ANTIMICROBIAL INTERVENION AND PROCESS VALIDATION

IN BEEF JERKY PROCESSING

by

WORAWUT RAKITI

(Under the Direction of Mark A. Harrison)

ABSTRACT

Beef jerky is a popular dried meat product because it has a high protein and low fat content and is tasty. Association of jerky products with foodborne illness outbreaks has raised questions concerning the microbial safety of the product. Antimicrobial interventions before and after marinating the strips are optional and offer the opportunity to increase the level of pathogen reduction greater than that achieved by heating and drying alone. The antimicrobial effect of chemical pretreatments with combination of either a horizontal-flow dehydrator or a commercial-type smokehouse in the inactivation of *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* on whole-muscle beef jerky strip was investigated. The populations of *Salmonella* were significantly reduced by more than 6.5 logs cfu/strip on jerky that were pretreated with the 1:2 acidic calcium sulfate:water and dried in the dehydrator (p=0.0044) and jerky pretreated with the 1,200 ppm concentration of acidified sodium chlorite and dried in the smokehouse (p=0.0081). The populations of *E. coli* O157:H7 were significantly reduced by at least 5 logs for all the treatments except for jerky pretreated with the 500 ppm concentration of acidified sodium chlorite and dried in the dehydrator. The populations of *L. monocytogenes* were reduced by 5 logs for all the treatments regardless of the drying method.

INDEX WORDS: Beef jerky, *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, acidic calcium sulfate, acidified sodium chlorite, dehydrator, smokehouse

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DEDICATION

To my great mother and my loving brother

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Jerky, among the oldest of meat products, has existed since the time of ancient Egypt. Traditionally, it was made by the use of the sun, wind and smoke from fires as a way to preserve and extend the shelf life of the meat. Today, jerky is a high demand snack food and widely available to consumers throughout North America because it has a rich nutrient content (high in protein and iron but low in fat). Jerky is classified by the U.S. Department of Agriculture (USDA) as a heat-treated, shelf-stable (can be stored without refrigeration) and ready-to-eat meat product (USDA-FSIS, 2004a). Jerky must have 0.75–1.0 moisture protein ratio (MPR) and water activity (a_w) <0.8 (USDA-FSIS, 2004b).

Jerky is produced by both consumers at home and industrial establishments. Numerous recipes for making jerky are based on using meat from several species such as beef, poultry, and wild animals, preparation procedures of meat such as ground beef, thick or thin slices, different marination techniques with various ingredients, volumes, times and temperatures, and drying processes such as dehydrator and smokehouse. Small and very small commercial processors of meat jerky products must show their processes are sufficient to provide a safe finished product. *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* are foodborne pathogenic bacteria of concern to the industry and regulators. The extent and duration of the survival of pathogens on jerky during storage may vary depending on the type of bacteria and the variety of antimicrobial hurdles such as low pH, low water activity, preservatives in the composition of the marinade, and drying temperature.

The Food Safety and Inspection Service of the USDA (FSIS/USDA) reported that for the period from 1990 to 1999, cumulative prevalence of *Salmonella* and *L. monocytogenes* in jerky produced in the U.S. federally inspected plants was 0.31% and 0.52%, respectively (Levine et al., 2001). Association of jerky products with foodborne disease outbreaks (CDC, 1995b, Keen et al, 1997, and Eidsen et al., 2000) has increased the significance of research that may improve the microbial safety of jerky.

In 2004, FSIS issued and revised several compliance guidelines for processing jerky, calling for the use of adequate humidity during heating and drying to ensure adequate pathogen kill. FSIS also issued the generic HACCP model for heat treated, shelf stable meat and poultry products to assist jerky processors in development of their HACCP plans. According to the compliance guideline, small and very small jerky processing establishments can develop customized lethality processes that achieve an appropriate reduction of pathogens throughout the product. Customized processes must be validated (9 CFR 417.4). Possible means to validate a process are to conduct challenge studies that are based on scientific rationale and provide the necessary data to determine the $\log_{10}$ reduction of the pathogen (USDA-FSIS, 2004b).

There are published studies on inactivation of foodborne pathogens during jerky processing. Numerous variations in the type of beef used (whole strip, ground), marinades, jerky preparation steps, and drying methods have been evaluated. A number of researchers and jerky processors have studied and developed a variety of methods to reduce numbers of pathogenic bacteria on jerky products and to improve microbiological safety.

The reduction of the pathogen populations is influenced by drying temperature and whether or not the meat received additional treatments designed to raise the internal temperature. A study by Harrison et al. (2001) investigated the effectiveness of heating inoculated beef jerky

strips at 71.1°C, post-drying heating at 135°C for 10 min, boiling strips in traditional marinade, and traditional marinade for reducing numbers of foodborne pathogens during drying at 60°C for 10 h. The authors reported that all preparation procedures yielded equal to or greater than 5.8, 4.6 and 3.9 log reductions of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes*, respectively. All alternative treatments resulted in a significant difference in color, saltiness, and texture from traditional jerky. Texture, however, only appeared to influence overall consumer acceptability (Harrison et al., 2001).

According to the C*ompliance Guideline for Meat and Poultry Jerky Produced by Small and Very Small Plants* (USDA-FSIS, 2004b) marination is an optional step. Jerky processors may use a variety of marinade ingredients and methods for application. The strips are marinated in a solution that often contains salt, sugar and other favoring ingredients. The use of various ingredients can reduce the pathogen levels on jerky products. The fate of the pathogens in reduced sodium home style beef jerky was investigated. *Salmonella*, *L. monocytogenes,* and *E. coli* O157:H7 populations in both of the samples heated to 71.1°C prior to drying and the unheated samples exhibited a greater reduction during drying when a cure mix containing salt and nitrite was added during ground beef jerky preparation. The effect of heating on the pathogens was enhanced by the inhibitory effects of the cure mix (Harrison et al., 1997 and 1998).

Antimicrobial interventions before and after marinating the strips are also optional and offer the opportunity to increase the level of pathogen reduction greater than that achieved by heating and drying alone. The treatments with various organic acids could help to decline or eliminate pathogens from the process.

Albright et al. (2003) examined the effect of 4 different pretreatments of whole beef jerky slices on *E. coli* O157:H7 prior to drying at 62.5°C for 10 h. The treatment marinated at 4°C for 24 h followed by submersion in pickle brine at 78°C for 90 s was shown to have the highest pre-pretreatment reduction (3.1-4.1 log cfu/cm^2) and the largest overall reduction (5.7-5.8 log cfu/cm^2). The other pretreatments consisting of; immersing in boiling water at 94°C for 15 s then marination at 4°C for 24 h; immersing in a 1:1 vinegar and water solution at 57.5°C for 20 s then marination at 4°C for 24 h; and marination at 4°C for 24 h and then immersing in a 1:1 vinegar and water solution at 57.5°C for 20 s resulted in a 4.3- 4.5, 4.9-5.2, and 4.7- 4.8 log cfu/cm^2 reduction, respectively (Albright et al., 2003).

Calicioglu et al. (2002a, 2002b, and 2003a) studied the inactivation of acid-adapted and unadapted *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* during the processing of beef jerky. The strips were subjected to different predrying marinade treatments and dried at 60°C for 10 h. The predrying treatments evaluated were traditional marinade (TM) and dipping into 1% Tween 20 (polysorbate 20 or polyoxyethylene-20-sorbitan monolaurate) for 15 min and then into 5% acetic acid for 10 min followed by traditional marinade (TWTM). The TM consisted of soy sauce, Worcestershire sauce, black pepper, garlic powder, onion power, and hickory-smoked salt. Results indicated that TWTM decreased the populations of *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* during drying by 4.9 to 6.7, 5.9 to 6.3, and 4.8 to 6.0 log cfu/cm^2, respectively (Calicioglu et al, 2002a, 2002b, and 2003a). The authors concluded that acid adaptation may not cause increased resistance of microbes to the microbial hurdles involved in jerky processing.

Calicioglu et al. (2003b, 2003c, and 2003d) also determined the effects on survival of acid-adapted and nonadapted bacteria inoculated post-drying on beef jerky strips. They used the

same marinade treatments as their previous studies. Then strips were inoculated post drying and aerobically stored at 25°C for 60 days. Results showed that TWTM had the highest pathogen population reduction and populations decreased faster on jerky inoculated with acid-adapted cultures than with nonadapted cultures for all marinade treatments. By the end of 60 days of storage, there was no significant difference in survival of acid-adapted and nonadapted cultures (Calicioglu et al., 2003b, 2003c, and 2003d).

Aqueous chemical treatments, such as organic acids, ozonated water, and chlorinated water can reduce pathogen levels on the surface of meat products. A highly acidic metalated calcium sulfate with a pH of ≤ 2.0 was developed by the Mionix Corporation for use as a food additive to reduce or eliminate microbial contaminants (Mionix, 2003, Kemp et al., 2003). When mixed with organic acids, such as acetic acid, propionic acid and/or lactic acid, acidic calcium sulfate (ACS) reduces the pH sufficiently to maintain more organic acid in its undissociated form. This form of the acid has the greatest antimicrobial activity (Zhao, 2004). ACS ingredients are affirmed as General-Recognized-as-Safe (GRAS) by the U.S. FDA 21 CFR 184,1230 (FDA, 2003). Although, GRAS chemicals are exempted from the Food and Drug Administration's testing and approval process, USDA-FSIS must approve any GRAS, additive or otherwise, for specific use and specify acceptable levels in meat products.

Zhao et al., (2004) determined the combined effect of freezing and addition of a mixture of 20% acidic calcium sulfate and 10% lactic acid (ACS-LA) on the thermal sensitivity of *E. coli* O157:H7 in ground beef. Results revealed that *D*-values (decimal reduction times) at 57°C obtained for microbes in previously refrigerated and frozen ground beef containing ACS-LA and ACS-LA diluted by half were significantly less than those obtained for ground beef with no ACS-LA added. The authors concluded that the addition of ACS-LA to ground beef, whether

previously frozen or refrigerated, can reduce the time and temperature required to eliminate *E. coli* O157:H7 during heating (Zhao et al., 2004).

A study by Nuñez de Gonzalez et al. (2004) investigated the effectiveness of acidic calcium sulfate with propionic and lactic acid and lactates as postprocessing dipping solutions to control *L. monocytogenes* inoculated onto the surface of frankfurters with or without potassium lactate (KL) and stored vacuum packaged at 4.5°C for up to 12 weeks. The incorporation of KL added as a primary ingredient in frankfurters combined with a surface application of acidic calcium sulfate with propionic and lactic acid could potentially afford protection against the growth of *L. monocytogenes* in or on frankfurters. After cooking, chilling, and peeling, the frankfurters, with or without KL, were inoculated and treated with saline solution (control), acidic calcium sulfate with propionic and lactic acid (ACS, 1:2 water), KL, or lactic acid (LA) for 30 sec. Results showed that *L. monocytogenes* populations over 12 weeks were reduced from 5.1 to 7.1 and from 5.1 to 5.9 log on frankfurters without KL or with KL and dipped in ACS and LA, respectively. These results revealed the potential effectiveness of ACS or LA (bactericidal effect) as postprocessing dipping solutions to inhibit or control the growth of *L. monocytogenes* on vacuum-packaged frankfurters stored at 4.5°C for up to 12 weeks (Nuñez de Gonzalez et al., 2004).

