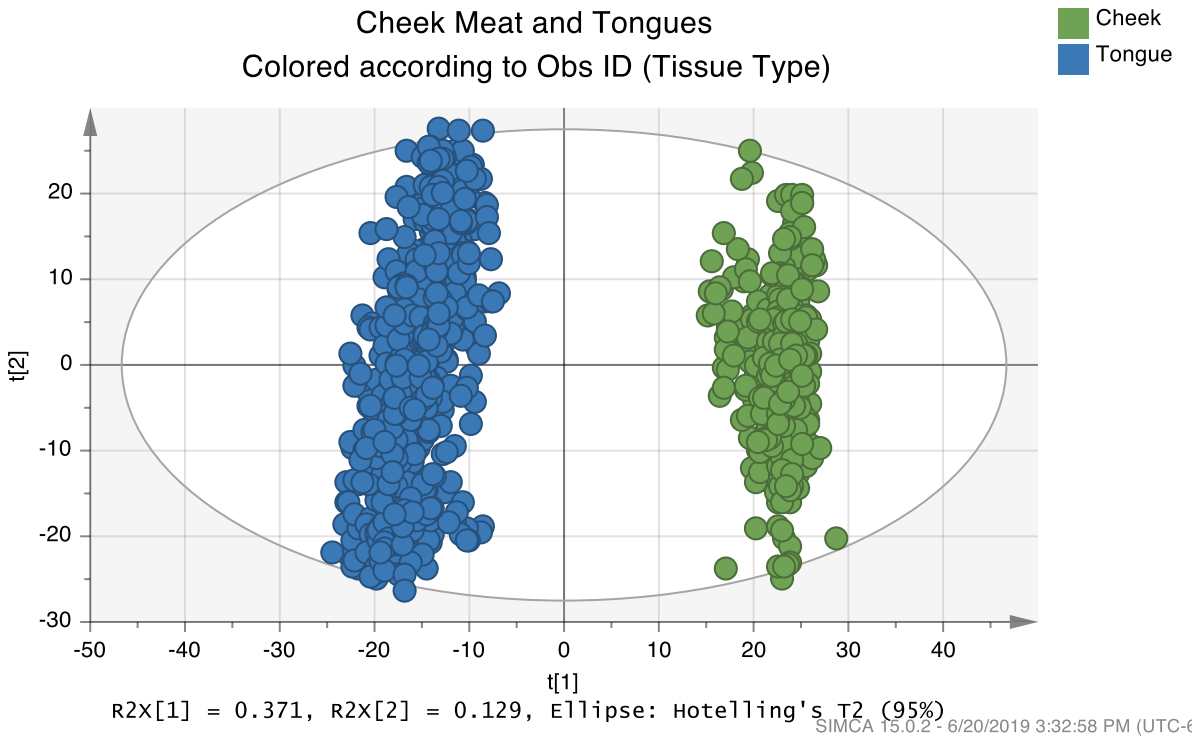

FIGURE 3. 6. Orthogonal partial least squares discriminant plot of all samples, classifying samples based on tissue type (i.e., cheek meat or tongues), across both storage temperatures (4°C or -20°C) and all storage days.

CHAPTER IV

Evaluating the Effects of Commonly Used Antimicrobial Intervention Chemical Spray Combinations on the Flavor Profile of Beef

Summary

Multiple hurdle technology has proven multiple intervention steps are more effective any single intervention; therefore, sequential decontamination steps are utilized (Bacon et al., 2000; Graves-Delmore et al., 1998). Extensive research has been conducted to determine the efficacy of antimicrobials, but traditionally, less focus has been aimed at the effects on quality especially sequential usage of antimicrobials.

The objective of this study was to characterize flavor profiles of beef treated with a combination of common pre-chilling and post-chilling antimicrobial interventions. Beef briskets collected from the harvest floor were utilized as the beef source. Briskets were not chilled until initial treatments were applied. Following collection, briskets were treated in a factorial arrangement of interventions commonly applied pre- and post-chilling. Whole beef briskets pre-chilling were treated to emulate the impact of harvest floor interventions. Briskets were spray treated (15 s, 15 psi) with lactic acid (4.5%; LA), peroxyacetic acid acidified with sulfuric acid and sodium sulfate blend (pH 1.2; aPAA) or left untreated (CONTROL). Briskets were chilled at 2°C for 24 h then divided into four equal sections, and randomly assigned to one of each of the four post-chilling treatments. Post-chilling treatments were spray treated (15s, 15 psi) with lactic acid (4.5%; LA), a commercial blend of lactic and citric acid (LAC), peroxyacetic acid acidified with sulfiuric acid and sodium sulfate blend (pH 1.2; aPAA) or left untreated (CONTROL).

Samples were then stored at 2°C for 48 to 72 h prior to processing. Each sample was ground twice and formed into 28 g patties and stored at -20°C until analysis. At the time of sensory analysis, patties were cooked to 71°C. A subset of samples (N = 72; n = 6) were analyzed for fatty acid composition and organic volatile compounds.

Each cooked sample was analyzed by a trained sensory panel to evaluate flavor attributes. Trained taste panelist ratings for sour and chemical were rated highest ($P < 0.01$) for the LA pre-chilling treatment, compared to the CONTROL and aPAA. Ratings for the browned attribute were greater ($P < 0.05$) for samples subjected to aPAA than CONTROL or LA. No differences ($P \geq 0.05$) were found for beef flavor ID, roasted, metallic, fat-like, sour, rancid, warmed over, or liver-like ratings due to pre-chilling treatments. Post-chilling treatments did not create any significant ($P \geq 0.05$) flavor attribute differences.

