EFFECTS OF DEHYDRATION ON BACTERIA LEVELS OF NON-HEAT TREATED, SHELF-STABLE, WHOLE-MUSCLE BEEF JERKY

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Coming to Angelo State University was certainly unexpected, however, I wouldn't change it for the world. I thought that I was ready to attend graduate school when I enrolled into Angelo State University, but I didn't realize I still had much to learn and to grow in maturity. My time here has been occupied with education, as well as a reflection on who I am as a person and as a professional. The decision to attend ASU was sudden; however, it is one that God put in my path as it afforded me endless opportunities.

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ABSTRACT

This study evaluated, aerobic bacteria and pathogen surrogate (Saga 200) levels of non-heat treated, shelf-stable, whole-muscle beef jerky over five processing days. Beef inside rounds (NAMP# 169A), were sliced into identical strips. Strips were assigned to either experiment I (n=72) or Experiment II (n=126/trial) and then assigned to one of three treatments: control, marinate, or dry rub. Experiment II strips were inoculated with Saga 200 prior to treatment application and dehydration. Strips were dehydrated on racks at 50±2°F (relative humidity of 65±5%). Samples were tested every 24 h for bacteria levels, water activity (A_w), and pH. Experiment I, showed an average 1.25 $\log_{10}$ reduction of APC over six days ($P \leq 0.05$). Experiment II showed a 1.5 $\log_{10}$ reduction of Saga 200 over six days ($P \leq 0.05$), and A_w ranged from 0.97-0.68 and pH ranged between 5.84 and 5.48. Results showed that simple dehydration without thermal treatment is insufficient to meet FSIS requirements.

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INTRODUCTION

Jerky has become a great source of convenience protein, as it is a shelf-stable, ready-to-eat (RTE) product (Harrison et al., 2006). In early civilization, jerky was produced by slicing meat into thin strips that were set to dry by the sun, fire, and wind. Jerky is typically in the form of whole muscle; however, ground and formed jerky is commonly available. Jerky is typically produced out of very lean cuts of beef, pork, poultry, or game meat and then marinated in brine or seasoned by a dry rub consisting of salt, pepper, and spices (Nummer et al., 2004). The United States Department of Agriculture, Food Safety and Inspection Services (USDA, FSIS) recommends jerky producers monitor water activity (A_w) as a requirement of safety (FSIS, 2014). Water activity is used to verify reduction of moisture content within the product as a tool to inhibit microbial growth and prolong shelf stability. Shelf stability is achieved at a water activity level of 0.85 or less if steps are taken to prevent mold growth (FSIS, 2014).

Microorganisms, including *Salmonella* species or *Escherichia coli* O157:H7 have been identify as causative sources in outbreaks linked to beef jerky and are considered adulterants in ready-to-eat products (RTE). As a result, USDA-FSIS established compliance guidelines to ensure pathogen lethality using a time-temperature combination. The guidelines require 90% humidity for at least one hour or 25% of the cooking cycle and the product must meet a water activity level of 0.85 or less (FSIS, 2014). The guidelines require processors to achieve a 5.0 log reduction in *Salmonella* during processing. The USDA does not require a regulation regarding *E. coli* O157:H7 lethality, since Salmonella is the most heat resistant once a 5 log reduction is met, lethality for other pathogens is considered met (FSIS, 2014).

Additional anti-microbial interventions approved by FSIS include placing jerky strips in a marinate and heating them until they reached a minimum internal temperature of 71°C, dipping the jerky strips in a 5% acetic solution for a period of 10 minutes before adding the marinate and drying, dipping the strips into a calcium sulfate solution, or acidified sodium chloride solution before drying (Calicioglu et al., 2002, 2003; Harrison et al., 2006; and Harrison and Harrison, 1996). However, some intervention options have demonstrated a potential negative impact on the product on the basis of undesirable texture and flavor (FSIS, 2014).

The FSIS compliance guidelines specify that unique processes capable of reducing 5.0 logs of bacteria can be validated by conducting a challenge study if sufficient scientific documentation is presented (FSIS, 2014). Currently, commercial production incorporates either an in-house plant validation or they must meet the components of the USDA-FSIS compliance guidelines.

In an effort to reinstate old time artesian recipes of non-heat treated, shelf-stable, products to the industry, the objectives of this study were to use a pathogen surrogate, as well as aerobic bacteria normally found on meat, to determine the efficacy of drying in absence of forced heat cooking to achieve a 5.0 log reduction of bacteria.

LITERATURE REVIEW

History of Jerky

Jerky is a unique ready-to-eat product (RTE), and its origin can be traced back to ancient civilizations who employed drying as a method of preservation. Meats were further preserved using sun, wind, and fire. Preserving meat provided a sustainable source of protein as large animals, such as buffalo and bear, could not be consumed all at once (FSIS, 2011). Practices of preservation and drying have been revolutionized over time. The addition of salt and spices, which provides extra flavors and microbial inhibition, have been utilized to expand markets and consumer demand while simultaneously adding value and convenience to lower end cuts of meats.