Chlorine dioxide (ClO_2) and ozone (O_3) are strong oxidizing and antimicrobial agents with numerous potential applications in the food industry. The method of action for an oxidant is to cause irreversible damage to the fatty acids in the cell membrane and to cellular proteins of the microorganisms (Luck and Jager, 1998). The FDA has allowed the use of aqueous chlorine dioxide as an antimicrobial agent in washing fruits and vegetables that are not raw agricultural commodities (RACs) in an amount not to exceed 3 ppm residual chlorine dioxide. However,

treatment of fruits and vegetables with chlorine dioxide shall be followed by a potable water rinse or by blanching, cooking, or canning (FDA, 1998). FDA amended the food additive regulations in 21 CFR 173 to provide for the safe use of ozone in gaseous and aqueous phases as an antimicrobial agent for the treatment, storage, and processing of food, including meat and poultry. The proposed use would include the use of this additive on raw agricultural commodities in the preparing, packing, or holding of such commodities for commercial purposes (FDA, 2001a). Recently, ozone has been declared as a GRAS substance by an expert panel for use in bottled water at a maximum residual concentration of 0.4 mg/liter (FDA, 2005).

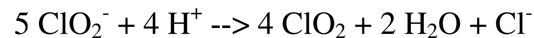
Research and commercial applications have revealed that chlorine dioxide and ozone can replace traditional sanitizing agents and provide other benefits (Graham, 1997; Cherry, 1999). Water containing 50-200 mg/L of chlorine is widely used in food processing plants to sanitize whole fruits and vegetables as well as fresh-cut produce. This treatment, however, only results in a reduction of bacterial population of less than 2 log cfu/g on fruits and vegetables (Beuchat, 1999; Cherry, 1999). Another study by Zhang and Farber (1996) reported that maximum log reduction of *L. monocytogenes* on fresh-cut lettuce and cabbage treated with 200 mg/L of chlorine was 1.3–1.7 and 0.9–1.2 log cfu/g, respectively. They also reported that chlorine dioxide solution treatment (5 mg/L, 10 min) at 4 and 22°C resulted in 1.1 and 0.8 log reduction of *L. monocytogenes*, respectively, on cut lettuce (Zhang and Farber, 1996). Han et al. (2000) studied the effects of washing and chlorine dioxide gas treatment on survival and attachment of *E. coli* O157:H7 on injured green pepper surfaces. They obtained 3.03 and 6.45 log reduction of *E. coli* O157:H7 after treatments with 0.62 and 1.24 mg/L chlorine dioxide, respectively, for 30 min at 22°C and 90–95% RH on surface-injured green peppers. Acidified chlorine dioxide significantly reduced *E. coli* O157:H7 populations on alfalfa seeds at concentrations $\geq$ 100 ppm and at 500

ppm of chlorine dioxide from 2.7 to <0.5 log cfu/g (Taormina and Beuchat, 1999). Singh et al. (2002) evaluated the efficacy of aqueous chlorine dioxide, ozone, and thyme essential oil alone or through their sequential washing in reducing *E. coli* O157:H7 on shredded lettuce and baby carrots. The results indicated that sequential washing treatments, with thyme oil followed by aqueous chlorine dioxide/ozonated water, or ozonated water/aqueous chlorine dioxide, were significantly more effective in reducing *E. coli* O157:H7 (3.75 and 3.99 log, and 3.83 and 4.34 log reduction) on lettuce and baby carrots, respectively (Singh et al., 2002). Another study by Singh et al. (2003) was to evaluate the efficacy of these sanitizers, alone or their sequential washing (thyme oil followed by ozonated water and aqueous chlorine dioxide) for eliminating *E. coli* O157:H7 from alfalfa seeds prior and during sprouting. Results also showed that sequential washing treatments (as irrigation water) during sprouting process have greater lethality than other sanitizer treatments alone (Singh et al., 2003). Therefore, a sequential washing may be an important component of the overall pathogen reduction process.

Antimicrobial intervention treatments in meat processing have also been investigated with treatments ranging from water washing and stream pasteurization (Phebus et al., 1997) to using organic acids, alkaline phosphates and other compounds (Kochevar et al., 1997). Pohlman et al. (2002) determined the effectiveness of chlorine dioxide and ozonated water as multiple antimicrobial interventions during the processing of ground beef on the reduction of microorganisms. For this, beef trimmings were inoculated with *E. coli* and *Salmonella* Typhimurium, treated with multiple interventions, ground, packaged and sampled at interval periods for microbial enumeration. The authors concluded that 1% ozonated water followed by 5% acetic acid treatment and 1% ozonated water followed by 0.5% cetylpyridinium chloride treatment reduced ($p<0.05$) populations of all bacterial types evaluated, whereas 200 ppm

chlorine dioxide followed by 10% trisodium phosphate treatment reduced ($p<0.05$) *E. coli,* coliforms and aerobic plate counts (Pohlman et al., 2002). Stivarius et al. (2002) also studied the effects of beef trimming decontamination with ozone and chlorine dioxide on ground beef microbial populations. Results revealed that 1% ozonated water for 15 min and 200 ppm chlorine dioxide reduced ($p<0.05$) populations of all bacterial types evaluated, while 1% ozonated water for 7 min reduced ($p<0.05$) aerobic plate counts and *Salmonella* Typhimurium (Stivarius et al., 2002).

Acidified sodium chlorite (ASC) is an antimicrobial intervention treatment for poultry carcasses, poultry carcass parts, red meat carcasses, red meat parts and organs, seafood, and raw agricultural commodities. ASC chemistry is principally that of chlorous acid ($HClO_2$), which is the metastable oxychlorine species, which forms acidification of sodium chlorite.



$$5\ ClO_2^- + 4\ H^+ \dashrightarrow 4\ ClO_2 + 2\ H_2O + Cl^-$$

Once formed, chlorous acid gradually decomposes to form chlorate ions, chlorine dioxide, and chloride ions. These reactive intermediates are broad-spectrum germicides that act by breaking oxidative bonds on cell membrane surfaces (Kross, 1984).

ASC is approved by the FDA (21 CFR 173.325) as a secondary direct food additive permitted in food for human consumption. ASC is used as an antimicrobial agent in accordance with current industry standards of good manufacturing practices in the processing of red meat, red meat parts, and organs as a component of a spray or a dip. Applied as a dip or spray, ASC is used at levels that result in sodium chlorite ($NaClO_2$) concentrations between 500 and 1,200 ppm in combination with any GRAS acid such as citric acid, phosphoric acid or hydrochloric acid at levels sufficient to achieve a solution pH of 2.5 to 2.9 (FDA, 2001b). ASC is often confused with chlorine dioxide (ClO_2), also approved by the FDA (21 CFR 173.300) as a secondary direct food

additive largely because solutions ASC can generate small quantities of chlorine dioxide under certain conditions.

Hajmeer et al. (2004) evaluated the effectiveness of spray application of 0.1% (v/v) acidified sodium chlorite against *E. coli* O157:H7 and *S. aureus* inoculated onto beef briskets. Treatments were applied for 10-60 s at 419 kPa. The results showed that ASC significantly reduced *E. coli* O157:H7 and resulted in improved removal with increased exposure time. ASC reduced *S. aureus* counts throughout exposure. The authors concluded that the spray duration of ASC was critical in its effectiveness as an antimicrobial agent, with 30 s, the optimal time for reducing *E. coli* O157:H7 and *S. aureus* counts (Hajmeer et al., 2004). Kemp et al. (2000) investigated the antimicrobial effects of acidified sodium chlorite on broiler carcasses. ASC treatment was shown to be an effective method for significantly reducing naturally occurring microbial contamination on carcasses. Reductions following immersion dipping were demonstrated at all disinfectant concentrations (500, 850, and 1,200 ppm) for total aerobes (82.9 to 90.7%), total coliforms (86.1 to 98.5%), and *Escherichia coli* (99.4 to 99.6%) (Kemp et al. 2000). Effects of postchill dip application of acidified sodium chlorite (the amount between 600 and 800 ppm) to control *Campylobacter* spp. and *E. coli* on commercial broiler carcasses was studied by Oyarzabal et al. (2004). The treatment demonstrated a significant reduction in microbial counts. The authors concluded that postchill systems may eventually be used in different applications, such as spray, mist, or bath, which could be applied closer to the final stages in processing (Oyarzabal et al., 2004). Bosilevac et al. (2004) evaluated acidified sodium chlorite spray at 300 and 600 ppm applied at a rate of 1.0 and 1.3 oz/lb, respectively, to determine its efficacy for reducing bacterial contamination on boneless beef trimmings used for production of raw ground beef products. The results indicated that ASC reduced contamination

and lengthened the shelf life of ground beef. Moreover, the 300 ppm ASC treatment reduced bacterial counts while maintaining desirable organoleptic ground beef qualities (Bosilevac et al., 2004). Beverly and Janes (2005) investigated acidified sodium chlorite as a possible method for the control of *L. monocytogenes* on the surface of various RTE meat products such as ham, turkey, and roast beef. After samples were inoculated on the surface and allowed to air dry, they were sprayed with ASC solutions at 500, 750, and 1,000 ppm, vacuum packaged, refrigerated at 4°C and then bacterial counts were examined at 0, 14, and 28 days. Results showed that on day 28, there were no significant differences in the reduction of *L. monocytogenes* counts between the different concentrations of ASC treated samples with the ham samples having 1.92 log cfu/g reductions, turkey 2.0 log cfu/g reductions, and roast beef 4.91 log cfu/g reductions as compared to the controls (non-treated samples) (Beverly and Janes, 2005 and Janes and Beverly, 2005).

Salmonella: *Salmonella* is a gram-negative, non-spore-forming, rod-shaped, facultative anaerobe, motile bacterium belonging to the *Enterobacteriaceae* family. This bacterium is named after the scientist who discovered it in 1885, Dr. Daniel Salmon, an American veterinary scientist. *Salmonella* can be widely distributed in environmental soil, water, kitchen and factory surfaces, animal feces, raw meats, raw poultry, and raw sea foods (U.S. Food and Drug Administration, 1992a). It has been known to cause illness for over 100 years (Centers for Disease Control and Prevention, 2005a).

Salmonella enterica are involved in causing diseases of the intestines. The three main serotypes of *S. enterica* are Typhi, Typhimurium, and Enteritidis. *S.* Typhi is the causative agent of typhoid fever but not widespread in the United States. Typhoid fever causes a serious, often fatal disease. The symptoms of typhoid fever include nausea, vomiting, fever and death. *S.* Typhi can only infect humans, and no other host has been identified. The main source of *S.* Typhi

infection is from swallowing infected water. Foods, however, may also be contaminated with *S.* Typhi if they are washed or irrigated with contaminated water (Centers for Disease Control and Prevention, 2005b).

S. Typhimurium and *S.* Enteritidis are the most common cause of food poisoning in the U.S. They cause an illness called salmonellosis. It is estimated that 40,000 cases are reported and confirmed yearly and approximately 600 deaths occur because of acute salmonellosis (Centers for Disease Control and Prevention, 2005a). However, many milder cases are never diagnosed nor reported, the actual incidence may be undoubtedly much higher. Salmonellosis is more common in the warmer months of the year (Centers for Disease Control and Prevention, 2005a).

The majority of salmonellosis outbreaks are caused by consuming raw or undercooked eggs, raw or unpasteurized milk and raw meat or poultry. After *Salmonella* is ingested it passes through the stomach to the intestine and binds to the wall of the small intestine. There is evidence that an enterotoxin may be produced, perhaps within the enterocyte (U.S. Food and Drug Administration, 1992a). The acute symptoms of salmonellosis include the sudden onset of nausea, vomiting, abdominal cramping, diarrhea, headache, and fever. The onset of symptoms normally occurs within 6 to 48 hours after the infection. The infectious dose is as few as 15 to 20 cells (U.S. Food and Drug Administration, 1992a). These symptoms usually persist for 1 to 2 days or may be prolonged depending on age and health of host, ingested dose, and strain characteristics. *Salmonella* infections can also develop a severe complication in a small number of patients. Reiter's syndrome, which includes and is sometimes referred to as "reactive arthritis", has been reported to occur generally after 3 weeks of infection. It is a disorder that causes at least two of three seemingly unrelated symptoms including conjunctivitis, an inflammation of the mucous membrane that covers the eyeball, urethritis, a urinary tract

inflammation, and reactive arthritis. Reactive arthritis generally occurs rapidly with joints becoming hot and swollen, large effusions or collections of fluid can develop in the knee joint (Barth and Segal,1999).