Fatty acid analysis showed very minimal differences due to use of any chemical interventions. Only C10:0 was affected by treatment; LA treatment post-chilling produced greater ($P < 0.05$) concentrations of C10:0 than LAC or CONTROL samples. When volatile acid compounds were assessed, relative abundance of pentanal was greater ($P < 0.05$) in LA-treated post-chilling intervention samples than in CONTROL, LAC, or aPAA samples. Similarly, relative abundance of hexanoic acid and pentanol were greater ($P < 0.05$) in samples treated with LA post-chilling than for samples from CONTROL or LAC treated samples. Overall, LA-treated samples resulted in various slight off-flavor attributes, with increased sour and chemical attributes. However, in general, minimal differences were associated with use of chemical antimicrobial interventions.

Introduction

Various chemical and physical decontamination systems are utilized throughout the beef production chain to reduce pathogen contamination on cattle hides, carcasses, and beef trim (Sofos, 2005). Numerous studies have evaluated antimicrobials such as lactic acid, acidified sodium chlorite, peroxyacetic acid, sodium metasilicate, potassium lactate and cetylpryidinum chloride for decontamination efficacy (Ellebrach et al., 1999; Stivarius et al., 2002, Ransom et al., 2003; Bosilevac et al., 2004; Pohlman et al., 2009;). Multiple intervention steps in a production system are more effective than any single intervention (Bacon et al., 2000). Therefore, sequential decontamination processes are commonly applied within the beef industry as a more effective method for controlling risk of pathogens. Although a variety of chemical interventions have been considered, lactic acid (LA), peroxyacetic acid (PAA), and lactic/citric acid blends (LAC) are among the most commonly utilized interventions with beef trimmings and primal cuts. The United States Department of Agriculture – Food Safety and Inspection Services (USDA-FSIS) requires that plants validate food safety critical control, which generally includes the intervention system(s) (FSIS, 1996a). Consequently, an abundance of research has been conducted on chemical interventions to evaluate efficacy in reducing pathogenic contamination to improve the safety of beef products. However, limited research has been devoted to determining the potential impact on flavor attributed to such use. The antimicrobial compounds of interest are predominantly acidic, and some are strong oxidants, thereby raising a concern for potential impacts on the flavor profile. Previous published research addressed effects of interventions on pH, texture, color, and odor, but not specifically on flavor impacts (Gill et al., 2004; McCarty et al., 2016; Pohlman et al., 2002; Quilo et al., 2009; Stivarius et al., 2002). Beef flavor is a major driver of consumer acceptance, and has become a growing focus (Hunt et al.,

2014). Therefore, the objective of this study was to evaluate effects of common antimicrobials, used in combination, on the flavor profile of beef.

Materials and Methods

Sample Collection, Fabrication and Treatment Design

Whole beef briskets were obtained from carcasses on the harvest floor and collected on two separate production days. For logistical and regulatory reasons, briskets could not be collected before antimicrobial treatments were applied on the harvest floor, so they were later trimmed to completely remove external surface. Briskets were immediately transported (< 30 min) hot in insulated coolers to the Colorado State University Meat Laboratory for treatment. Upon arrival, briskets were trimmed using a Whizard Quantum® Trimmer (Quantum Q1400, Bettcher Industries, Birmingham, OH). The entire external surface, sternum fat and deckle fat of each brisket was trimmed to eliminate any potential antimicrobial treatment residues from processing during harvest in the plant, and to achieve a minimal, uniform external fat level.

This study was designed as a 3 x 4 factorial with three pre-chilling treatments and four post-chilling treatments as described in Table 4.1. Trimmed whole briskets were randomly assigned to one of three pre-chilling treatments: an untreated control, lactic acid (4.5%; LA), or solution of aPAA. The aPAA was comprised of 400 ppm PAA (Kroff, Pittsburg, PA) that was acidified to a pH of 1.2 with a commercial blend of sulfuric acid and sodium sulfate (Centron; Zoetis, Parsippany, NJ). Treatments were applied to individual briskets using a custom-built, pilot-sized spray cabinet (Chad Co., Olathe, KS) designed to simulate a commercial beef spray cabinet (Figure 4.1). The cabinet had 18 total floodjet spray nozzles (0.1 gallons per minute; Grainger Industrial Supplies, Fort Collins, CO). Ten nozzles were above the product belt and eight nozzles below. Solutions were applied at a pressure of 15 psi with a contact time of

approximately 15 s. Following treatment, briskets were placed on individual drying racks and allows to drip for 10 min. Briskets were held on drying racks uncovered at 2°C for approximately 24 h, to stimulate carcass chilling.