Due to a series of outbreaks of Salmonellosis linked to beef jerky in New Mexico in 2003, 22,000 pounds of beef jerky was voluntarily recalled (FSIS, 2003). The USDA FSIS integrated a set of compliance guidelines to establish a safe level of water activity and an effective combination of time and temperature levels for sufficient pathogen reduction. The method to achieve these levels of lethality requires maintaining high temperatures and relative humidity prior to drying. The outbreak in New Mexico was found to be due to the high altitude where the production facility was located (Eidson et al., 2000). High altitude allows for fast evaporation resulting in evaporative cooling. Evaporative cooling prevented the jerky strips from reaching lethality temperatures and allowed *Salmonella* to become dry heat resistant (Allen et al., 2007). To prevent evaporative cooling, it is important that jerky is cooked with high humidity to allow temperatures to rise and reach lethality. However, cooking jerky with high humidity can impact sensory properties negatively which can affect consumer appeal (Allen et al., 2007).

Pathogens of Concern

Jerky is a dried, but not fermented, ready-to-eat, shelf-stable product that its safety is challenged given its typical room temperature storage environment (FSIS, 2001). The lists of pathogens of concern for jerky processors created by the FSIS consist of *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Trichinella spiralis*; however, *Trichinella spiralis* is only problematic in porcine and game meats (FSIS, 2001). Since this researched focused on whole muscle beef jerky, *Trichinella* was not a pathogen of concern. The compliance guidelines require that such pathogens be reduced following a validated study that achieves a ≥5.0 log CFU reduction of *Salmonella* as it is the most heat resistant pathogen (FSIS, 2014). Some of the symptoms related to these pathogens are severe vomiting and diarrhea resulting in multiple severe health issues and potentially death if not treated properly. Therefore producers need to meet these regulations to ensure a safe product.

Listeria monocytogenes

Listeria monocytogenes is an anaerobic, non-spore forming, gram-positive bacteria generally associated with unsanitary food manufacturing and storage facilities (FSIS, 2001). It is tolerant to cold temperatures ranging as low as 1 – 45°C, making it a hazard for refrigerated products (Jay et al., 2005b). Furthermore, *Listeria monocytogenes* grows best at pH levels near neutral. However, it can easily adapt, survive and grow at pH levels ranging from 4.1 - 9.6 (Jay et al., 2005b). According to Vermeulen et al. (2007), *Listeria monocytogenes* strains are not able to grow at water activity levels (A_w) of < 0.93.

The disease related to *Listeria monocytogenes* is known as listeriosis. Although there are a low number of cases reported annually, it is one of the foremost causes of foodborne

illness that leads to death (FDA, 2012a). Individuals infected with listeriosis can contract two types of disease. One that is self-limiting and includes symptoms such as vomiting, diarrhea, nausea, and fever, and the other is a more severe illness that can occur when the infection spreads to the nervous system. The most severe version of listeriosis can result in meningitis and potentially death (FDA, 2012a). Mild cases of listeriosis have a very short incubation time and an onset of a few hours, whereas, more severe cases are characterized by an incubation time of three days to three months (FDA, 2012a).

Jerky production is subject to FSIS ready-to-eat regulations. These regulations require the production establishment to have thorough sanitation standard operating procedures (SSOP) as part of their HACCP (Hazard Analysis Critical Control Points) plan, for post-lethality exposed RTE products to prevent contamination with *L. monocytogenes* (FSIS, 2007). The Food Safety Inspection Service labels *L. monocytogenes* as an adulterant in RTE meat products and has a zero tolerance for this pathogen in RTE products (Jay et al., 2005b). Products that contain an adulterant are subject to recalls and cannot be distributed. Food manufacturers have to spend thousands of dollars setting up a recall plan and personnel to deal with customer complaints. Moreover, producers lose millions of dollars when RTE products have to be recalled as they cannot be reworked or reprocessed, as they were a fully cooked product before leaving the production facility.

Salmonella spp.

Salmonella is identified as a gram-negative, non-spore-forming bacterium that is known to cause foodborne infections and is composed of two species; *S. bongori* and *S. enterica* (FDA, 2012b). *Salmonella* foodborne infection, salmonellosis, occurs when an individual consumes food containing live pathogens that will later grow in their intestinal

tract. Of the two species of *Salmonella*, *S. enterica* is the main species of concern. This specie can cause severe illness consisting of vomiting, diarrhea, and possible arthritis symptoms (FSIS, 2012b). *Salmonella enterica* is comprised of six subspecies, *S. enterica* subsp. *indica, houtenae, arizonae, salamae, diarizonae, and enterica* (FDA, 2012b). In addition, these six subspecies consist of over 2,500 serotypes (Coburn et al., 2007). Foodborne illnesses in humans are associated to serovars within the subsp. *enterica*. Serovars *S.* Enteritidis and *S.* Typhimurium are the most commonly reported foodborne infections in humans (FDA, 2012b). *Salmonella* is a pathogen that adapts to its environment very easily, and if given a prolonged amount of time, it is possible that it can adapt and survive in dry meat products (Andino and Hanning, 2015). *Salmonella* can grow in temperatures ranging from 5 to 47°C, and favors an optimum pH around neutral (Jay et al., 2005a).