Salmonella infections can be diagnosed by isolation of the bacterium from the stools of an infected person. The diagnosis, however, requires 5 days for results with conventional methods. Several rapid methods are now available to test for the presence of *Salmonella* which require only 2 days. Treatment with antibiotics, such as ampicillin, gentamicin, trimethoprim/sulfamethoxazole, or ciprofloxacin, is used when the infection spreads from the intestines into the bloodstream (Centers for Disease Control and Prevention, 2005a). Symptomatic treatment of Reiter's syndrome is accomplished with high doses of a potent non-steroidal anti-inflammatory drug, such as indomethacin (Barth and Segal, 1999).

In 1985, there was the largest outbreak of foodborne salmonellosis in the U.S. involving 16,000 confirmed cases in 6 states, caused by low fat and whole milk from one Chicago dairy. During 1985-1990, *S. enteritidis* outbreaks continued to occur in the U.S. The Centers for Disease Control (CDC) estimates that 75% of those outbreaks were associated with the consumption of raw or inadequately cooked Grade A whole shell eggs (U.S. Food And Drug Administration, 1992a). There have also been several salmonellosis outbreaks in recent years involving meat and meat products. In 1995, there was an outbreak of salmonellosis first associated with beef jerky in New Mexico, which sickened 93 people. *S.* Typhimurium, *S.* Motevideo, and *S.* Kentucky were isolated in this single outbreak (Centers for Disease Control and Prevention, 1995b). Another outbreak of *S.* Typhimurium involving raw ground beef occurred in Wisconsin in 1994, which resulted in 17 illnesses (Centers for Disease Control and Prevention, 1995c).

To prevent salmonellosis, people should cook poultry, ground beef, and eggs thoroughly before eating. Persons also should not consume raw or unpasteurized milk or other dairy products. Cross-contamination of foods should be avoided. Produce, cooked foods, and ready-to-eat foods should be kept separate from uncooked meat. Hands, kitchen work surface, knives, and other utensils should be washed with soap and water immediately after handling uncooked foods (Centers for Disease Control and Prevention, 2005a).

***Escherichia coli* O157:H7:** *Escherichia coli* O157:H7 is one of the hundreds of strains of the bacterium *Escherichia coli*, which belongs to *Enterobacteriaceae* family referred to as the enteric bacteria. *E. coli* is a Gram-negative, rod-shaped bacterium propelled by long, rapidly rotating flagella. Normally *E. coli* serves a useful function in the intestinal tract by suppressing the growth of pathogenic bacteria and by synthesizing appreciable amounts of vitamins (U.S. Food and Drug Administration, 1992b). Although the majority of *E. coli* strains are harmless and normal inhabitants of the intestines of all animals and humans, *E. coli* O157:H7 strains produce powerful toxins and cause severe illness.

E. coli O157:H7 was first isolated in 1975 from a woman having gross bloody diarrhea (Padhye and Doyle, 1992). It was first identified as a human pathogen in 1982 during an outbreak of bloody diarrhea; the outbreak was traced to contaminated hamburgers. An estimated 73,000 cases of *E. coli* O157:H7 infection and 61 deaths occur every year in the U.S. (Centers for Disease Control and Prevention, 2005c).

E. coli O157:H7 can live in the gastrointestinal tract of such healthy ruminants as cattle (Elder et al., 2000), deer (Keene, 1997), sheep (Kudva et al., 1996), and goats (Shukla et al., 1995). However, the animals are only the reservoir for the bacteria. The majority of foodborne illness outbreaks associated with *E. coli* O157:H7 have involved undercooked or raw

hamburger (ground beef); however, the outbreaks have also involved contaminated alfalfa sprouts, lettuce, cheese curds, unpasteurized fruit juices (Cody et al., 1999), unpasteurized milk, dry-cured salami, and drinking or swimming in sewage-contaminated water (Friedman et al., 1999). Meat can become contaminated during the slaughtering process and *E. coli* O157:H7 can be eventually mixed into the meat when it is ground into hamburger. Contaminated meat looks and smells normal. Although the number of organisms required to cause an infection is not known, it is suspected to be very small.

After oral consumption, *E. coli* O157:H7 travels through the stomach and small intestine and then attaches to the inside surface of the large intestine. It produces large quantities of one or more related verotoxins or shiga-like toxins that cause damage to the lining of the intestine. Hemorrhagic colitis (HC) is the symptom characterized by the sudden onset of severe cramping and abdominal pain and diarrhea within 24 hours. The diarrhea is initially watery but becomes grossly bloody. Vomiting can also occur but fever is either low-grade or absent. The illness typically lasts 3 to 9 days with an average duration of 8 days. In some patients, particularly children under 5 years of age and the elderly, the infection can also cause a severe, life-threatening complication called hemolytic uremic syndrome (HUS). In patients with HUS, symptoms include the destruction of red blood cells, lack of urine formation (anurina), swelling and kidney failure (Buchanan and Doyle, 1997). Although most people recover from an *E. coli* O157:H7 infection, about 2-7% of infections lead to this complication. In the U.S., HUS is the principal cause of acute renal failure in children, and most cases of HUS are caused by *E. coli* O157:H7 (Centers for Disease Control and Prevention, 2005b). In adults, thrombotic thrombocytopenic purpura (TTP), a clinical syndrome defined by the presence of thrombocytopenia (low blood platelet counts) and microangiopathic hemolytic anemia, can

develop along with neurological and renal abnormalities and fever. This involves the central nervous system and patients may develop blood clots in the brain (Meng et al., 2001). TTP is also characterized by purplish or brownish red discoloration, easily visible through the epidermis, caused by hemorrhages in the tissue (U.S. Food and Drug Administration, 1992a).

Infections with *E. coli* O157:H7 can be diagnosed by isolation of the bacterium from diarrheal stools and being tested with sorbitol MacConkey (SMAC) agar containing d-sorbitol instead of lactose as the carbon source for the presence of *E. coli* O157:H7. SMAC can distinguish generic *E. coli* from *E. coli* O157:H7. *E. coli* O157:H7 and most other hemorrhagic *E. coli* strains can not utilize the d-sorbitol so colonies will remain colorless rather than appear bright pink which are characteristic of non-hemorrhagic strains (Feng, 1995). Confirmation can be obtained by isolation of *E. coli* O157:H7 from the incriminated food involving direct plating on hemorrhagic colitis agar (U.S. Food and Drug Administration, 1992b). Most individuals recover without antibiotics or other specific treatment within two weeks. Treatment with certain antibiotics does not improve the course of disease, and may increase the risk of kidney complications. Some antibiotics can cause the release of shiga-like toxin from injured bacteria in the intestine, making the toxin more available for absorption (Wong et al., 2000). Hemolytic uremic syndrome is usually treated in an intensive care unit. Blood transfusions and kidney dialysis are often required (Centers for Disease Control and Prevention, 2005c).

In 1982, the initial outbreaks of *E. coli* O157:H7 were associated with eating undercooked hamburgers at restaurants of the same fast-food chain. Stool isolates containing this bacterium have been identified by the CDC from specimens obtained from four patients in Oregon and Michigan and was reported in the Morbidity and Mortality Weekly Report (MMWR) (Centers for Disease Control and Prevention, 1997). Since this report, there has been

many other *E. coli* O157:H7 outbreaks linked to ground beef including a large outbreak in the Western United States in 1993 (Centers for Disease Control and Prevention, 1993) and an outbreak at a summer camp in Virginia in 1994 (Centers for Disease Control and Prevention, 1995a).

E. coli O157:H7 infection can be prevented by cooking ground beef and hamburger thoroughly. Ground beef can turn brown before disease-causing bacteria are killed, so it is recommended that a digital instant read meat thermometer be used to ensure thorough cooking. Ground beef should be cooked until a thermometer inserted into several parts of the patty, including the thickest part, reads at least 160ºF. Furthermore, the infection can be prevented by avoid spreading harmful bacteria in the kitchen; keep raw meat separately from ready-to-eat foods; wash hands, counters, and utensils with hot soapy water after they touch raw meat (Centers for Disease Control and Prevention, 2005c).

Listeria monocytogenes: *Listeria monocytogenes* is a facultative anaerobic, non-spore-forming, psychrotrophic, motile by means of flagella, non-fastidious, rod shaped, intracellular gram-positive bacterium. *Listeria* can contaminate foods during any part of the process of food production because *L. monocytogenes* is ubiquitous in the environment (Cossart and Bierne, 2001). It can be found in soil, water, vegetables, fish and shellfish, birds, and wild and domestic animals. Since *Listeria* is found in soil and vegetation, it is easily contracted and transmitted by herd animals. It is found in grazing areas, stale water supplies, and poorly prepared animal feed.

L. monocytogenes causes an illness called listeriosis. It is acquired by the ingestion of contaminated foods. *L. monocytogenes* has been associated with foods including raw milk, pasteurized milk, cheeses, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, and raw meats (all types) (U.S. Food and Drug Administration, 1992c).

Listeria is able to survive and multiply at refrigeration temperatures even though its optimal temperature range is 30-37°C and can tolerate low pH and high NaCl concentrations. As a result, *Listeria* may be transmitted in ready-to-eat (RTE) foods that have been kept at refrigeration temperature. On the basis of the characteristics of this microorganism and the severity of reported cases of listeriosis, the FDA and USDA/FSIS have specified a "zero tolerance" for *L. monocytogenes* in RTE foods (Ryser and Marth, 1999).

Symptoms of listeriosis can be similar to those of influenza, including persistent fever and muscle aches with occasional gastrointestinal illness such as nausea, vomiting and diarrhea. If infection spreads to the nervous system, symptoms such as headache, stiff neck, loss of balance, confusion, decreased consciousness or convulsions can occur (Centers for Disease Control and Prevention, 2005d). The onset time to serious forms of disease may range from a few days to three weeks with gastrointestinal symptoms appearing after more than 12 hours. Most healthy people are not at increased risk for developing listeriosis but there are some people who are considered at high risk because they are more susceptible to listeriosis. These high risk groups include pregnant women and their unborn babies, newborns, elderly people and people with weakened immune systems caused by cancer treatments, AIDS, diabetes, and kidney disease. Pregnant women are 20 times more likely than other healthy adults to get listeriosis; about one-third of listeriosis cases happen during pregnancy. Listeriosis can be transmitted to the fetus through the placenta. This can lead to premature delivery, miscarriage, stillbirth, or serious health problems for the newborn (U.S. Food and Drug Administration, 2001a). The infectious dose is unknown but believed to be as few as 1,000 cells of *Listeria* bacteria to cause an illness. Listeriosis can only be positively diagnosed by culturing the organism from blood, cerebrospinal fluid, or stool. Antibiotics such as ampicillin and penicillin are given to treat listeriosis.

The first documented outbreak of listeriosis happened in Canada in 1981 which was due to coleslaw and involved 34 perinatal cases and seven adults (Francis et al., 1999). The annual incidence of listeriosis in the U.S. has been estimated to be 1,850 cases resulting in 425 deaths. (U.S. Food and Drug Administration, 2001b). In 2002, there was a multi-state outbreak of *L. monocytogenes* infections with 46 culture-confirmed cases, seven deaths, and three stillbirths or miscarriages in eight states linked to eating sliceable turkey deli meat (Centers for Disease Control and Prevention, 2002). The general guidelines recommended for the prevention of listeriosis include: thoroughly cook raw food from animal sources; keep uncooked meats separate from cooked and ready-to-eat foods and from vegetables; avoid consuming unpasteurized milk or foods made from unpasteurized milk; wash raw vegetables thoroughly before eating; consume perishable and ready-to-eat foods as soon as possible; and wash hands, knives, and cutting boards after handling uncooked foods (Centers for Disease Control and Prevention, 2005d).