After 24 h of chilling, each brisket was divided into four equal parts and each brisket portion was randomly assigned to one of four post-chilling intervention treatments; an untreated control, lactic acid (4.5%; LA), a commercial blend of lactic and citric acid (2.5%; LAC; Beefxide; Birko, Henderson, CO), peroxyacetic acid acidified with sulfiuric acid and sodium sulfate blend (pH 1.2; aPAA). Post-chilling treatments were spray-applied following the same procedure as the pre-chilling treatments. Brisket portions were the stored uncovered on drying racks at 2°C for approximately 72 h before processing. Samples were individually twice ground (Model# 1781, LEM Big Bite #22 Stainless Steel Grinder, West Chester, OH). First, briskets were coarse ground (9.5 mm plate), and then subsequently each sample was homogenized in a hand mixer for 3 min. After mixing, samples were ground again using the same grinder, equipped with a fine grind plate (4.5 mm). Plates were cleaned between every sample, and equipment was thoroughly rinsed between treatments. The finely ground product was then formed into 28 g patties (Patty-O-Matic® Eazy Slider, Farmingdale, NJ). Patties were placed, uncovered on metal sheet pans in the freezer (-20°C) to hold form and thickness of patty while packaged for approximately 15 min. Patties were placed in 15.24 x 20.32 cm vacuum pouch bags (3-mil. standard barrier; Clarity Vacuum Pouches; Kansas City, MO), vacuum packaged and held at -20°C until further analysis.

Cooking Procedures.

Frozen patties were tempered for approximately 12 h at 0 to 2°C to attain raw internal temperatures of 0 to 4°C at the time of cooking. Internal temperatures of the raw patties were

measured using a calibrated, type K thermocouple thermometer (AccuTuff 340, model 34040, Cooper-Atkins Corporation, Middlefield, CT) placed in the geometric center of each patty. Patties were evenly spaced on a griddle and cooked in a combination oven at (Model SCC WE 61 E; Rational, Landsberg am Lech, Germany). Patties were cooked at 204°C, 0% relative humidity for 7 min which allowed the internal temperature to reach 71°C. Internal temperature was monitored with a Type 5 Thermocouple Thermometer (AccuTuff 340, model 34040, Cooper-Atkins Corporation, Middlefield, CT). Immediately following cooking, samples were placed in a vacuum pouch bag, vacuum packaged, and held in the combination oven (Model SCC WE 61 E; Rational, Landsberg am Lech, Germany) at 55°C, 100% relative humidity, until the start of sensory analysis. At the time of sensory analysis, patties were held in a circulating water bath (Fisher Scientific™ Isotemp™ Heated Immersion Circulators: Model 6200 H24) set at 57.5°C until served.

Trained Sensory Evaluation

Trained sensory analysis of flavor was conducted at Colorado State University. Patties from each brisket (N = 360; n = 30) were evaluated by a trained sensory panel consisting of 6 qualified panelists. Samples for sensory analysis were randomly assigned to panel sessions to have a representation of each treatment group in every panel for a total of 12 samples per panel. Patties were cut equally into fourths, allowing each panelist to receive 2-3 pieces, and served warm in individual booths equipped with a red incandescent light. For palate cleansing between samples, panelists were given unsalted saltine crackers, apple juice and distilled water.

Panelists were trained to objectively quantify 11 flavor attributes from the Beef Lexicon (Adikari et al., 2011), including beef flavor, browned, roasted, fat-like, sour, bitter, metallic, warmed-over flavor, liver-like, chemical and rancid (Table 4.2). Panelists objectively quantified

attributes using an unstructured line scale anchored at both ends (0 = absence or low intensity of specified attribute, 100 = extreme intensity of specified flavor attribute). Panelist ratings were captured using an electronic ballot produced by an online survey software (Qualtrics, Provo, UT) and a single average for each sample was obtained.

Sample Preparation and Chemical Analysis

Vacuum packaged ground beef patties were held at -20°C until analysis. For homogenization, samples were frozen by liquid nitrogen, transferred into a blender (NutriBullet Lean, Pacoima, CA, USA), ground into a uniform fine powder, placed in an individual bag and stored at -80°C. Lipid content was extracted using a modified Folch method (Folch et al., 1957), as described by Phillips et al. (2010). Crude fat levels were determined for all (N = 360) ground beef patty samples. A subset (N = 72; n = 6) of samples randomly selected from each treatment group were designated for fatty acid analysis. Samples allotted to fatty acid analysis were methylated as described by Morrison and Smith (1964). Fatty acid methyl esters (FAME) were analyzed using an Agilent Model 6890 Series II (Avondale, PA) gas chromatograph equipped with 00-m x 0.25-mm fused silica capillary column (SP-2560 Supelco Inc. Bellefonte, PA). Identification and quantification of FAME was accomplished by an internal calibration comparing to FAME standards. Percentage of fatty acids were calculated based on total FAME analyzed.

Volatile Compound Analysis

A subset (N = 72; n = 6) of samples randomly selected for volatile analysis corresponded with the subset of samples utilized for fatty acids. Five grams of homogenized ground beef were weighed into 20mL headspace vial and stored at -80°C until analysis. In order to minimize oxidation degradation during data acquisition, the autosampler sample tray was insulated by a

custom-built circulation system which maintains the sample temperature at below 10°C. Samples were first incubated at 40°C for 30 min, and then the headspace volatiles were extracted by a Carboxen/PDMS fiber (85µm, Stableflex, Sigma-Aldrich) for 40 min following the method of (Pérez et al., 2008), and injected into a DB-WAXUI column (30 m x 0.25 mm x 0.25 µm, Agilent) in a Trace1310 GC (Thermo Scientific, Waltham, MA) coupled to an ISQ-LT mass spectrometer (Thermo Scientific, Waltham, MA). Solid-phase microextraction (SPME) fiber desorbed at injection port (250°C) for 3 min, and then at fiber conditioning port (270°C) for 10 min. Gas chromatography (GC) inlet was operated under splitless mode during fiber desorption. The oven program started at 35°C for 5 min, with the first ramp to 100°C at a rate of 8°C/min, and the second ramp to 240°C at a rate of 12°C/min, and a final hold at 240°C for 5 min. Data were acquired under electron impact mode, with full scan 35-350 amu and a scan rate 10 scans/second. Transfer line and source temperatures were 250°C.