Jerky consumption has increased in the last century as food trends for many members of society have moved towards convenience food items. Jerky is a convenient ready-to-eat product that is shelf stable and is nutrient dense. The presence of *Salmonella* in jerky can be devastating as the Center for Disease Control and Prevention (CDC) has determined *Salmonella* as the causative pathogen of most foodborne illness in the United States (CDC, 2012).

Staphylococcus aureus

Staphylococcus aureus is a non-spore forming, gram-positive bacterium that is readily found on the skin and in nasal tracts of healthy humans and animals (CDC, 2011). Food products that are heavily handled after the lethality process can have a high risk for *S. aureus*. *Staphylococcus aureus* produces a toxin that can cause gastrointestinal discomfort. It

has a high tolerance to salt and can survive in temperatures from 7 – 47.8°C. Moreover, it can grow at pH levels between 4.5 to 9.3 (FDA, 2012c). *S. aureus* is the most resistant foodborne pathogen to A_w and is able to survive in a dry state for extended periods of time (FDA, 2012c). Although A_w of 0.86 is recognized as the minimum, growth has been observed in A_w as low as 0.83 under ideal conditions (Jay et al., 2005c). These findings elevate a concern for jerky processors that focus on certain texture and quality specifications for their product. FSIS guidelines require jerky to have a A_w below 0.86 to be safe for packaging and human consumption (FSIS, 2014).

Escherichia coli O157:H7

Escherichia coli O157:H7 (*E. coli*) is considered to be an adulterant in foods, beef products in particular. *Escherichia coli* O157:H7 is a gram-negative, zoonotic, pathogenic bacterium commonly found in the gastrointestinal tract of ruminant animals, and can cause extreme illness in humans and even death (Wells et al., 2014). *Escherichia coli* O157:H7 falls under the subgroup classification of enterohemorrhagic *E. coli* (EHEC) (Wells et al., 2014). This particular pathotype is associated with Shiga-toxigenic *E. coli*, which produces shiga toxins. Shiga toxins can cause severe illness in humans such as hemorrhagic colitis, with hemolytic uremic syndrome (HUS) being the most extreme illness (Wells et al., 1991). Approximately 10% of *E. coli* O157:H7 infections will develop to HUS and those infected with HUS may develop permanent renal failure, which may be fatal (Tserenpuntsag et al., 2005). The most outbreaks of *E. coli* O157:H7 related to beef were associated with ground beef, therefore, FSIS recommends consumers to only eat ground beef and ground beef products that have been cooked to 160° F (CDC, 2014; FSIS, 2016). Outbreaks linked to whole muscle beef jerky are rare. However, since cattle are asymptomatic carriers, the

likelihood of a potential outbreak still exists. *E. coli* O157:H7 can become acid resistant and can grow in pH levels from 4.0-8.0, although, it would not grow at a A_w below 0.95 (Fu et al., 2003).

Use of Surrogates

According to FSIS (2002b), a surrogate microorganism can be utilized in validation lethality studies when it has a similar material characteristic to foodborne pathogens. Surrogates are used in circumstances where the risk of using a true pathogen could compromise the safety of the production of such facility. Other options to validate studies also exist. One example is the utilization of an indicator organism or a non-pathogenic organism. An indicator organism works to indicate that the product is or has been exposed to an ideal environment for a product to be contaminated with a pathogen (FSIS, 2002a). However, the utilization of an indicator or a nonpathogenic organism of the same family could result in a false positive when sanitation and product quality are tested to verify HACCP (Scott, 2005). Therefore, it is beneficial to use a good surrogate that is non-pathogenic, has similar characteristics to the target pathogen, behaves similarly when exposed to similar processing environments (pH, temperature, A_w, oxygen, etc), that is stable and is easy to prepare (FSIS, 2002c; Rodriguez et al., 2006). The present study utilized a commercial lactic acid producing bacteria (Saga 200; *Pediococcus acidilictici*; Kerry Inc, Beloit, WI) as a surrogate. Saga 200 is in the list of generally recognized as safe (GRAS) food ingredients, and has been validated in previous jerky research as an ideal surrogate (Borowski et al., 2009; FDA; Buege et al., 2006; Dierskey, 2010; FDA, 2016). The use of a pathogen surrogate such as Saga 200 is a safe way for validating cooking methods for small and very small jerky processors, as it would not result in false positives.

Outbreaks Linked to Jerky

Escherichia coli and *Salmonella* have been found on jerky products, causing recalls in recent years due to zero tolerance of adulterants in ready-to-eat products (FSIS, 2014). Such adulterations have been found to be related to inadequate lethality treatment (FSIS, 2014). Therefore FSIS compliance guidelines requires achieving a 5.0 $\log_{10}$ reduction or greater of *Salmonella* which will also achieve lethality of *Escherichia coli* (FSIS, 2014). The FSIS (2014) Compliance Guidelines for Meat and Poultry Jerky produced by small and very small establishment recommends a time-temperature combination, along with adequate water activity instead of moisture: protein ratio (MPR) to ensure proper lethality. The guidelines also recommend that the process of cooking jerky is done with a 90% relative humidity. This prevents the surface from drying too fast and potentially allowing bacteria to become heat resistant and jerky strips from reaching lethality (FSIS, 2014).