Physical properties: Intrinsic properties in food such as water activity, moisture content, pH, and oxidation-reduction potential are important factors in food quality and safety issues. Some of these properties and their roles in predicting the survival of microorganisms in a food product will be described.

-*Water Activity*, (a_w) represents the ratio of the water vapor pressure of the food (p) to the water vapor pressure of pure water (p_o) at a given temperature. Multiplication of water activity by 100 gives the percent equilibrium relative humidity (ERH) of the atmosphere in equilibrium with the food (Fontana, 1998). It is expressed as a fraction, $a_w = p/p_o = \%ERH/100$. The water activity of a food describes the energy state or escaping tendency of water in the food, and hence its availability to act as a solvent and participate in chemical and biochemical reactions and growth

of microorganisms (Fontana, 2000a). Water activity is used to predict food safety and stability with respect to microbial growth, chemical/ biochemical reaction rates, and physical properties. By measuring and controlling the water activity of foodstuffs, it is possible to predict which microorganisms will potentially contribute to spoilage and infection. Microorganisms have a limiting water activity level below which they will not grow. Water activity determines the lower limit of available water for microbial growth. The lowest a_w levels at which the majority of food spoilage bacteria will grow is about 0.90 (Fontana, 1998). The water activity of raw meat is around 0.98 which means meat has a high level of water available for microbial growth. Meat products with a water activity of 0.85 or less are considered shelf stable (21 CFR 110.8). Since water activity and moisture content of a product are not related, it is not practical to assume that beef jerky is shelf stable based on such sensory evaluation as touch, sight, and taste. The only way to know the water activity of a product is through testing with a calibrated water activity meter.

Water activity is dependent on temperature. Although most high water activity foods (0.75 – 1.0) have negligible change with temperature, some products have increased water activity with increasing temperature and vice versa (Fontana, 2000a). Measuring the water activity of foodstuff can use either chilled-mirror dew point technology or relative humidity with sensors detecting changes in electrical resistance or capacitance. Both methods vary in accuracy, repeatability, stability in calibration, speed of measurement, and convenience of use (Fontana, 2000b).

-*Moisture Content* is one of the most commonly measured properties of foodstuffs. The moisture content of a food material is defined by the given equation, % moisture = (mass of the water/mass of the sample) × 100. Therefore, the moisture content of a food can be

determined accurately by measuring the mass of water molecules present in a known mass of sample. However, it is not possible to directly measure the number of water molecules present in a sample because of the huge number of molecules involved.

A number of analytical techniques used to measure moisture are based on the fact that the water in a food can be distinguished from the other components. The basic principle of this technique is that water has a lower boiling point than the other major components within foods, *e.g.,* carbohydrates, proteins, lipids, and minerals. Examples of techniques developed to measure the moisture content of foods include evaporation methods such as convection and forced oven draft, vacuum oven, microwave oven, and infrared drying. The moisture content is determined by measuring the mass of a food sample before and after the water is removed by evaporation;

$$\% \text{ Moisture} = \frac{\text{(wt. of initial sample - wt. of dried sample)}}{\text{wt. of initial sample}} \times 100$$

-*pH* is a method to express differences in acidity or alkalinity of a solution. The pH is a measure of the molar concentration of hydrogen ions $[H^+]$ in the solution. Pure water dissociates to give equal numbers of hydrogen and hydroxyl $[OH^-]$ ions. At 25°C the concentration of $[H^+]$ and $[OH^-]$ in solution is 10^{-7} mol/l. The pH is defined as the negative logarithm to base 10 of hydrogen ion activity or concentration ($pH = -\log [H^+]$). Acids are substances defined as hydrogen ion donors and bases are defined as hydrogen ion acceptors. When acids are added to water they produce large amounts of hydrogen ions. In contrast, bases produce large amounts of hydroxyl ions, when they are added (Warriss, 2000). The pH of meat can be measured directly by placing the glass pH electrode onto the surface of meat samples. However, there is a consideration of contaminating the sensitive glass with fat.

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CHAPTER 2

ANTIMICROBIAL INTERVENION AND PROCESS VALIDATION IN BEEF JERKY PROCESSING

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ABSTRACT

The objectives of this paper were to conduct beef jerky processing validation studies using chemical pretreatments, marination, and drying procedures to determine the effectiveness of antimicrobial intervention on whole-muscle beef jerky strips inoculated with *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes.* Jerky strips were exposed to either an acidic calcium sulfate (1:3 and 1:2 calcium sulfate:water ratios) or acidified sodium chlorite (500 and 1,200 ppm) pretreatment, stored overnight in a marinade containing water, salt, sugar, vinegar, sodium erythrobate, Worchestershire sauce, monosodium glutamate, garlic powder, thyme, and sodium nitrite and either dried in a horizontal-flow dehydrator or a commercial-type smokehouse. Acidic calcium sulfate was the most effective in reducing the populations of *Salmonella* regardless of both concentration while the lower concentrations of acidified sodium chlorite were least effective. For *E. coli* O157:H7 and *L. monocytogenes*, there was no significant difference ($p>0.05$) in the effectiveness of the chemical pretreatments on reducing the populations. However, the results showed the higher concentrations of acidic calcium sulfate were the most effective and the lower concentrations of acidified sodium chlorite were the least effective. Statistical analysis indicated that the populations of *Salmonella* were reduced ($p<0.05$) by more than 6.5 logs cfu/strip on jerky that was pretreated with the 1:2 acidic calcium sulfate:water ratios and dried in a dehydrator and jerky pretreated with the 1,200 ppm concentration of acidified sodium chlorite and dried in a smokehouse. Populations were reduced ($p=0.0557$) also on jerky pretreated with both concentrations of acidic calcium sulfate and dried in the smokehouse. For *E. coli* O157:H7, at least 5 log population reductions were noted for all the treatments except for jerky pretreated with the 500 ppm concentration of acidified sodium chlorite and dried in the dehydrator. The populations of *L. monocytogenes* were reduced by

5 logs for all treatments and both drying methods. The results from this study support the importance of the antimicrobial effect of a chemical pretreatment coupled with using a dehydrator or a smokehouse in jerky products on the pathogens used in this experiment.

INTRODUCTION

Jerky, one of the oldest of meat products, has existed since ancient Egyptian time. Humans made jerky from animal meat as a way to preserve the meat before it spoiled. It has been produced by salt preserving, smoking, and drying by the sun to extend the shelf life of meat. Today beef jerky is a common dried meat product in North America which is widely available. It is produced from either ground and formed meat or strips of meat and by both the industry and people drying meat at home. There are numerous recipes available for producing jerky based on different types of meat, preparation procedures, different marinade formulations and drying processes.

Salmonella, Escherichia coli O157:H7, and *Listeria monocytogenes* contamination of jerky products are of concern to the industry and regulators (Centers for Disease Control and Prevention, 1995, Keen et al., 1997, and Eidsen et al., 2000). A report by the Food Safety and Inspection service of the U.S. Department of Agriculture (FSIS/USDA) also indicated that for the period from 1990 to 1999, cumulative prevalence of *Salmonella* and *L. monocytogenes* in jerky produced in the U.S. inspected plants was 0.31% and 0.52%, respectively (Levine et al., 2001).

In 2004, FSIS issued and revised the Compliance Guidelines for Meat and Poultry Jerky Produced by Small and Very Small Plants to provide updated food microbiological safety information to jerky processors. Small and very small jerky processors can develop customized lethality processes that achieve an appropriate reduction of pathogens throughout the product. Customized processes must be validated (9 CFR 417.4). A number of researchers have studied a variety of methods to reduce numbers of pathogenic bacteria on beef jerky products to improve microbiological safety. The pathogen population reduction is influenced by marination, drying

temperature, and whether or not the meat receives additional treatments. Antimicrobial intervention is an option to increase the level of pathogen reduction on beef jerky greater than that achieved by heating and drying alone. Non-thermal pre-drying treatment will be used to determine the effectiveness of these pretreatments to increase the reduction of pathogen population on beef jerky. In recent years, an acidic calcium sulfate (ACS) with a pH of ≤ 2.0 has been used as a food additive to reduce or eliminate microbial contaminations (Kemp et al., 2003, Mionix, 2003). When mixed with organic acids, ACS reduces the pH sufficiently to maintain more organic acid in its undissociated form. This form of the acid has the greatest antimicrobial activity (Zhao, 2004). ACS ingredients are affirmed as General-Recognized-as-Safe (GRAS) under the FDA (21CFR 184.1230) (FDA, 2003). Acidified sodium chlorite (ASC) is another antimicrobial intervention treatment for food processing products. It is approved by the FDA (21 CFR 173.325) as a secondary direct food additive permitted in food for human consumption. ASC is used as an antimicrobial agent in accordance with current industry standards of good manufacturing practice in the processing of red meat products as a component of a spray or a dip. It is used at levels that result in sodium chlorite ($NaClO_2$) concentrations between 500 and 1,200 ppm in combination with any GRAS acids at levels sufficient to achieve a solution pH of 2.5 to 2.9 (FDA, 2001). For these reasons, ACS and ASC may have potential as effective antimicrobial against pathogens when applied to surface of beef jerky. Furthermore, the validation of the overall beef jerky processing methods to reduce pathogen population in the product in a simulated processing environment should be carried out.

The objectives of this research were to conduct beef jerky processing validation studies evaluating the use of chemical pretreatments (acidic calcium sulfate and acidified sodium chlorite) and drying procedures using either a dehydrator or smokehouse to evaluate the

effectiveness of antimicrobial intervention on whole-muscle beef jerky inoculated with *Salmonella, E. coli* O157:H7, and *L. monocytogenes.*

MATERIALS AND METHODS

Using whole strip beef jerky, experiments examined the effect of two drying methods (drying with a horizontal dehydrator and with a commercial-type smokehouse) and antimicrobial pretreatments (acidic calcium sulfate and acidified sodium chlorite pretreatments) on three different organisms (*Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*). The trials were conducted separately with three replications on three different days.

Bacterial strains and inoculum preparation. *Salmonella* (*S*. Typhimurium 654, *S*. Typhimurium DT 104 H3380, *S*. Typhimurium DT 104 H3402, *S*. California and *S*. Enteritidis) were obtained from USDA/ARS, Athens, GA. *E. coli* O157:H7 932 (clinical), E009 (beef), 204 P (pork), E0019 (cattle feces), and 380-94 (salami) and *L. monocytogenes* Brie (cheese isolate), Scott A (clinical isolate), LCDC #81-861 (coleslaw outbreak isolate), V7 (milk isolate) and 301 (cheddar cheese isolate) were obtained from the Center for Food Safety, The University of Georgia, Griffin, GA. All strains were preserved on Microbank™ beads (Pro-Lab Diagnostic, Austin, TX) frozen at -80°C. Each strain was activated in 9 ml portions of tryptic soy broth (TSB; Difco Labs, Division of Becton Dickinson and Co., Sparks, MD) statically at 32 ± 2°C for 18-20 h. Each culture was centrifuged for 20 min at 7,000 x g and the pellet re-suspended in 10 ml 0.1% peptone water (Difco Labs). The five strains for each bacterial pathogen were combined just prior to inoculation to make a bacterial cocktail for the 3 pathogens used in this study. Each strip was inoculated with 1 of the 3 pathogen cocktails by inoculating 500 µl of it on 1 side of the strip.