An non-targeted processing method was used in Chromeleon software (ThermoFisher Scientific, Waltham, MA). Twelve compounds were identified, and their retention times and peak width were built into the processing method. Chromeleon software was used to export the peak area of compounds of interest. Gas chromatography-mass spectrometry (GC-MS) spectra were annotated by matching unknown spectra to the NIST v12 EI spectral database. Additionally, an alkane mix of C:8-C:20 was injected at the end of the sequence and could be used for further confirmation of compound identities if needed.

Statistical Analysis

Individual panelist ratings were averaged to obtain a single sensory rating for each flavor attribute of each sample. Data from trained sensory panel were analyzed as a 2-way factorial, using pre- and post-chilling treatments as fixed effects. Panel number, feed order, and collection

day were included as random variables in all models. Crude fat was used as a covariate in the model to analyze flavor attributes. To more accurately reflect production practices, only samples with crude fat levels of 5-20% were included in the analysis (N = 298). In further analysis, samples were grouped into three crude fat levels, and data were analyzed using crude fat level as a main effect along with pre- and post-chilling treatments. Data from fatty acid and volatile compound analysis were analyzed a 2-way factorial, using pre- and post-chilling as fixed effects. Data analyses were performed using the procedures of SAS (Version 9.4; SAS Inst. Inc., Cary, NC). Least squares means are reported by treatment for all sensory attributes. For each analysis, interaction and main effect comparisons were tested for significance using PROC GLIMMIX with $\alpha = 0.05$ and the denominator degree of freedom was calculated using the Kenward-Roger method.

Results and Discussion

Trained Sensory Analysis

Effects of pre-chilling treatments on beef flavor attributes assessed by trained panelists are presented in Table 4.3. Treatments applied pre-chilling, represent common interventions applied on the harvest floor. Trained panelist ratings for sour and chemical were rated highest ($P < 0.05$) for the LA pre-chilling treatment compared to the CONTROL and aPAA. Of the off-flavor notes, sour was the most notable, detectable difference. Chemical notes were rated highest ($P < 0.05$) for LA treatment applied pre-chilling, but the quantitative measure still represented low levels of detection. Ratings for the browned descriptor were greater ($P < 0.05$) for aPAA than CONTROL or LA pre-chilling. No differences ($P > 0.05$) due to pre-chilling treatments were identified for beef flavor ID, roasted, metallic, fat-like, sour, rancid, warmed over, or liver-like. Table 4.4 shows flavor attribute intensities by post-chilling treatments. No differences ($P >$

0.05) were found for any flavor attribute due to post-chilling treatments. While post-chilling treatments did not create any significant differences, similar results were observed with LA-treated samples having great numerical ratings for metallic, sour, warmed over, liver-like and chemical attributes.

To determine the role fat played in differences in flavor, crude fat levels were used as an interaction with pre- and post-chilling treatments. The only significant ($P < 0.05$) 3-way interaction was for fat-like. This was expected as crude fat level should be an indicator of fat-like flavors. Therefore, the main effect of crude fat level was then evaluated for each flavor attribute. Three levels of crude fat percentages were used to categorize samples; LOW (5-10%), MED (10-15%), and HIGH (15-20%). Results of the main effect of fat level are presented in Table 4.5. LOW fat level samples were found to have higher levels of off-favor notes. Flavor attributes, including metallic, sour, warmed over, and chemical, were rated higher ($P < 0.05$) in the LOW fat level group, compared to the MED and HIGH levels. However, brown flavor ratings were lower ($P < 0.05$) in the LOW fat level than the MED and HIGH levels. As expected, fat-like, and beef flavor ID ratings were strengthened ($P < 0.05$) with increasing fat levels, with LOW levels receiving the lowest intensity rating ($P < 0.05$) and HIGH receiving the highest ratings ($P < 0.05$). No differences ($P \geq 0.05$) in roasted, bitter, rancid, or liver-like were found due to crude fat levels.

Fatty Acid Analysis

Fatty acid analysis showed minimal differences due to intervention treatments (Table 4.7 and Table 4.8). Treatment only affected C10:0, as samples treated with LA post-chilling produced greater ($P < 0.05$) concentrations than treatment with LAC or in comparison to negative CONTROL samples (Table 4.8). Also referred to as capric acid or decanoic acid, C10:0

is not a main saturated fatty acid found in beef. No other differences ($P \geq 0.05$) in fatty acid concentrations were found due any pre- or post-chilling treatments. Fatty acid concentrations did not appear to be influenced by treatment in the present study.