Government Regulations

Jerky is a dry slice of meat that utilizes water activity to maintain shelf stability. According to the FSIS (2014) guidelines, water activity should be <0.85 under aerobic conditions. For that reason, an establishment should verify that sufficient drying is met by measuring water activity instead of moisture protein ration (MPR). Nevertheless, all product needs to meet an MPR of 0.75:1or less in order to be label jerky (FSIS, 2005). Water activity is a better measurement of available water for bacteria growth. The reduction of this available water would improve shelf stability, however, other steps need to be taken into account to prevent mold growth such packaging modifications or adjustment of pH levels (FSIS, 2014).

Texas Cottage Food Laws

The Department of State Health Services (DSHS) is the authority that monitors the Texas Cottage Food production law. Under this law, an individual can operate a food production operation out of their own home kitchen, and products can be sold at the individual's home, farmers market, a county fair, a non-profit fair festival or event, if their annual gross income on such products do not exceed over $50,000.00 on annual sales (DSHS, 2015). An individual is allowed to sale a variety of baked goods, candy, nuts, dehydrated fruit or vegetables, canned jams or jellies, as well as a pickle, etc (DSHS, 2015). On the other hand, dairy products, baked goods that require refrigeration, fresh or dried meats or any meat product, including jerky cannot be sold under the Texas Cottage Food law (DSHS, 2015). Since meat is an excellent medium for microorganisms as it is very high in nutrients, has and slightly neutral pH, and high moisture content (Velasco, 2007). The improper preparation of meat products can cause severe illness and therefore they cannot be sold under the Texas Cottage Food law. No permits or license are required to start your own cottage food operation, however, it is very important that one is aware of the potential hazard these foods may present. Therefore such foods need to be packaged as to prevent contamination and labels need to include information such as: Name and address of production establishment, name of the product, label any of the eight major allergens, with this statement "This food is made in a home kitchen and is not inspected by the Department of State health Services or a local health department" (DSHS, 2015).

Benefits of Artesian Jerky

Artesian jerky is produced by slicing meat into thin lean strips, spiced by a dry rub of salt and pepper, or marinated by either sweet or salty brine with addition of other spices.

Some producers utilize the heat of the sun when the humidity is low to sun dry their jerky strips and some hang their strips in a cellar. Other producers utilize small dehydrators, or a kitchen oven at very low temperatures to dry their jerky strips. These drying methods result in a ready-to-eat meat snack that can be stored without refrigeration for elongated periods of times. The removal of moisture prevents enzymatic action and microbial growth (Velasco, 2007). The benefits of producing artesian jerky are the improved quality without the addition of additives. Jerky that has no additives and has to meet lethality tends to be very bridle in texture. The addition of additives such as maltodextrin can improve the texture of meat, as it improves the springiness, gumminess, chewiness and decreases the hardness in the absent of fat (Crehan et al., 2000). Some desirable characteristics of jerky are its texture and chewiness that is affected by the drying time (Konieczny et al., 2007).

Consumption of ready-to-eat snack such as jerky has increased drastically in the United States (Allen et al., 2007; Miller et al., 1988). This increase in the demand has been acknowledged and processors have expanded their lines of convenience snacks (Miller et al., 1988). The possibility of using artesian methods and the utilization of old-time recipes could expand ready-to-eat snacks even further. These artesian methods could even tap into the all-natural niche market. Producing jerky following old methods can increase savings in production as less electricity would be required run a smoke house or a commercial dehydrator, as they will not be utilized heavily.

MATERIALS AND METHODS

Approval was not required from the Animal Care and Use Committee for this study as no live animals were used. All product was purchased from a federally inspected abattoir.

Experiment I

Strip Formation

Experiment one investigated the use of basic dehydration (with no thermal application) of beef jerky, on aerobic bacteria enumerating naturally found on the meat. Denuded beef inside rounds (NAMP# 169A; n=3) from a commercial abattoir were selected. Each inside round (10 ± 2 lb.) was trimmed leaving only the *biceps femoris*, then cut with the grain into identical strips measuring 5.08 cm x 15.24 cm x 0.6 cm. Strips (n=72) were randomly assigned to one of three treatments (n=24/treatment) consisting of control, rub, and marinated. The average weight of each strip was 35.0g. Care was taken to utilized strips that had similar lean to fat ratio and devoid of defects to minimize the variation that affects the drying process.

Treatment Application

The treatments consisted of; dry rub of salt and coarse black pepper (RUB), liquid marinate consisting of salt, coarse black pepper, distilled water and vinegar (MARINATE), and fresh meat with the absence of spices, water or vinegar (CONTROL) (Table 1). Samples consisting of the RUB and MARINATE were placed in a small 15-25 lb. vacuum drum tumbler (Doug Care Equipment, Springville, CA). The strips were tumbled at 30.5 Hg for 20 minutes. Samples were then placed on a flat stainless steel, rack and laid to dry in a drying room in the Angelo State University Food Safety and Product Development Laboratory

(FSPD), at 50 ± 2°C with a controlled relative humidity of 60 ± 5%. The humidity was monitored and controlled using a dehumidifier (whirlpool corporation, Benton Harbor, MI).