Inoculation was done in a laminar air flow hood starting about 2.0-2.5 cm from each end of the beef strip. Samples were stored at 4 ± 2°C for 24 h to allow for microbial adhesion to the beef. One ml of each combination was placed in 9 ml tubes containing 0.1% peptone and serially diluted to determine initial inoculum levels for each bacterial combination.

Beef strip preparation. Vacuum-packaged beef was purchased fresh from a local meat distributor and stored at -18°C. The frozen beef was sliced into approximately 0.48 x 2.5 x 30 cm size strips and then placed at 4 ± 2°C overnight (12 ± 2 h) to slightly thaw. The strips were placed in sterilized stainless steel pans prior to inoculation.

Antimicrobial solutions preparation. Different pre-drying antimicrobial washing treatments were evaluated. These treatments included exposing beef strips to two concentrations of aqueous acidified sodium chlorite (500 and 1,200 mg/L) and two concentrations of acidic calcium sulfate with lactic acid solution (1:3 and 1:2 water). Acidified sodium chlorite solution was freshly prepared from a 3.35 % stock solution of sodium chlorite (Keeper™, Bio-Cide International Inc., Norman, OK) mixed with citric acid anhydrous (Mallinckrodt Baker Inc., Phillipsburg, NJ) and clean, potable water. The final pH value of the solution was 2.7-2.9. The acidified sodium chlorite concentration (mg/L) in the aqueous solution was determined using a Keeper® test kit (Bio-Cide International Inc.). Acidic calcium sulfate (ACS) with lactic acid ($Safe_2O$™ RTE-01), supplied by Mionix Corp., Rocklin, CA. was diluted with tap water. The final pH value of the solution was 1.4-1.7 (Accumet® Model AB15 pH meter, Fisher Scientific, Suwanee, GA).

Marinade ingredients. Pretreated strips were marinated by applying the marinade ingredients to the strips. The marinade consisted of 106 g salt, 34.2 g sugar, 107 ml vinegar, 8.8 ml Worchestershire sauce, 8.8 g sodium erythrobate, 4.28 g monosodium glutamate, 3.42 g

garlic powder, 4.28 g thyme, and 0.86 g sodium nitrite / 2850 g of meat. Marinated strips were placed in sterilized stainless steel pans, covered with lids and stored at 4 ± 2°C for 21 ± 3 h before drying.

Drying. A horizontal air flow food dehydrator (model # 3936T, Excalibur® Products, Sacramento, CA) and a commercial style smokehouse (model #450, Alkar-Rapidpak, Inc., Lodi, WI) were used to dehydrate marinated strips. The dehydrator and shelves with empty mesh were preheated to 62 ± 2°C (air) for at least 15 min and then loaded with the strips. During drying, the circulating air temperature within the dehydrator and internal temperature of a strip were monitored and recorded continuously by a data recorder (model RD106 A, Omega®, Stamford, CT) equipped with copper/constantan thermocouples (5TC-TT-T, Omega®). The conditions of the smoke house were 33% R.H., dry-bulb temperature 63°C (145°F) and wet-bulb temperature 43°C (110°F).

Experiment procedure. Six beef strips of meat were placed in stainless steel pans. Three pans were prepared with 6 strips in each pan for the 3 treatments (control and two concentration levels). Each beef strip was inoculated with 500 µl of each bacterial combination and stored at 4 ± 2°C for 22 ± 2 h. The different concentrations of chemical solutions and the water control were freshly prepared and poured in the sterilized stainless steel pan in order for the strips to be dipped into the solution. This was done by placing 6 strips (1 pretreatment) of beef on hardware cloth and immersing the strips into the antimicrobial solution for 30 s. One strip was immediately sampled after immersing for enumeration of surviving organisms. Following the treatments, each pan of strips had 1,000 ml of marinade added and stored at 4 ± 2°C for 21 ± 3 h. Treated and marinated strips were dried in the horizontal dehydrator or the commercial smokehouse for

8-9 h. After drying, the strips were placed into a Cryovac® bag, vacuum packed, and stored in an incubator at 25 ± 2°C and sampled at 1, 2, and 3 mos.

Microbiological analysis and enrichment. Samples were taken and analyzed after inoculation, after each antimicrobial pre-drying treatment, after marination, after drying, and after storage at 25 ± 2°C over 3 mos. For experiments requiring bacterial enumeration, sampling was done by placing a strip in a sterile bag with 225 ± 5 ml of nutrient broth (NB; Difco Labs), modified EC broth (mEC; Difco Labs), and *Listeria* enrichment broth base (UVM; Oxoid; Basingstoke, Hampshire, England) for *Salmonella, E. coli* O157:H7 and *L. monocytogenes* enumeration, respectively. Each sample was pummeled in a stomacher (TekMar model 400, Cincinnati, OH) for 2 min on high speed. Serial dilutions were made by using 0.1% peptone buffer. Portions were spiral plated (Autoplate® 4000; Spiral Biotech, Norwood, MA) on plate count agar (PCA; Difco Labs) for total aerobic bacteria enumeration and on bismuth sulfite agar (BSA; Difco Labs), sorbitol MacConkey agar (SMAC; Oxoid), and modified Oxford agar (MOX; Oxoid) for *Salmonella, E. coli* O157:H7, and *L. monocytogenes* enumeration, respectively. The plates were incubated at 37 ± 2°C for 22 ± 2 h before colony forming units were counted. The plates were then re-incubated and re-examined after an additional 24 h incubation. All counts were converted to log cfu/cm^2.

Subcultures of all pummeled samples were enriched in the event that the populations of the three pathogens were reduced to levels not detectable (4.0×10^1) by direct plating. All three enrichment broths were incubated at 37 ± 2°C for 22 ± 2 h. After incubation, portions of the mEC cultures were streaked onto SMAC plates. Plates were incubated at 37 ± 2°C for 18-24 h and examined for the presence of representative colonies.

Subcultures were also made from the nutrient broth into Rappaport-Vassiliadis R10 broth (Difco Labs) and TT broth Hajna (Difco Labs) and from UVM *Listeria* enrichment broth into Fraser broth (Difco Labs) and then incubated at 42 ± 2°C for 22-24 h and 37 ± 2°C for 26 ± 2 h, respectively. After incubation of the broths, portions were streaked onto BSA, XLD (Difco Labs) and XLT4 (Difco Labs) for possible *Salmonella* isolates and onto MOX for possible *L. monocytogenes* isolates. Plates were incubated at 35 ± 2°C for 22 ± 2 h and examined for the presence of representative colonies.

The identification of representative, presumptive isolates from the enrichment steps above were tested. Presumptive *Salmonella* and *E. coli* O157:H7 isolates were randomly identified using the Micro-ID® identification system for *Enterobacteriaceae* (Remel, Lenexa, KS) as per manufacturer's instructions. *Salmonella* and *E. coli* O157:H7 isolates were confirmed using the appropriate latex agglutination assays (Oxoid). *Listeria* isolates were randomly identified using the Micro-ID® *Listeria* system (Remel) as per manufacturer's instructions.

Physical analysis. pH and water activity (a_w) of beef jerky strips were determined before and after antimicrobial treatments, drying, and storage. pH was measured by the surface method using a surface electrode and a Accumet® Model AB15 pH meter. Water activity (a_w) was measured at 25 ± 2°C with a model CX2 Aqua Lab water activity meter (Decagon Devices, Inc., Pullman, WA).

Statistical analysis. The average bacterial plate counts of three separate replications were transformed to logarithmic counts and analyzed at each sampling time for significant differences ($p<0.05$). The minimum detection level was 40 cfu/strip. To facilitate the statistical analysis of these data, samples with bacterial counts below the minimum detection were assigned a value of 40 cfu/strip. Data was evaluated by using a 2x4x3x3x7x2 factorial design (drying

methods x chemical treatments x different pathogens x replications x sampling times x agar media). Analysis of variance of main effects (organism, pretreatment, marination, drying methods and agar media), as well as all the interactions were conducted using the PROC GLM procedure in the Statistical Analysis System (SAS, 2001). To compare the 6 pretreatments with each other for a fixed time difference, one-way Analysis of variance (ANOVA) was performed for testing the null hypothesis that the mean of $\log_{10}$ of the response variable for the 6 pretreatments was the same vs. the alternative that there was some difference.

RESULTS

Table 2.1 shows the bacterial populations on beef jerky processing pretreated with acidic calcium sulfate with lactic acid (ACS) (Mionix $Safe_2O^{TM}$RTE-01) and processed in the horizontal dehydrator. The trials were conducted separately with three organisms, three replications on three different days for each experiment. Treatment of the inoculated strips with the ACS-1:2 water and the ACS-1:3 water significantly reduced ($p<0.05$) the log populations of *Salmonella* by 1.98 and 1.61, respectively when enumerated on a nonselective medium (PCA) and by 2.22 and 1.69, respectively when enumerated on a selective medium (BSA), with the higher concentration of ACS yielding the greatest reduction even though it was not significantly different. The water control treatment did not reduce the population.

E. coli O157:H7 populations with the ACS-1:2 water and ACS-1:3 treatments were reduced ($p>0.05$) by 1.06 and 0.77 logs when enumerated on PCA, and by 1.23 and 0.97, when enumerated on selective SMAC. The ACS-pretreatments (ACS 1:2 and 1:3) reduced populations of *L. monocytogenes* by 0.5 and 0.29 logs when enumerated on PCA, and by 0.82 and 0.63, when enumerated on selective MOX, but they were not significantly different ($p>0.05$). As with

Salmonella, use of the greater concentration of ACS reduced the populations of *E. coli* O157:H7 and *L. monocytogenes* to a greater degree than did the lower concentration even though it was not significantly different ($p>0.05$). The average pH of the strips (5.81) dropped slightly to 4.58 and 4.74 after the ACS pretreatment at the concentration of 1:2 and 1:3 water, respectively (Table 2.2).

In all cases, the samples were plated onto both plate count agar and selective agar for each pathogen. The selective agar yielded lower counts compared to the less restrictive plate count agar. The remaining results below are from selective for each pathogen.

After drying with the dehydrator, the actual population reduction of the ACS-1:2 pretreated *Salmonella* was significantly reduced by 7.18 logs ($p<0.05$), and the reduction surpassed the desired 6.5 logs. Population reductions of 6.72 and 4.91 logs were achieved for the ACS-1:3 treated and the water treated strips, respectively, but the desired 6.5 log reduction was not significantly achieved ($p>0.05$). The populations of *E. coli* O157:H7 and *L. monocytogenes* were reduced by the ACS pretreatments to levels below the detection limit (1.6 log cfu/strip) for the enumeration of pathogens by at least 6.68 and 6.88 logs, respectively. On strips treated with water, *E. coli* O157:H7 populations were reduced by at least 6.88 logs and *L. monocytogenes* populations by 6.36 logs. Therefore, the population reduction of the *E. coli* O157:H7 and *L. monocytogenes* significantly achieved ($p<0.05$) the desired 5.0 logs on jerky pretreated with both concentrations of acidic calcium sulfate (Mionix) and the control treatment on the strips dried with the dehydrator.