Volatile Compounds

Relative abundance of volatile organic compounds are presented in Tables 4.9 and 4.10. As expected, hexanal was a dominant component, as it is a major contributor to meat products and also has been found to be a main volatile indicator of lipid oxidation (Fernando et al., 2003; Shahidi & Pegg, 1994). No statistical differences ($P \geq 0.05$) due to any treatment were identified for abundance of hexanal However, abundances were numerically highest for CONTROL samples compared to any pre- or post-chilling treatment. Pentanal abundance was greater ($P < 0.05$) in post-chilling LA-treated samples than CONTROL, LAC, or aPAA samples (Table 4.9). Similarly, hexanoic acid and pentanol abundances were greater ($P < 0.05$) in samples treated post-chilling with LA, compared to CONTROL or LAC-treated samples. Stetzer et al. (2008) reported livery off-flavor being positively correlated with pentanal and hexanoic acid. Hexanoic acids aroma has been described as pungent, blue cheese and sour (Table 4.11; Lecanu et al., 2002). For pre-chilling interventions, LA and aPAA-treated samples produced lower ($P < 0.05$) concentrations of acetoin than CONTROL samples. Acetoin has been demonstrated to have butter or cream like aroma characteristics (Table 4.11; Acree & Arn, 2004).

Conclusions

With multiple interventions being utilized, it is inevitable that beef primals and trimmings will be exposed to chemical interventions. This study showed that spray-application of antimicrobials that resemble interventions during the slaughter process, before chilling of carcasses had an impact on flavor. Lactic acid applied as a pre-chilling treatment resulted in

higher ($P < 0.05$) ratings of sour and chemical. This indicated that usage of antimicrobial interventions applied on the harvest floor may slightly increase off-flavors in ground beef products. Post-chilling treatments used to simulate effects of antimicrobial interventions following carcass chilling did not significantly ($P \geq 0.05$) impact flavor attributes. Eastwood et al. (2018) reported similar findings of antimicrobials (acidified sodium chloride, Beefxide, and lactic acid) having minimal impact on ground beef quality.

When considering the fat level of the samples, pre- and post-chilling treatments had a greater influence in leaner samples. Samples in the LOW-fat group (5-10% crude fat) had overall higher ($P < 0.05$) intensities of off-flavor attributes including metallic, sour, warmed-over, and chemical. This perhaps could be due to the HIGH levels having more fat to mask off-flavors, or the fat repelling the antimicrobial. Potentially there was less antimicrobial residuals in higher fat samples as fat tissue tends to retain less surface moisture than lean tissues (Dickson, 1992). Pre- and post-chilling treatments had negligible impacts on fatty acid composition or relative abundance of volatile compounds. Although, minimal differences were observed due to combinations of antimicrobial interventions, lactic acid as a pre-chilling treatment increased the off-flavor attribute ratings of sour and chemical, and post-chilling treatments may have the potential to create an impact on off-flavor. This study has shown the implications this standard production practice has on the end product's flavor profile.

TABLE 4. 1. Pre-chilling and post-chilling treatments to be utilized in complete combination with one another.

Pre-Chilling Treatment	Post-Chilling Treatment
CON	CON
	aPAA
	LA
	LAC
aPAA	CON
	aPAA
	LA
	LAC
LA	CON
	aPAA
	LA
	LAC

CON: untreated control; aPAA: Peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend; LA: Lactic acid at 4.5% in solution; LAC: Commercial lactic/citric acid blend at 2.5% in solution.

TABLE 4. 2. Definition and reference standards for beef descriptive flavor aromatics and basic taste sensory attributes and their intensities based on Adikari et al. (2011) where 0 = none and 100 = extremely intense.

Attribute	Definition	Reference
Beef Flavor	Amount of beef flavor identity in the sample.	Swanson's beef broth = 35 80% lean ground beef = 4 Beef brisket (160 °F) = 75
Bitter	The fundamental taste factor associated with a caffeine solution.	0.01% caffeine solution = 15 0.02% caffeine solution = 25
Browned	Aromatic associated with the outside of grilled or broiled meat; seared but not blackened or burnt.	Steak cooked at high temperature (internal 137 °F, seared on outside)
Chemical	The aromatics associates with garden hose, hot Teflon pan, plastic packaging and petroleum-based product such as charcoal liter fluid	Clorox in water = 45
Fat - Like	The aromatics associated with cooked animal fat	Hillshire farms Lit'l beef smokies = 45 Beef suet = 80
Liver - Like	The aromatics associated with cooked organ meat/liver	Beef Liver = 50
Metallic	The impression of slightly oxidized metal, such as iron, copper, and silver spoons.	0.10% Potassium Chloride solution = 10 Select strip Steak (60 °C internal) = 25 Dole Canned Pineapple Juice = 40
Rancid	The aromatics commonly associated with oxidized fat and oils. These aromatics may include carboard, paint, varnish and fishy.	Microwaved Wesson vegetable oil (3 min at high) = 45 Microwaved Wesson vegetable oil (5 min at high) = 60
Roasted	Aromatic associated with roasted meat.	Precooked Roast
Sour	The fundamental taste factor associated with citric acid.	0.015% citric acid solution = 10 0.050% citric acid solution = 25
Warmed-Over Flavor	Perception of a product that has been previously cooked and reheated	80% lean ground beef (reheated) = 40

TABLE 4. 3. Trained sensory ratings[1] for attributes of ground beef representing three pre-chilling treatments.