Sample Collection

Initial aerobic bacterial load was measured as well as water activity (A_w) and pH after the marination process. Samples were analyzed at 0 h, 24 h, 38 h, 72 h, 96 h, and 120 h. At each sampling period, one strip/treatment was randomly selected for pH/A_w analysis and three strips for aerobic plate count (APC). Water activity was measured to evaluate the dehydration process of the strips and pH was measured to ensure the product fell within normal range. One strip per treatment was selected for pH and A_w, and was diced into smaller pieces and mixed to facilitate blending to test for A_w using an AquaLab Series 3 water activity meter (Decagon Devices, Inc., Pullman, WA). Manufacture procedures guidelines for the AquaLab Series 3 water activity meter were followed. Water activity was evaluated to determine the effect of the treatments on the degree of dryness, and to determine finish product water activity. The rest of the sample was used determined pH by combining 10.0g of the dice sample with 100 ml of sterile deionized water in a Nutribullet grinder (Nutribullet LLC, Los Angeles, CA). Samples were ground for 30 seconds and pH was read immediately using a pH meter (Hanna Instruments, Woonsocket, RI), following manufacture guidelines.

The three random strips selected from each treatment, were left whole and placed separate in filtered stomacher bags. Samples were soaked in 99.0 mL of buffered peptone water (BPW) for 5 min and then stomached for 2 minute at 230 revolutions per minute (RPM) in a Stomacher 400 Circulator (Seward Stomacher, England). Serial dilutions were

performed and duplicates of each dilution were plated onto aerobic plate count petrifilms (APC; 3M, St Paul, MN).

Samples were then incubated by placing them in an incubator at 37 °C for 48 h. The counts (log CFU/cm^2) for each petrifilm were calculated using a colony counter (Bantrex, Taiwan) and recorded. The bacteria that was measured in this experiment was any aerobic bacteria that is found on the beef jerky strips. Typical colony appearance on the petrifilm was small red dots with well-defined edges.

Experiment II

Strip Formation

Denuded beef inside rounds (NAMP# 169A; n=8) from a commercial abattoir were selected. Each inside round (10 ± 2 lb.) was trimmed leaving only the *biceps femoris*, then cut with the grain into identical strips measuring 5.08 cm x 15.24 cm x 0.6 cm. Strips (n=252) were randomly grouped, into groups of ten, vacuum packaged and randomly assigned into two trials. Strips for trial-I (n=126) were utilize raw and not frozen, while strips for trial-II (n=126) were frozen at -4°C and stored to be utilized at a later date. The average weight of each strip was 35.0g. Care was taken to utilize strips that had similar lean to fat ratio and were devoid of defects to minimize the variation that affects the drying process.

Inoculum Preparation and Application

The frozen inoculum for the samples consisted of a generally recognized as safe (GRAS) lactic acid bacteria (LAB) starter culture, Saga 200, utilized as a pathogen surrogate (LAB- *Pediococcus acidilactici*; Kerry Bioscience, Rochester, MN). Saga 200 was thawed under a class II biological safety cabinet. After it was thawed completely, 0.05 mL of culture were suspended in 9.0 mL of sterile buffered peptone water (Becton, Dickinson and

Company, Sparks, MD) and mixed in a circular motion as to prevent the mixture from foaming. The same process was repeated for the second trial. The inoculum was continuously agitated during sample inoculation to ensure consistent inoculation levels.

Strips were randomly selected and taken to the microbiology laboratory to be inoculated. The inoculation process follows the inoculation protocol from Dierschke. (2010) the concentrate inoculate was pipetted onto the strips (0.4 mL/strips surface) in a class II biological safety cabinet and was evenly distributed with a sterile L-shape spreader. The surrogate was allowed to attach for 30 minutes before the process was repeated on the reverse side of the strip. The initial inoculation culture levels were approximately 10^9 CFU/mL.

Treatment Application

After the samples were inoculated, the strips (n=42/trt) were taken to the Food Science and Product Development drying room where they were randomly assigned to one of three treatment groups; control, rub, and marinated. The treatments consisted of; dry rub of salt and coarse black pepper (RUB), liquid marinate consisting of salt, coarse black pepper, distilled water and vinegar (MARINATE), and fresh meat with the absence of spices, water or vinegar (CONTROL) (Table 1). Samples consisting of the RUB and MARINATE were placed in a small 15-25 lb. vacuum drum tumbler (Doug Care Equipment, Springville, CA). The strips were tumbled at 30.5 Hg for 20 minutes. Samples were then placed on a flat, stainless steel rack and laid to dry in a drying room in the FSPD laboratory, at 50 ± 2°C with a controlled relative humidity of 60 ± 5%. The humidity was monitored and controlled using a dehumidifier (whirlpool corporation, Benton Harbor, MI).The process was repeated for the second trial.