Stored beef jerky was sampled monthly over a 3 month period. All samples treated with the ACS-1:2 water were negative for all three pathogens after enrichment for months 1, 2 and 3 sampling times. A positive *Salmonella* sample was detected from one replication treated with the

ACS-1:3 mix after 1 month but no samples receiving this treatment were positive after 2 or 3 months storage. Neither *E. coli* O157:H7 nor *L. monocytogenes* was enriched from samples treated with the ACS-1:3 water after 1, 2 or 3 months of storage. In comparison, some strips were positive for *Salmonella* from all or some of the replications for strips pretreated with water at all monthly sampling times. *E. coli* O157:H7 samples treated with water were positive at 1 month but negative after 2 and 3 months of storage. No *L. monocytogenes* positive samples were detected by enumeration or after enrichment for any of the monthly sampling times. Thus, the ACS pretreatment was more effective in eliminating the pathogens than water alone. There was no noticeable change in either the product pH or a_w during storage. The relative humidity in the dehydrator at the start of the drying process for the ACS experiments with *Salmonella, E. coli* O157:H7 and *L. monocytogenes* was 55.4%, 72.5 %, 61.1%, respectively.

Table 2.3 shows the populations during the beef jerky processing with the ACS pretreatments (Mionix $Safe_2O^{TM}$RTE-01) using a commercial smokehouse for the drying process rather than a dehydrator. The trials were conducted separately with three organisms, three replications on three different days for each experiment. The results of the effectiveness of the ACS pretreatments in population reduction of the 3 pathogens were similar to the results using the dehydrator for the drying process. The average pH of the strips (5.52) decreased to 4.39 and 4.63 after the ACS pretreatment at the concentration of 1:2 and 1:3 water, respectively (Table 2.2).

After the smokehouse drying process, the populations of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* were reduced by the ACS 1:2, ACS 1:3, and water pretreatments to level below the detection limit (1.6 log) resulting in at least a 6.83, 6.71, and 6.92 log reduction, respectively. Statistical analysis showed the population reduction of *Salmonella, E. coli*

O157:H7, and *L. monocytogenes* significantly achieved ($p<0.05$) the desired 6.5 log reduction for *Salmonella* and 5.0 log reduction for *E. coli* O157:H7 and *L. monocytogenes*, after pretreatment with either concentration of ACS and the control water treatment and then dried in the smokehouse.

Even though the processed strips treated with water were below the detection limit of 1.6 log, the strips were positive for all 3 pathogens after enrichment. All the dried ACS treated samples were negative after enrichment. The conclusion is that the ACS pretreatments are more effective in eliminating pathogens than water alone. Jerky dried in the smokehouse was stored and sampled monthly for 3 months like the strips dried in the dehydrator. During storage, only one positive *Salmonella* sample was detected from one replication treated with the water after 1 month. All other samples were negative for all 3 pathogens after 1, 2 and 3 months storage. These results indicate that the commercial smokehouse is more effective in eliminating the pathogens than the dehydrator.

There was also no noticeable change in either the pH or a_w during storage. The relative humidity in the smokehouse was constant at 33 ± 2 % with a dry-bulb temperature of 63°C (145°F) and a wet-bulb temperature of 43°C (110°F).

Tables 2.4 and 2.5 show the bacterial populations on beef jerky pretreated with acidified sodium chlorite (ASC) (Keeper™) and processed in the horizontal dehydrator and on ASC pretreated jerky dried in the commercial smokehouse. For each experiment, enumerations of all three pathogens were determined and the trials were conducted separately with three replications on three different days. The results showed that there was little difference in the effectiveness of the 3 pretreatments of ASC. After the pretreatments 1,200 ppm and 500 ppm ASC, the *Salmonella* populations were decreased at most by 0.67 log and 0.33 log, respectively.

As with *Salmonella,* the ASC pretreatments decreased *E. coli* O157:H7 and *L. monocytogenes* populations to levels similar to the control water pretreated samples prior to the dehydrator and smokehouse drying. The pH of the strips was not changed by the ASC pretreatments (Table 2.6).

Pretreatment with 1,200 ppm and 500 ppm concentrations of ASC and water pretreatment decreased the *Salmonella* population by 5.34, 4.94, and 4.75 logs, respectively, at the end of the drying process in a dehydrator and by 6.97, 6.71 and 5.78 logs respectively, at the end of the smokehouse drying process. Statistical results indicated that the population of *Salmonella* was reduced by less than 6.5 logs (p>0.05) for the two concentrations of ASC pretreatments with the dehydrator. The population of *Salmonella* was significantly reduced by more than 6.5 logs (p<0.05) for the 1,200 ppm ASC pretreatment but was not significantly reduced (p>0.05) for the 500 ppm ASC with smokehouse.

After drying the strips in the dehydrator, the log reduction of *E. coli* O157:H7 populations for the 1,200 ppm-ASC, 500 ppm-ASC pretreated, and water pretreated samples were 6.73, 5.68, and 6.63, respectively. After drying in the smokehouse, the reduction of *E. coli* O157:H7 populations from all pretreated samples was at least 6.89 logs (under the detection limit of 1.6). The log reduction of the *E. coli* O157:H7 populations significantly achieved (p<0.05) the desired 5.0 log reduction for all the treatments except for strips treated with 500 ppm concentration of ASC and dried in the dehydrator.

Cultural enrichment of the samples did recover the pathogen from samples for most of the replications. Positive *E. coli* O157:H7 samples were detected from some of the replications that received the 500 ppm-ASC and water pretreatment followed by the dehydrator drying process during 1 and 2 months storage but no positives were found after 3 months. With the

smokehouse drying process, there were no samples that were positive for month 1, 2 or 3 sampling times, when enriched.

The ASC and water pretreatments with the dehydrator drying process reduced the *L. monocytogenes* populations by at least 6.55 logs. The reduction in the *L. monocytogenes* populations of all pretreated samples with the smokehouse drying process was reduced to levels below the detection limit (at least 6.89 log reductions) but positive samples were detected by enrichment. The log reduction of the *L. monocytogenes* populations significantly achieved ($p<0.05$) the desired 5.0 log reduction for all the treatments regardless of the drying method. After 2 and 3 months, there were no positive samples for *L. monocytogenes* after samples were enriched. There was no noticeable change in pH or a_w values in the samples during storage. The relative humidity in the dehydrator at the start of drying process for the ASC experiments with *Salmonella, E. coli* O157:H7 and *L. monocytogenes* was 57.0%, 80.3%, 59.3%, respectively.

DISCUSSION

The results of this study indicated that *Salmonella* was more easily inactivated than *E. coli* O157:H7 and *L. monocytogenes* when whole-muscle strips were pretreated with acidic calcium sulfate (Mionix Safe$_2$O™RTE-01). Both concentrations of the acidic calcium sulfate used were effective in reducing the populations of *Salmonella* with the greater concentration being more effective. There was no statistical difference in the effectiveness of the acidic calcium sulfate in contributing to the reduction of *E. coli* O157:H7 and *L. monocytogenes*, although it is possible also to rank them with the greater concentration being more effective. Acidified sodium chlorite (Keeper™) had little antimicrobial effect on the pathogens even at the highest concentration (1,200 ppm) approved by the FDA (21 CFR 173.325) (FDA, 2001).

Heating and drying strips in the commercial smokehouse was more effective in reducing pathogen populations than with the horizontal dehydrator. The commercial smokehouse was constantly controlled with internal conditions at 43°C (110°F) wet-bulb temperature, 63°C (145°F) dry-bulb temperature and 33% relative humidity. The horizontal dehydrator was set to an internal temperature of 62.7°C (145°F) and the initial relative humidity varied by the actual relative humidity in the processing room. Figure 2.1 shows the average internal temperature of beef strips and % relative humidity in the horizontal dehydrator and in the commercial smokehouse. The temperature of beef strips reached 62.7°C at 5-6 h. The temperature profile for both the dehydrator and the smokehouse were the same. The relative humidity in the smokehouse was a constant 33% but in the dehydrator the relative humidity decreased to a level below 33% after 1 h and less than 10% after 5 h. The final relative humidity after drying was around 6%. The higher humidity held in the smokehouse compared to the dehydrator caused greater effectiveness of the smokehouse in killing the microorganisms studied in this project. Another explanation for the difference seen between the smokehouse and the dehydrator is that within the dehydrator, evaporating moisture absorbs most of the heat. The meat does not begin to rise in temperature until most of the moisture has evaporated. When the dried meat temperature starts to increase, the organisms have become more heat resistant and are more likely to survive (USDA-FSIS, 2004). Therefore, humidity has been identified as an important processing factor in jerky processing (Harrison et al., 2006). While the humidity can be controlled and measured in a smokehouse, the humidity in a dehydrator can not be easily controlled and is greatly affected by the relative humidity in the surrounding atmosphere. The average relative humidity in the dehydrator at the start of the drying process was 64.3 %.

Reduction in *E. coli* O157:H7 and *L. monocytogenes* of at least 5.0 log cfu can be significantly achieved ($p<0.05$) in the production of whole muscle beef jerky by using the acidic calcium sulfate pretreatments at the concentrations of Mionix 1:2 and 1:3 water with the drying process by horizontal dehydrator or commercial smokehouse. Furthermore, the reduction in *Salmonella* of at least 6.5 log cfu can be achieved in the jerky production by using the acidic calcium sulfate pretreatments at the high concentration of Mionix (1:2 water) with the dehydrator ($p<0.05$) or by using the acidified sodium chlorite pretreatments at the high concentrations of Keeper™ (1,200 ppm) with the smokehouse ($p<0.05$). Therefore, while processing jerky in a smokehouse was more effective, effective treatments may be attained using a dehydrator coupled with an antimicrobial pretreatment. In general, the acidic calcium sulfate was a more effective antimicrobial pretreatment than acidified sodium chlorite.

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Table 2.1. Survival of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* (log cfu/strip) on marinated, whole strip beef jerky pretreated with Mionix Safe$_2$O™ RTE-01 (acidic calcium sulfate with lactic acid) or not pretreated and dried in a horizontal dehydrator at 62°C (143.6°F). Enumeration was on plate count (PCA) and selective media (bismuth sulfite agar (BSA), sorbitol MacConkey agar (SMAC) and modified Oxford agar (MOX) for each pathogen type, respectively).

Organisms / media	1 : 2 water				1 : 3 water				Control			
	Inoculated	After pretreated[a]	After marinated[b]	After drying[c]	Inoculated	After pretreated	After marinated	After drying	Inoculated	After pretreated	After marinated	After drying
Salmonella PCA	8.79	6.81	6.23	<1.6[d]	8.79	7.18	6.93	2.89	8.79	8.49	7.69	4.29
Salmonella BSA	8.78	6.56	6.15	1.6	8.78	7.09	6.85	2.06	8.78	8.43	7.62	3.87
E. coli O157:H7 PCA	8.39	7.33	7.24	<1.6[d]	8.39	7.62	7.41	<1.6[d]	8.39	8.36	7.57	<1.6[d]
E. coli O157:H7 SMAC	8.28	7.05	7.02	<1.6[d]	8.28	7.31	7.22	<1.6[d]	8.28	8.16	7.50	<1.6[d]
L monocytogenes PCA	8.54	8.04	7.57	<1.6[d]	8.54	8.25	7.93	<1.6[d]	8.54	8.38	7.86	2.31
L monocytogenes MOX	8.48	7.66	7.27	<1.6[d]	8.48	7.85	7.74	<1.6[d]	8.48	8.25	7.77	2.12

[a] Pretreatment: Dipped in 1 part Mionix with 2 water, 1 part Mionix with 3 water, and control water for 30 sec

[b] Marinade composed of: water, salt, sugar, vinegar, Woecestershire sauce, sodium erythrobate, MSG, thyme, garlic powder, and sodium nitrite

[c] Drying time approximate 8-9 h and drying temperature at 62°C (143.6°F); no humidity control

[d] Enumeration on plating below detection level (4.0 x 10^1 cfu/strip); positive identification of organisms after enrichment

Table 2.2. Average a_w and pH values of marinated beef jerky that were pretreated with Mionix $Safe_2O^{TM}$ RTE-01 (acidic calcium sulfate) or not pretreated and dried in a horizontal dehydrator at 62°C (143.6°F) and in a commercial smokehouse.