	Pre-Chilling Treatment[2]				
Attribute	CON	aPAA	LA	SEM[3]	*P* - Value
Beef Flavor ID	44.72	45.19	44.16	0.86	0.36
Browned	35.81[a]	37.21[b]	35.70[a]	0.85	0.03
Roasted	42.70	42.64	42.14	0.86	0.63
Metallic	6.86	6.63	7.76	0.44	0.11
Fat-Like	16.12	16.28	16.41	0.62	0.92
Sour	7.57[a]	8.34[a]	10.56[b]	0.60	< 0.01
Bitter	2.13	2.31	2.45	0.33	0.74
Rancid	1.50	1.59	2.13	0.40	0.30
Warmed Over	5.04	6.72	4.86	0.71	0.08
Liver-Like	1.30	1.75	1.01	0.32	0.18
Chemical	2.10[a]	1.76[a]	3.32[b]	0.37	< 0.01

[a-b] Least square means in the same row without a common superscript differ ($P < 0.05$)

[1]Attributes were scored using a 100mm unstructured line scale, anchored at both ends: 0 = absence, not present 100 = extreme intensity of specified flavor attribute

[2] Treatments applied pre-chilling, untreated control (CON; no interventions applied); peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA); lactic acid at 4.5% in solution (LA).

[3]Standard error (largest) of the least squares means

TABLE 4. 4. Trained sensory ratings[1] for attributes of ground beef representing four post-chilling treatments.

	Post-Chilling Treatment[2]					
Attribute	CON	aPAA	LA	LAC	SEM[3]	*P* - Value
Beef Flavor	44.45	44.15	45.46	44.70	0.91	0.36
Browned	35.61	36.13	36.05	37.19	0.88	0.17
Roasted	42.27	42.18	43.04	42.47	0.90	0.57
Metallic	6.78	7.16	7.31	7.10	0.49	0.85
Fat-Like	16.04	15.40	16.56	17.10	0.70	0.20
Sour	8.22	8.14	10.00	8.94	0.70	0.16
Bitter	2.27	2.84	2.02	2.06	0.37	0.28
Rancid	1.96	1.26	1.95	1.78	0.43	0.47
Warmed Over	5.07	5.59	5.78	5.73	0.80	0.89
Liver-Like	1.27	1.34	1.49	1.32	0.36	0.96
Chemical	2.04	2.24	2.75	2.55	0.42	0.53

[1]Attributes were scored using a 100mm unstructured line scale, anchored at both ends: 0 = absence, low intensity, not present 100 = extreme intensity of specified flavor attribute
[2] Untreated control (CON; no interventions applied); peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA); lactic acid at 4.5% in solution (LA); lactic/citric acid blend at 2.5% in solution (LAC).
[3]Standard error (largest) of the least squares means

TABLE 4. 5. Trained sensory ratings[1] for attributes of ground beef across all treatments, separated into three fat levels.

	Fat Level[2]				
Attribute	Low	Med	High	SEM[3]	*P* - Value
Beef Flavor ID	43.12[a]	45.06[b]	46.51[c]	0.85	< 0.01
Browned	35.43[a]	36.42[b]	37.52[b]	0.81	< 0.01
Roasted	41.88	42.88	42.80	0.89	0.07
Metallic	8.28[a]	6.79[b]	5.98[b]	0.43	< 0.01
Fat-Like	12.77[a]	16.67[b]	19.86[c]	0.62	< 0.01
Sour	10.06[a]	8.69[b]	7.48[b]	0.68	< 0.01
Bitter	2.62	2.53	1.90	0.33	0.08
Rancid	1.93	1.54	1.38	0.37	0.20
Warmed Over	6.87[a]	5.07[b]	3.82[b]	0.73	< 0.01
Liver-Like	1.57	1.47	1.34	0.30	0.79
Chemical	2.96[a]	2.12[b]	2.39[b]	0.38	0.04

[a-c] Least square means in the same row lacking a common superscript differ ($P < 0.05$)
[1]Attributes were scored using an unstructured line scale, anchored at both ends: 0 = absence, low intensity, not present 100 = extreme intensity of specified flavor attribute
[2]Samples were divided into three crude fat levels: Low = 5-10%; Med = 10-15 %; High = 15-20%
[3]Standard error (largest) of the least squares means

TABLE 4. 6. Mean values for crude fat percentages according to pre- and post-chilling treatments.

Pre-Chilling Treatment	Post-Chilling Treatment	Mean	Standard Deviation	Minimum	Maximum
CON	CON	11.5	3.50	6.0	18.9
	aPAA	10.5	3.64	5.4	18.3
	LA	9.4	3.52	5.0	18.5
	LAC	9.7	4.00	5.3	19.4
aPAA	CON	12.6	4.04	6.5	19.4
	aPAA	11.2	4.01	5.8	19.7
	LA	11.6	3.92	5.9	18.2
	LAC	11.0	4.29	5.5	18.4
LA	CON	11.9	3.35	7.2	18.9
	aPAA	12.5	4.86	5.7	19.9
	LA	13.0	4.33	5.4	19.2
	LAC	12.7	3.83	5.6	19.4

[1]Untreated control (CON; no interventions applied); peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA); lactic acid at 4.5% in solution (LA); lactic/citric acid blend at 2.5% in solution (LAC).

TABLE 4. 7. Percentages of neutral fatty acid identified for ground beef patties representing three pre-chilling treatments.