Sample Collection

Initial bacterial load was measured as well as A_w and pH after the marination process. Samples were analyzed at 0 h, 24 h, 38 h, 72 h, 96 h, and 120 h. The samples were split into Saga 200 analysis, and pH/A_w analysis. At each sampling period, two strips/treatment were randomly selected for pH/A_w analysis and five strips for Saga 200. The strips selected for pH and A_w were diced into smaller pieces and mixed together to facilitate blending to test for A_w using an AquaLab Series 3 water activity meter (Decagon Devices, Inc., Pullman, WA). Manufacture procedures guidelines for the AquaLab Series 3 water activity meter were followed. Water activity was evaluated to determine the effect of the treatment on the degree of dryness, and to determine finish product water activity. Sample pH was determined by combining 10.0g of the mixture of the two strips and 100 mL of sterile deionized water in a Nutribullet grinder (Nutribullet LLC, Los Angeles, CA). Samples were ground for 30 seconds and pH was read immediately using a pH meter (Hanna Instruments, Woonsocket, RI), following manufactures guidelines.

The five random strips from each treatment were left whole and placed in filtered stomacher bags. Samples were soaked in diluent (99.0 mL BPW) for 5 minutes and then stomached for 2 min at 230 (RPM) in a Stomacher 400 Circulator (Seward Stomacher, England). Serial dilutions were performed. Selective plating was performed by adding 0.5ml of De Man-Rogosa-Sharpe broth (MRS; 3M, St Paul, MN) on aerobic count petrifilm plates (APC; 3M, St Paul, MN), followed by 0.5 ml of sample. The samples were mixed by aspirating and dispensing the sample on the APC petrifilm to ensure proper mixture of sample and MRS broth. Duplicates of each dilution were plated.

Samples were then incubated by placing them into GasPak EZ large incubation containers (Becton, Dickinson and Company, Sparks, MD). Before sealing the incubation container three GasPak EZ gas generating sachet were placed inside the container, to achieve an anaerobic atmosphere with less than 1.0% oxygen. Once the chamber was sealed the sample was placed in an incubator at 37°C for 48 h. The counts (log CFU/cm^2) for each petrifilm were evaluated using a colony counter (Bantrex, Taiwan) and recorded. Typical colony appearance on the petrifilm was small red dots with well-defined edges.

Statistical Analysis

Data was analyzed with version 9.1.3 of SAS (SAS Institute, Inc., Cary, NC). Bacteria population was converted to a log base 10. The Mixed Procedure of SAS was utilized to evaluate the bacterial load response and to determine significant differences between treatment, day, and trial at a predetermined $\alpha < 0.05$. In addition, the Means Procedure was generated to evaluate water activity and pH.

RESULTS AND DISCUSSION

Experiment I

Bacteria Response

In experiment I control and rub had no significant difference ($P \leq 0.05$). Bacterial response to treatments can be found in Table 2. Samples (n=54) from experiment I were analyzed for aerobic bacterial levels. A gradual decrease was observed from day zero (6.18 $\log_{10}$ CFU/cm^2) trough day two (5.14 $\log_{10}$ CFU/cm^2). However, bacteria levels remain consistent from day two trough day five (Figure 1). Microbial data from Experiment I showed an average reduction of 1.25 $\log_{10}$ CFU/cm^2 across the three treatments (Figure 1). The results from Experiment I reaffirm the need for another intervention in addition to single dehydration. Harrison et al. (2001) found that heating the dry product in an oven at 275 °F for 10 minutes results in 2 $\log_{10}$ reduction of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*. Heating the jerky strips after drying is something to consider in the production of artesian jerky, as the time temperature combination will not affect consumer acceptability (Harrison et al., 2001). Although the water activity (A_w) met was lower than 0.85, there was an insufficient reduction aerobic bacteria to meet the FSIS regulations.

Water Activity & pH

Due to the small sample size, A_w and pH were not analyzed for statistical differences; however, pH of samples ranged between 5.14 and 5.65 and A_w ranged between 0.99 on day zero and 0.63 on day five. These ranges fall within normal pH of meat and expected water activity of dehydration (Kerth, 2013).

At the completion of Experiment I, adjustments were made to the experimental design in Experiment II to ensure statistical accuracy. Sample quantity was increased to have

a better representation of the reaction of the pathogen surrogate using only dehydration (with no thermal application) as a means of lethality.

Experiment II

Bacterial Response

In trial-I Saga 200 levels of control, rub, and marinate had a significant interaction ($P \geq 0.05$) (Table 2). These could of have been due to the ingredients of the treatments as they could have inhibited bacterial growth. On the other hand trial-II had no significant levels of Saga 200 across all three treatments ($P \leq 0.05$) (Table 2). These could of been a result of the faster dehydration due to the freezing and thawing step, as Saga 200 could of have adapted to the dryer environment.