Processing step / Drying method		1 : 2 water		1 : 3 water		Control (water)	
		a_w	pH	a_w	pH	a_w	pH
Before pretrerated / dehydrator	1[a]	0.992	5.68	0.992	5.68	0.992	5.68
	2[b]	0.995	5.85	0.995	5.85	0.995	5.68
	3[c]	0.994	5.90	0.994	5.90	0.994	5.90
Before pretrerated / smokehouse		0.990	5.52	0.990	5.52	0.990	5.52
After pretrerated / dehydrator	1[a]	0.994	4.55	0.995	4.74	0.994	5.72
	2[b]	0.992	4.56	0.993	4.73	0.993	5.85
	3[c]	0.994	4.63	0.994	4.76	0.995	5.94
After pretrerated / smokehouse		0.990	4.39	0.991	4.63	0.993	5.52
After marinated / dehydrator	1[a]	0.982	4.55	0.982	4.70	0.982	5.30
	2[b]	0.984	4.80	0.983	4.83	0.983	5.25
	3[c]	0.983	4.63	0.984	4.78	0.983	5.09
After marinated / smokehouse		0.979	4.45	0.982	4.55	0.981	5.03
After drying / dehydrator	1[a]	0.683	4.54	0.690	4.74	0.697	4.83
	2[b]	0.680	4.61	0.683	4.68	0.681	4.90
	3[c]	0.678	4.58	0.684	4.73	0.676	5.12
After drying / smokehouse		0.677	4.58	0.697	4.81	0.667	5.08

[a] results from *Salmonella* experiment

[b] results from *E.coli* O157:H7 experiment

[c] results from *Listeria monocytogenes* experiment

Table 2.3. Survival of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* (log cfu/strip) on marinated, whole strip beef jerky pretreated with Mionix $Safe_2O^{TM}$ RTE-01 (acidic calcium sulfate with lactic acid) or not pretreated and dried in a commercial-type smokehouse. Enumeration was on plate count (PCA) and selective media (bismuth sulfite agar (BSA), sorbitol MacConkey agar (SMAC) and modified Oxford agar (MOX) for each pathogen type, respectively).

Organisms / media	1 : 2 water				1 : 3 water				Control			
	Inoculated	After pretreated[a]	After marinated[b]	After drying[c]	Inoculated	After pretreated	After marinated	After drying	Inoculated	After pretreated	After marinated	After drying
Salmonella PCA	8.50	6.71	6.26	<1.6	8.50	7.02	6.46	<1.6	8.50	8.48	7.21	<1.6[d]
Salmonella BSA	8.43	6.67	6.32	<1.6	8.43	6.79	6.51	<1.6	8.43	8.48	7.10	<1.6[d]
E. coli O157:H7 PCA	8.36	7.01	6.37	<1.6	8.36	7.14	6.87	<1.6	8.36	8.25	7.25	<1.6[d]
E. coli O157:H7 SMAC	8.31	6.62	6.14	<1.6	8.31	6.75	6.61	<1.6	8.31	8.20	7.11	<1.6[d]
L monocytogenes PCA	8.55	7.44	6.81	<1.6	8.55	7.58	7.23	<1.6[d]	8.55	8.47	7.28	<1.6[d]
L monocytogenes MOX	8.52	7.16	6.52	<1.6	8.52	7.19	6.98	<1.6[d]	8.52	8.44	7.18	<1.6[d]

[a] Pretreatment: Dipped in 1 part Mionix with 2 water, 1 part Mionix with 3 water, and control water for 30 sec
[b] Marinade composed of: water, salt, sugar, vinegar, Woecestershire sauce, sodium erythrobate, MSG, thyme, garlic powder, and sodium nitrite
[c] Drying time approximate 8-9 h and the conditions of smokehouse: 33% relative humidity, dry-bulb temperature 63°C (145°F) and wet-bulb temperature 43°C (110°F)
[d] Enumeration on plating below detection level (4.0×10^1 cfu/strip); positive identification of organisms after enrichment

Table 2.4. Survival of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* (log cfu/strip) on marinated, whole strip beef jerky pretreated with Keeper™ (acidified sodium chlorite) or not pretreated and dried in a horizontal dehydrator at 62°C (143.6°F). Enumeration was on plate count (PCA) and selective media such as bismuth sulfite agar (BSA), sorbitol MacConkey agar (SMAC) and modified Oxford agar (MOX), respectively.

Organisms / media	1,200 ppm				500 ppm				Control			
	Inoculated	After pretreated[a]	After marinated[b]	After drying[c]	Inoculated	After pretreated	After marinated	After drying	Inoculated	After pretreated	After marinated	After drying
Salmonella PCA	8.28	7.77	7.35	3.46	8.28	8.14	7.35	3.61	8.28	8.18	7.40	3.83
Salmonella BSA	8.29	7.68	7.28	2.95	8.29	8.17	7.33	3.35	8.29	8.27	7.37	3.54
E. coli O157:H7 PCA	8.57	7.97	7.24	3.38	8.57	8.43	7.75	3.44	8.57	8.29	7.49	3.56
E. coli O157:H7 SMAC	8.49	7.86	7.17	1.76	8.49	8.36	7.65	2.81	8.49	8.26	7.33	1.86
L monocytogenes PCA	8.28	8.17	7.56	2.93	8.28	8.25	7.79	2.82	8.28	8.39	7.83	3.03
L monocytogenes MOX	8.15	8.03	7.40	<1.6[d]	8.15	8.11	7.57	1.6	8.15	8.31	7.69	<1.6[d]

[a] Pretreatment: Dipped in 1,200 ppm, 500 ppm of $NaClO_2$ and water control for 30 sec

[b] Marinade composed of: water, salt, sugar, vinegar, Woecestershire sauce, sodium erythrobate, MSG, thyme, garlic powder, and sodium nitrite

[c] Drying time approximate 8-9 h and drying temperature at 62°C (143.6°F); no humidity control

[d] Enumeration on plating below detection level (4.0×10^1 cfu/strip); positive identification of organisms after enrichment

Table 2.5. Survival of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* (log cfu/strip) on marinated, whole strip beef jerky pretreated with Keeper™ (acidified sodium chlorite) or not pretreated and dried in a commercial-type smokehouse. Enumeration was on plate count (PCA) and selective media such as bismuth sulfite agar (BSA), sorbitol MacConkey agar (SMAC) and modified Oxford agar (MOX), respectively.

Organisms / media	1,200 ppm				500 ppm				Control			
	Inoculated	After pretreated[a]	After marinated[b]	After drying[c]	Inoculated	After pretreated	After marinated	After drying	Inoculated	After pretreated	After marinated	After drying
Salmonella PCA	8.54	7.77	7.10	<1.6[d]	8.54	8.17	7.31	2.23	8.54	8.38	7.37	2.98
Salmonella BSA	8.57	7.90	6.94	<1.6[d]	8.57	8.24	7.13	1.86	8.57	8.38	7.24	2.79
E. coli O157:H7 PCA	8.53	7.73	7.05	<1.6[d]	8.53	8.12	7.15	<1.6[d]	8.53	8.19	7.10	<1.6[d]
E. coli O157:H7 SMAC	8.49	7.59	6.87	<1.6[d]	8.49	8.02	7.01	<1.6[d]	8.49	8.23	6.95	<1.6[d]
L monocytogenes PCA	8.52	8.21	6.94	<1.6[d]	8.52	8.32	7.31	<1.6[d]	8.52	8.50	7.15	<1.6[d]
L monocytogenes MOX	8.49	7.79	6.83	<1.6[d]	8.49	8.27	7.19	<1.6[d]	8.49	8.40	7.31	<1.6[d]

[a] Pretreatment: Dipped in 1,200 ppm, 500 ppm of $NaClO_2$ and water control for 30 sec

[b] Marinade composed of: water, salt, sugar, vinegar, Woecestershire sauce, sodium erythrobate, MSG, thyme, garlic powder, and sodium nitrite

[c] Drying time approximate 8-9 h and the conditions of smokehouse: 33% relative humidity, dry-bulb temperature 63°C (145°F) and wet-bulb temperature 43°C (110°F)

[d] Enumeration on plating below detection level (4.0×10^1 cfu/strip); positive identification of organisms after enrichment

Table 2.6. Average a_w and pH values of marinated beef jerky that were pretreated with Keeper™ (acidified sodium chlorite dioxide) or not pretreated and dried in horizontal dehydrator at 62°C (143.6°F) and in a commercial smokehouse.

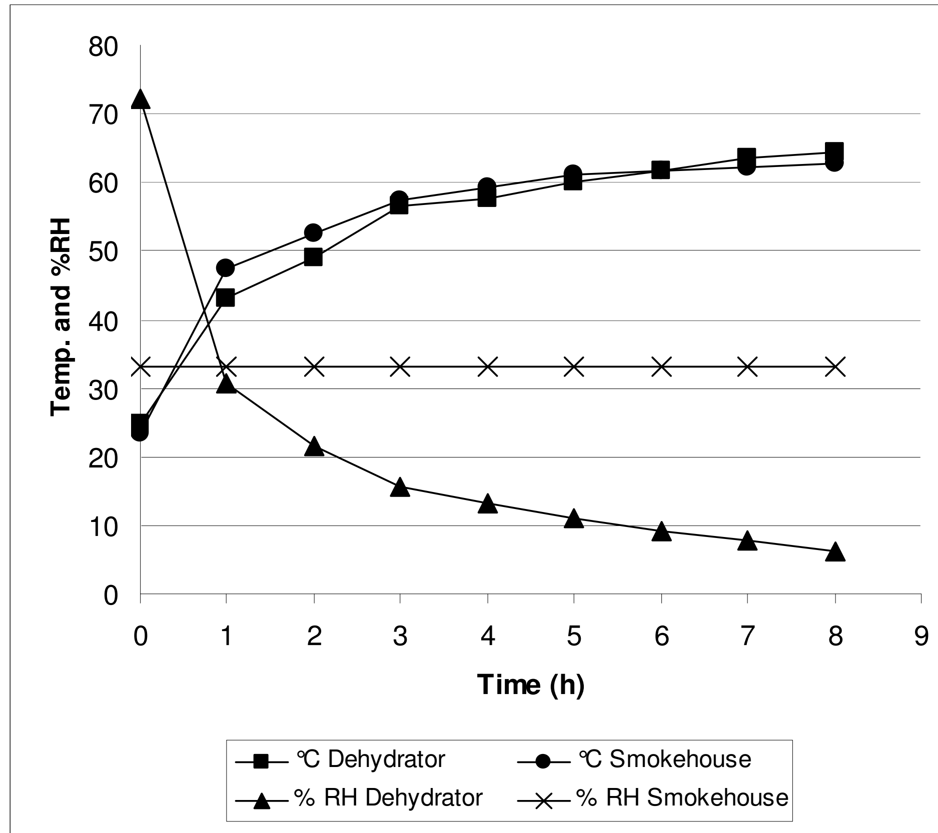
Processing step / Drying method		1,200 ppm		500 ppm		Control (water)	
		a_w	pH	a_w	pH	a_w	pH
Before pretrerated / dehydrator	1[a]	0.997	5.92	0.997	5.92	0.997	5.92
	2[b]	0.993	5.89	0.993	5.89	0.993	5.89
	3[c]	0.994	5.84	0.994	5.84	0.994	5.84
Before pretrerated / smokehouse		0.995	5.94	0.995	5.94	0.995	5.94
After pretrerated / dehydrator	1[a]	0.995	5.83	0.995	5.81	0.994	5.90
	2[b]	0.993	5.53	0.995	5.57	0.995	5.85
	3[c]	0.996	5.60	0.994	5.74	0.995	5.78
After pretrerated / smokehouse		0.997	5.76	0.997	5.96	0.998	5.76
After marinated / dehydrator	1[a]	0.984	5.08	0.982	5.26	0.983	5.19
	2[b]	0.984	5.06	0.984	5.19	0.983	4.98
	3[c]	0.983	5.05	0.984	5.05	0.983	5.01
After marinated / smokehouse		0.985	5.07	0.984	5.08	0.985	5.05
After drying / dehydrator	1[a]	0.685	5.11	0.681	5.03	0.687	5.36
	2[b]	0.690	5.05	0.683	5.07	0.706	4.99
	3[c]	0.695	5.02	0.674	5.11	0.689	5.11
After drying / smokehouse		0.661	5.04	0.665	5.13	0.675	5.09

[a] results from *Salmonella* experiment

[b] results from *E.coli* O157:H7 experiment

[c] results from *Listeria monocytogenes* experiment

Figure 2.1. Average internal temperature (°C) of beef jerky strips and % relative humidity in a horizontal dehydrator at 62°C (143.6°F) and in a commercial smokehouse with dry bulb/wet bulb temperatures of 63°C (145°F) and 43°C (110°F).