	Pre-Chilling Treatment[1]				
Fatty Acid	CON	aPAA	LA	SEM[2]	*P* - Value
C10:0	0.10	0.13	0.12	0.01	0.28
C12:0	0.09	0.09	0.09	< 0.01	0.96
C12:1	0.04	0.04	0.04	< 0.01	0.62
C14:0	2.04	1.91	1.98	0.04	0.11
C14:1	0.68	0.68	0.69	0.04	0.94
C16:0	22.69	22.36	22.51	0.13	0.17
C16:1	5.14	4.88	5.15	0.19	0.48
C17:0	1.28	1.30	1.27	0.03	0.76
C17:1	0.86	0.84	0.86	0.01	0.28
C18:0	13.78	14.37	13.91	0.29	0.32
C18:1 t6	0.41	0.39	0.40	0.02	0.27
C18:1 t8	0.42	0.40	0.42	0.01	0.58
C18:1 t10	3.76	3.63	3.68	0.12	0.76
C18:1 trans vaccenic	0.63	0.70	0.61	0.04	0.23
C18:1 c9	36.61	39.45	40.01	0.43	0.63
C18:1 c11	1.91	1.87	1.93	0.04	0.50
C18:2	0.75	5.06	4.57	0.20	0.21
C18:3	0.16	0.15	0.15	0.01	0.44
C18:2 c9 t 11	0.30	0.30	0.30	< 0.01	0.54
C18:2 t10 c12	0.03	0.03	0.03	< 0.01	0.92
C20:0	0.03	0.03	0.03	< 0.01	0.78
C20:1	0.09	0.07	0.08	0.02	0.81
C20:2	< 0.01	0.01	0.01	< 0.01	0.43
C20:4	0.91	1.02	0.89	0.06	0.22
C20:5	0.02	0.01	0.01	< 0.01	0.91
C22:6	0.91	1.02	0.89	0.06	0.22
C24:0	0.16	0.18	0.16	0.01	0.22
unknown	0.11	0.10	0.10	0.01	0.90

[1]Untreated control (CON; no interventions applied); lactic acid at 4.5% in solution (LA); peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA).
[2]Standard error (largest) of the least squares means

TABLE 4. 8. Percentages of neutral fatty acid identified for ground beef patties representing four post-chilling treatments.

	Post-Chilling Treatment[1]					
Fatty Acid	CON	aPAA	LA	LAC	SEM[2]	*P* - Value
C10:0	0.13[ab]	0.09[bc]	0.08[c]	0.15[a]	0.17	0.02
C12:0	0.09	0.08	0.09	0.08	0.01	0.67
C12:1	0.04	0.04	0.04	0.04	< 0.01	0.50
C14:0	1.98	1.94	2.01	1.98	0.05	0.74
C14:1	0.64	0.72	0.67	0.70	0.05	0.53
C16:0	22.57	22.44	22.52	22.55	0.15	0.93
C16:1	4.85	5.32	4.94	5.11	0.23	0.48
C17:0	1.30	1.24	1.31	1.28	0.04	0.53
C17:1	0.84	0.88	0.85	0.85	0.02	0.35
C18:0	14.30	13.49	14.23	14.06	0.34	0.34
C18:1 t6	0.39	0.39	0.44	0.39	0.02	0.27
C18:1 t8	0.38	0.41	0.44	0.41	0.01	0.07
C18:1 t10	3.37	3.48	3.89	3.67	0.15	0.28
C18:1 trans vaccenic	0.61	0.63	0.68	0.67	0.04	0.56
C18:1 c9	39.54	40.46	39.06	39.71	0.51	0.27
C18:1 c11	1.85	1.95	1.90	1.90	0.46	0.52
C18:2	4.89	4.63	4.97	4.67	0.24	0.67
C18:3	0.16	0.16	0.16	0.16	< 0.01	0.98
C18:2 c9 t 11	0.31	0.28	0.30	0.31	0.01	0.24
C18:2 t10 c12	0.03	0.03	0.03	0.03	< 0.01	0.93
C20:0	0.04	0.03	0.03	0.03	< 0.01	0.31
C20:1	0.09	0.07	0.08	0.07	0.02	0.93
C20:2	0.01	0.01	< 0.01	0.01	< 0.01	0.52
C20:4	0.95	0.94	0.98	0.89	0.06	0.80
C20:5	0.02	0.02	0.02	0.01	< 0.01	0.58
C22:6	0.95	0.94	0.98	0.89	0.07	0.80
C24:0	0.17	0.16	0.18	0.16	0.01	0.68
unknown	0.11	0.09	0.10	0.11	0.01	0.18

[a-c] Least square means in the same row without a common superscript differ ($P < 0.05$)
[1]Untreated control (CON; no interventions applied); lactic acid at 4.5% in solution (LA); lactic/citric acid blend at 2.5% in solution (LAC); peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA).
[2]Standard error (largest) of the least squares means.

TABLE 4. 8. Percentages of neutral fatty acid identified for ground beef patties representing four post-chilling treatments.