Results showed a reduction over five days across all trials and treatments of 1.64 $\log_{10}$ CFU/cm^2 of Saga 200 (Figure 1). Saga 200 bacteria levels were reduced gradually over the 5 days of dehydration. While levels decreased from day zero to day two, there was no significant decrease from day two to day three and from day four to day five. Bacterial reduction can be found in Figure 1. Reduction of water activity alone is not sufficient to reduce bacteria more than 5 $\log_{10}$ CFU/cm^2. Research has shown high bacterial levels of *L. monoctogenes*, *S. aureus*, *E. coli* O157:H7, and *Salmonella* even after water activity regulations have been met. Dierschke. (2010), found that samples reach A_w <0.85 in a period of four hours utilizing four different home style dehydrators, and although the dehydrators reached drying temperatures (between 57-68°C) the log reduction was only a decrease of 3.0 $\log_{10}$ CFU. Dierschke did however, find administering a post-dehydration heating phase, improved the reduction of bacterial levels (Dierschke, 2010).

Although the minimum water activity (A_w) of 0.85 for preventing *S. aureus* to thrive and growth and produce toxin was met, sufficient reduction of bacteria was not met. However, pathogens such as *Salmonella* can adapt to certain dry environments if given enough time (Andino and Hanning, 2015). Buege et al. (2006) found that Saga 200 is more resistant than *E. coli* and *Salmonella,* and that E. *coli* is capable of surviving a heating and drying step in whole muscle beef jerky. Even though, pathogens such as the ones described in this study have been linked to foodborne outbreaks related to beef jerky, not many studies have investigated the microbial safety of this highly priced snack (Eidson et al., 2000).

Water Activity & pH

Dehydration of the jerky strips inoculated with Saga 200 gradually increased over the five days of processing for each trial. Water activity and pH levels of inoculated jerky strips are found in Table 3. The average A_w for day zero for trial-I was 0.98 and the average A_w for day five was 0.75. In addition, trial-II saw the same gradual decrease in A_w, day zero was 0.98 and day five was 0.68. Due to slight differences in sample preparation trials were analyzed independent from each other and cannot be compared. Trial-II utilized inside round strips, which were frozen and required thawing before being inoculated and later set to dry, while Trial-I samples were never frozen. The freezing-thawing cycle created small ice crystals that disrupt muscle fibers of each inside round strip, releasing some of the water that was previously bound and immobilized resulting in drip loss, or purge as it is most commonly known (Lawrie and Ledward, 2006; Leygonie et al., 2012). This could have impacted the dehydration parameters as water that was once bound was easily dehydrated. However, there were very few samples to have a representative answer that would show that the differences in dehydration rate were due to the freezing-thawing cycle. Along with the

freezing and thawing, a problem with the dehumidifier was encounter during trial-I. The dehumidifier was not functioning properly from day zero to day one and humidity was not monitored. However, the dehumidifier was fixed by the end of day one and humidity was controlled continuously through the rest of the trial. Inadequate quantities of pH readings for the inoculated strips were collected for statistical analysis; however, the pH levels were near the average pH of fresh meat which is 5.6-5.8 (Kerth, 2013) (Table 3). The average pH for both treatments throughout the six consecutive readings was as follows: Control had an average pH of 5.8, while Rub had an average pH of 5.74, and Marinate had an average pH of 5.43. The pH readings of the Marinate treatment were expected to be more acidic due to the addition of vinegar to the recipe. Reduction in water activity and pH levels were not sufficient on their own to meet bacterial reduction requirements, however, it is possible that with additional interventions further reduction can be achieved.

CONCLUSION

Upon evaluating aerobic bacteria and Saga 200 response to basic dehydration (with no thermal application), an insufficient reduction of bacteria to meet FSIS guidelines was found. Nonetheless, it can help achieve a further log reduction after a heating process. Both experiments had a similar reduction of bacteria levels. Furthermore, a short cooking time at extremely hot temperatures could possibly be utilized as a method of achieving a higher reduction of bacteria.

There are many other interventions without forced heat treatments, which can increase the reduction of bacterial levels. These interventions can be used in many combinations, and need to be evaluated to understand other methods of bacterial inhibition. Therefore, more studies are required to achieve a higher log reduction. The utilization of UV light as a kill step that can be considered for further projects to determine if these interventions can suppress pathogen growth to survival and achieve sufficient reduction of bacteria levels. In addition, a high acidic marinade or hot dipping step before drying should be further evaluated.

Table 1. Marinate and Rub treatment formulation for experiment I and experiment II

Treatment	Ingredients	Ounces[1]
Marinate	Salt	2.5
	Course Black Pepper	1.0
	Water	2.9
	Vinegar	2.9
Rub	Salt	2.5
	Course Black Pepper	1.0

[1] Inclusion rate per treatment batch

Table 2. Least squares means ± SEM within experiment, trial, and treatments of bacterial levels (log_{10} CFU/cm^2) for non-heat treated, shelf-stable, whole-muscle beef jerky strips dehydrated over a five day period

Experiment #	Control	Rub	Marinate
APC[1]	5.25±0.08[a]	5.25±0.08[a]	5.50±0.08[b]
Trial 1 Saga 200[2]	8.81±0.09[a]	8.35±0.09[b]	7.99±0.09[c]
Trail 2 Saga 200[2]	7.96±0.09[a]	7.85±0.09[a]	7.72±0.09[a]