CHAPTER 3

CONCLUSIONS

Beef jerky, solely on heating and drying in the dehydrator and the smokehouse, may not be as safe as previously thought. Experimental data has shown that an antimicrobial intervention step, in which the meat exposes in acidic calcium sulfate or acidified sodium chlorite, is safer from food pathogens. The chemical pretreatments offer the opportunity to increase the level of pathogen reduction greater than that achieved by heating and drying alone. The data from these experiments showed that the relative humidity is an important processing factor in the drying process. The higher humidity held in the smokehouse compared to the dehydrator caused greater effectiveness of the smokehouse in killing the pathogens.

APPENDICES

Appendix A. T-statistic and p-value of the testing log population reduction from the time after inoculation to the time after drying meeting USDA regulation [a].

Dehydrator				K0[b]	K1[c]	K2[d]	M0[e]	M1[f]	M2[g]
				Pretreatment					
PCA	E[h]	5	t	0.03	0.40	0.68	39.15	39.15	39.15
			p	0.4906	0.3642	0.2833	0.0004	0.0004	0.0004
	L	5	t	0.31	0.69	45.95	3.47	50.33	50.33
			p	0.3924	0.2801	0.0003	0.0358	0.0002	0.0002
	S	6.5	t	-15.73	-14.87	-7.50	-1.86	-0.30	11.67
			p	1.0000	0.9999	0.9983	0.7974	0.2057	0.0037
Selective agar	E	5	t	6.09	0.73	10.1	40.17	40.17	40.17
			p	0.0019	0.2692	0.0003	0.0003	0.0003	0.0003
	L	5	t	20.58	20.58	20.58	22.89	44.38	44.38
			p	0.0012	0.0012	0.0012	<0.0001	0.0003	0.0003
	S	6.5	t	-7.71	-4.51	-5.87	-1.14	0.66	10.66
			p	0.9985	0.9893	0.9958	0.6296	0.2736	0.0044

Smokehouse				K0	K1	K2	M0	M1	M2
				Pretreatment					
PCA	E	5	t	25.71	25.71	25.71	16.04	16.04	16.04
			p	0.0008	0.0008	0.0008	0.0020	0.0020	0.0020
	L	5	t	13.55	13.55	13.55	20.02	20.02	20.02
			p	0.0027	0.0027	0.0027	0.0013	0.0013	0.0013
	S	6.5	t	-2.65	-0.59	6.637	4.08	4.08	4.08
			p	0.9430	0.4117	0.0109	0.0276	0.0276	0.0276
Selective agar	E	5	t	43.53	43.53	43.53	17.96	17.96	17.96
			p	0.0003	0.0003	0.0003	0.0016	0.0016	0.0016
	L	5	t	15.5	15.5	15.5	19.44	19.44	19.44
			p	0.0021	0.0021	0.0021	0.0013	0.0013	0.0013
	S	6.5	t	-1.84	1.37	7.77	2.74	2.74	2.74
			p	0.7982	0.1219	0.0081	0.0557	0.0557	0.0557

[a] At least a 5-log reduction for *E. coli* O157:H7 and *L. monocytogenes* and at least a 6.5-log reduction for *Salmonella*

[b] water control for Keeper® treatment

[c] 500 ppm of acidified sodium chlorite (Keeper®) treatment

[d] 1200 ppm of acidified sodium chlorite (Keeper®) treatment

[e] water control for Mionix; Safe$_2$O™ RTE-01 treatment

[f] 1:3 acidic calcium sulfate:water (Mionix; Safe$_2$O™ RTE-01) treatment

[g] 1:2 acidic calcium sulfate:water (Mionix; Safe$_2$O™ RTE-01) treatment

[h] E : *E. coli* O157:H7, L : *L. monocytogenes,* S : *Salmonella*

Appendix B. Survival of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* (log cfu/strip) during storage time, whole strip beef jerky pretreated with Mionix Safe$_2$O™RTE-01 (acidic calcium sulfate with lactic acid) or not pretreated and dried in a horizontal dehydrator at 62°C (143.6°F). Enumeration was on plate count (PCA) and selective media (bismuth sulfite agar (BSA), sorbitol MacConkey agar (SMAC) and modified Oxford agar (MOX) for each pathogen type, respectively).

Processing step/treatment (trt)	*Salmonella*		*E. coli* O157:H7		*L. monocytogenes*		Meat pH	Meat a_w
	PCA	BSA	PCA	SMAC	PCA	MOX		
Pretrt A[a] after 1 m storage[d]	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.89	0.686
Pretrt B[b] after 1 m storage	<1.6[d]	<1.6[d]	<1.6	<1.6	<1.6	<1.6	5.04	0.687
Control pretrt[c] after 1 m storage	<1.6[d]	<1.6[d]	<1.6[d]	<1.6[d]	<1.6	<1.6	5.42	0.675
Pretrt A after 2 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.75	0.679
Pretrt B after 2 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.90	0.685
Control pretreatment after 2 m storage	<1.6[d]	<1.6[d]	<1.6	<1.6	<1.6	<1.6	5.27	0.676
Pretrt A after 3 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.87	0.683
Pretrt B after 3 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.98	0.687
Control pretreatment after 3 m storage	<1.6[d]	<1.6[d]	<1.6	<1.6	<1.6	<1.6	5.33	0.689

[a] Pretreatment A: 1 part Mionix concentrate with 2 parts water, dipped for 30 sec
[b] Pretreatment B: 1 part Mionix concentrate with 3 parts water, dipped for 30 sec
[c] Control pretreatment: Dipped in tap water for 30 sec
[d] Enumeration on plating below detection level (4.0 x 10^1 cfu/strip); positive identification of organisms after enrichment
[e] Product was stored at 25°C and sampled for pathogen by enumeration plating and enrichment

Appendix C. Survival of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* (log cfu/strip) during storage time, whole strip beef jerky pretreated with Mionix Safe$_2$O™RTE-01 (acidic calcium sulfate with lactic acid) or not pretreated and dried in a commercial smokehouse. Enumeration was on plate count (PCA) and selective media (bismuth sulfite agar (BSA), sorbitol MacConkey agar (SMAC) and modified Oxford agar (MOX) for each pathogen type, respectively).

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Processing step/treatment (trt)	*Salmonella*		*E. coli* O157:H7		*L. monocytogenes*		Meat pH	Meat a_w
	PCA	BSA	PCA	SMAC	PCA	MOX		
Pretrt A[a] after 1 m storage[d]	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.62	0.695
Pretrt B[b] after 1 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.83	0.677
Control pretrt[c] after 1 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.88	0.647
Pretrt A after 2 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.72	0.647
Pretrt B after 2 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.75	0.657
Control pretreatment after 2 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.94	0.657
Pretrt A after 3 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.89	0.676
Pretrt B after 3 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	4.75	0.652
Control pretreatment after 3 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.03	0.657

[a] Pretreatment A: 1 part Mionix concentrate with 2 parts water, dipped for 30 sec
[b] Pretreatment B: 1 part Mionix concentrate with 3 parts water, dipped for 30 sec
[c] Control pretreatment: Dipped in tap water for 30 sec
[d] Enumeration on plating below detection level (4.0×10^1 cfu/strip); positive identification of organisms after enrichment
[e] Product was stored at 25°C and sampled for pathogen by enumeration plating and enrichment

Appendix D. Survival of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* (log cfu/strip) during storage time, whole strip beef jerky pretreated with Keeper™ (Acidified sodium chlorite) or not pretreated and dried in a horizontal dehydrator at 62°C (143.6°F). Enumeration was on plate count (PCA) and selective media (bismuth sulfite agar (BSA), sorbitol MacConkey agar (SMAC) and modified Oxford agar (MOX) for each pathogen type, respectively).

Processing step/treatment (trt)	*Salmonella*		*E. coli* O157:H7		*L. monocytogenes*		Meat pH	Meat a_w
	PCA	BSA	PCA	SMAC	PCA	MOX		
Pretrt A[a] after 1 m storage[d]	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.25	0.695
Pretrt B[b] after 1 m storage	<1.6	<1.6	<1.6[d]	<1.6[d]	<1.6	<1.6	5.29	0.682
Control pretrt[c] after 1 m storage	<1.6	<1.6	<1.6[d]	<1.6[d]	<1.6	<1.6	5.46	0.690
Pretrt A after 2 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.22	0.690
Pretrt B after 2 m storage	<1.6	<1.6	<1.6[d]	<1.6[d]	<1.6	<1.6	5.18	0.679
Control pretreatment after 2 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.22	0.696
Pretrt A after 3 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.17	0.693
Pretrt B after 3 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.11	0.688
Control pretreatment after 3 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.14	0.710

[a] Pretreatment A: 1,200 ppm $NaClO_2$, dipped for 30 sec

[b] Pretreatment B: 500 ppm $NaClO_2$, dipped for 30 sec

[c] Control pretreatment: Dipped in tap water for 30 sec

[d] Enumeration on plating below detection level (4.0×10^1 cfu/strip); positive identification of organisms after enrichment

[e] Product was stored at 25°C and sampled for pathogen by enumeration plating and enrichment

Appendix E. Survival of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* (log cfu/strip) during storage time, whole strip beef jerky pretreated with Keeper™ (Acidified sodium chlorite) or not pretreated and dried in a commercial smokehouse. Enumeration was on plate count (PCA) and selective media (bismuth sulfite agar (BSA), sorbitol MacConkey agar (SMAC) and modified Oxford agar (MOX) for each pathogen type, respectively).

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Processing step/treatment (trt)	*Salmonella*		*E. coli* O157:H7		*L. monocytogenes*		Meat pH	Meat a_w
	PCA	BSA	PCA	SMAC	PCA	MOX		
Pretrt A[a] after 1 m storage[d]	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.03	0.676
Pretrt B[b] after 1 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.15	0.671
Control pretrt[c] after 1 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.24	0.658
Pretrt A after 2 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.01	0.655
Pretrt B after 2 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.32	0.664
Control pretreatment after 2 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.22	0.661
Pretrt A after 3 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.24	0.651
Pretrt B after 3 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.27	0.642
Control pretreatment after 3 m storage	<1.6	<1.6	<1.6	<1.6	<1.6	<1.6	5.40	0.649

[a] Pretreatment A: 1,200 ppm $NaClO_2$, dipped for 30 sec

[b] Pretreatment B: 500 ppm $NaClO_2$, dipped for 30 sec

[c] Control pretreatment: Dipped in tap water for 30 sec

[d] Product was stored at $25^{o}C$ and sampled for pathogen by enumeration plating and enrichment