	Post-Chilling Treatment[1]					
Fatty Acid	CON	aPAA	LA	LAC	SEM[2]	*P* - Value
C10:0	0.13[ab]	0.09[bc]	0.08[c]	0.15[a]	0.17	0.02
C12:0	0.09	0.08	0.09	0.08	0.01	0.67
C12:1	0.04	0.04	0.04	0.04	< 0.01	0.50
C14:0	1.98	1.94	2.01	1.98	0.05	0.74
C14:1	0.64	0.72	0.67	0.70	0.05	0.53
C16:0	22.57	22.44	22.52	22.55	0.15	0.93
C16:1	4.85	5.32	4.94	5.11	0.23	0.48
C17:0	1.30	1.24	1.31	1.28	0.04	0.53
C17:1	0.84	0.88	0.85	0.85	0.02	0.35
C18:0	14.30	13.49	14.23	14.06	0.34	0.34
C18:1 t6	0.39	0.39	0.44	0.39	0.02	0.27
C18:1 t8	0.38	0.41	0.44	0.41	0.01	0.07
C18:1 t10	3.37	3.48	3.89	3.67	0.15	0.28
C18:1 trans vaccenic	0.61	0.63	0.68	0.67	0.04	0.56
C18:1 c9	39.54	40.46	39.06	39.71	0.51	0.27
C18:1 c11	1.85	1.95	1.90	1.90	0.46	0.52
C18:2	4.89	4.63	4.97	4.67	0.24	0.67
C18:3	0.16	0.16	0.16	0.16	< 0.01	0.98
C18:2 c9 t 11	0.31	0.28	0.30	0.31	0.01	0.24
C18:2 t10 c12	0.03	0.03	0.03	0.03	< 0.01	0.93
C20:0	0.04	0.03	0.03	0.03	< 0.01	0.31
C20:1	0.09	0.07	0.08	0.07	0.02	0.93
C20:2	0.01	0.01	< 0.01	0.01	< 0.01	0.52
C20:4	0.95	0.94	0.98	0.89	0.06	0.80
C20:5	0.02	0.02	0.02	0.01	< 0.01	0.58
C22:6	0.95	0.94	0.98	0.89	0.07	0.80
C24:0	0.17	0.16	0.18	0.16	0.01	0.68
unknown	0.11	0.09	0.10	0.11	0.01	0.18

[a-c] Least square means in the same row without a common superscript differ ($P < 0.05$)
[1]Untreated control (CON; no interventions applied); lactic acid at 4.5% in solution (LA); lactic/citric acid blend at 2.5% in solution (LAC); peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA).
[2]Standard error (largest) of the least squares means.

TABLE 4. 9. Relative abundance (thousands) of volatile compounds identified ground beef patties representing three Pre-Chilling treatments.

	Pre-Chilling Intervention[1]				
Compound	CON	aPAA	LA	SEM[2]	*P* - Value
Pentanal	422	433	509	131	0.58
Hexanal	9086	8296	7193	2359	0.55
Propanol	31	34	29	4	0.63
P-xylene	26	15	16	13	0.51
Pentanol	597	544	586	63	0.82
Acetoin	1216[a]	708[b]	979[ab]	105	< 0.01
Octanedione	45	37	46	9	0.72
Acetic Acid	773	851	731	120	0.41
Butanic Acid	75	55	66	13	0.12
Benzaldehyde	6	9	9	4	0.56
Pentanoic Acid	12	11	12	2	0.77
Hexanoic Acid	21	19	22	4	0.71

[a-b] Least square means in the same row without a common superscript differ ($P < 0.05$)
[1] Negative control (CON; no interventions applied); lactic acid at 4.5% in solution (LA); peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA).
[2]Standard error (largest) of the least squares means

TABLE 4. 10. Relative abundance (thousands) of volatile compounds identified ground beef patties representing four post-chilling treatments.

	Post-Chilling Intervention[1]					
Compound	CON	aPAA	LA	LAC	SEM[2]	*P* - Value
Pentanal	405[b]	431[b]	649[a]	333[b]	131	0.02
Hexanal	9943	5399	7739	9685	2380	0.09
Propanol	27	29	36	35	5	0.48
P-xylene	24	23	24	8	13	0.31
Pentanol	512[b]	575[ab]	755[a]	459[b]	75	0.02
Acetoin	941	890	1055	984	124	0.79
Octanedione	36	37	63	34	10	0.12
Acetic Acid	727	883	844	685	123	0.21
Butanic Acid	63	59	75	64	13	0.47
Benzaldehyde	5	8	12	6	4	0.11
Pentanoic Acid	10	11	14	12	2	0.28
Hexanoic Acid	17[b]	21[ab]	26[a]	18[b]	4	0.05

[a-b] Least square means in the same row lacking a common superscript differ ($P < 0.05$)
[1] Negative control (CON; no interventions applied); lactic acid at 4.5% in solution (LA); lactic/citric acid blend at 2.5% in solution (LAC); peroxyacetic acid (400 ppm) acidified to a pH of 1.2 with a sulfuric acid and sodium sulfate blend (aPAA).
[2]Standard error (largest) of the least squares means

TABLE 4. 11. Characteristic aromas of volatile compounds.

Compound	Description	Source
Pentanal	Pungent, acrid, malt, almond	(Calkins et al., 2007)
Hexanal	Woody, cut grass, chemical-winey, fatty, fruity, weak metallic	(Calkins et al., 2007)
Propanol	Alcoholic	(Calkins et al., 2007)
P-xylene	Plastic	(Acree et al., 2004)
Pentanol	Mild odor, fusel oil, fruit balsamic	(Calkins et al., 2007)
Acetoin	Butter, cream	(Acree et al., 2004)
Octanedione	Alkane	(Acree et al., 2004)
Acetic Acid	Vinegar, pungent	(Lecanu et al., 2002)
Butanic Acid	Rancid, cheese, sweat	(Acree et al., 2004)
Benzaldehyde	Volatile almond oil, bitter almond, burning aromatic taste	(Calkins et al., 2007)
Pentanoic Acid	Sweat	(Acree et al., 2004)
Hexanoic Acid	Pungent, blue cheese, sour	(Lecanu et al., 2002)

FIGURE 4. 1. Custom-built, pilot-sized spray cabinet designed to simulate a commercial beef spray cabinet.

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