[1] Aerobic Plate Count bacteria; n=18/treatment

[2] Generally, Recognize as Safe pathogen surrogate (Saga 200; *Pediococcus acidilictici*); n=30/treatment and trial

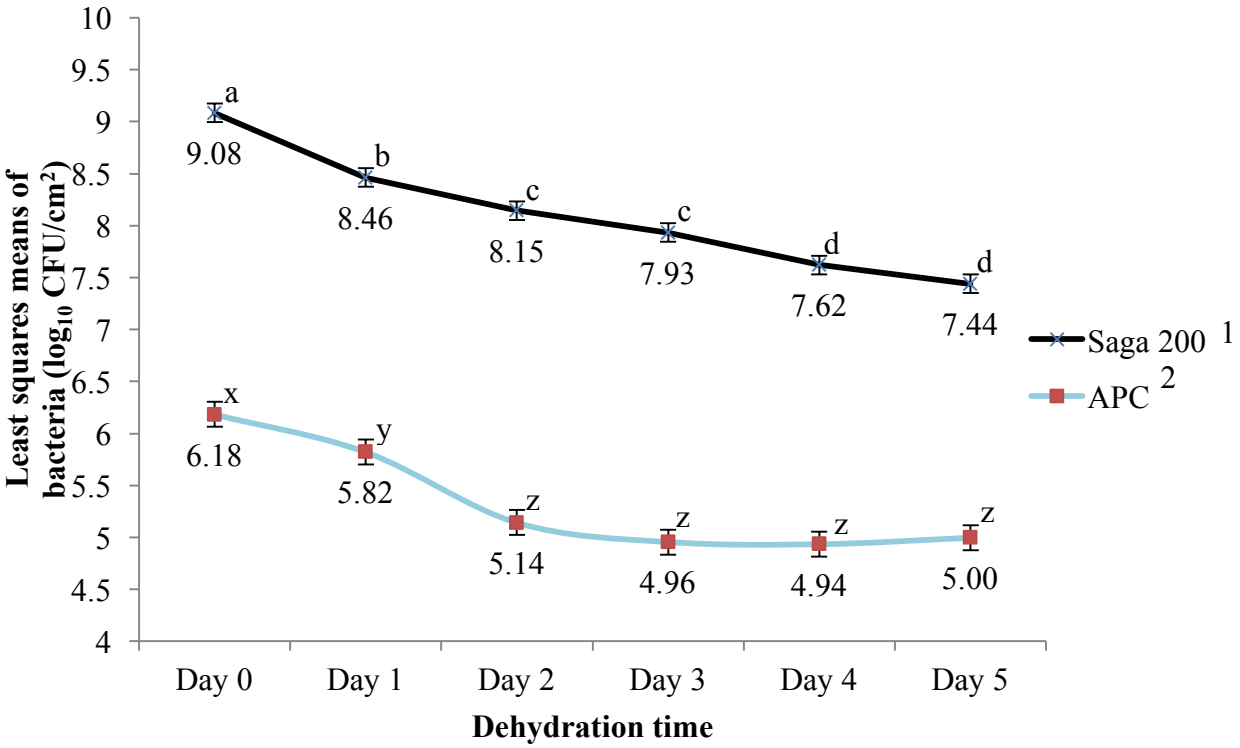
[abc] Means with differing superscripts within a row differ ($P<0.05$)

Table 3. Means of water activity (Aw) and pH by day and treatment for non-heat treated, shelf-stable, whole-muscle beef jerky strips inoculated with Saga 200[1] dehydrated over a five day period

		Day 0		Day 1		Day 2		Day 3		Day 4		Day 5	
Experiment II	Treatment	pH	Aw	pH	Aw	pH	Aw	pH	Aw	pH	Aw	pH	Aw
Trial I	Control	6.52	0.98	5.54	0.98	6.14	0.97	6.38	0.95	5.60	0.80	5.76	0.79
	Marinate	5.15	0.96	5.32	0.96	5.30	0.91	5.49	0.84	5.58	0.77	5.58	0.73
	Rub	5.76	0.97	5.85	0.96	5.53	0.92	5.66	0.75	6.22	0.76	5.99	0.73
Trial II	Control	5.66	0.98	5.65	0.97	5.59	0.92	5.41	0.76	6.18	0.72	5.68	0.71
	Marinate	5.24	0.96	5.45	0.92	5.25	0.85	5.70	0.75	5.62	0.66	5.53	0.67
	Rub	5.67	0.97	5.71	0.92	5.60	0.82	5.59	0.69	5.70	0.68	5.64	0.66

[1] Generally, Recognize as Safe pathogen surrogate (Saga 200; *Pediococcus acidilictici*)

Figure 1. Least squares means of bacteria counts by day of aerobic plate count and Saga 200 for non-heat treated, shelf-stable, whole-muscle beef jerky strips dehydrated over a five day period



[1] Generally, Recognize as Safe pathogen surrogate (Saga 200; *Pediococcus acidilictici*)
[2] Aerobic Plate Count bacteria
[abcd] Means with different superscripts within Saga 200 differ ($P \leq 0.05$)
[xyz] Means with different superscripts within APC differ ($P \leq 0.05$)

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