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Pretreatment effect on inactivation of *Escherichia coli* O157:H7 inoculated beef jerky

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Abstract

Changes in inoculated *Escherichia coli* O157:H7 populations were determined during drying (62.5°C, 10 h) of whole muscle beef jerky slices pretreated by: (1) immersing in boiling water (94°C, 15 s), then marinating (4°C, 24 h); (2) seasoning (4°C, 24 h), then immersing in a pickling brine (78°C, 90 s); (3) immersing in a vinegar/water (750/750 mL) solution (57.5°C, 20 s), then marinating (4°C, 24 h); and (4) marinating (4°C, 24 h), then immersing in a vinegar/water (750/750 mL) solution (57.5°C, 20 s). Samples were analysed (bacterial enumeration with selective and nonselective agar media, pH, and a_w) following inoculation, each preparation step, and at 0, 4, 6, 8 and 10 h of drying. While all pre-drying treatments resulted in significant ($P<0.05$) bacterial reductions, treatment 2 resulted in the greatest pre-drying reduction (3.1–4.1 log cfu/cm^2) and the highest overall reduction at 10 h drying (5.7–5.8 log cfu/cm^2). Total reductions for treatments 1, 3 and 4 after 10 h drying were 4.3–4.5, 4.9–5.2 and 4.7–4.8 log cfu/cm^2, respectively. Bacterial populations declined to <1.0 log cfu/cm^2 after 30 d storage and remained at this level throughout 90 d storage. These results should be useful in developing guidelines for jerky preparation by consumers and processors.

Keywords: *E. coli*; Beef; Jerky; Dried meat; Acetic acid; Marination

1. Introduction

Escherichia coli O157:H7 presents a problem to current preventive strategies for food safety protocols because it has a low infectious dose and can result in severe disease characteristics (hemorrhagic colitis and hemolytic uremic syndrome) (Buchanan & Doyle, 1997). In addition, the pathogen is unevenly distributed in raw ingredients, which makes testing unreliable as a method of control (Tilden et al., 1996). Thus, the most desirable approach for processors and consumers may be a lethal processing step that successfully minimizes the risk of *E. coli* O157:H7 contamination. Outbreaks in the mid-1990s associated with *E. coli* O157:H7 in dried and fermented meats (Alexander et al., 1995; Keene et al., 1997) prompted federal action mandating that manufacturers of such products demonstrate a 5-log reduction of *E. coli* O157:H7 during processing (Reed, 1995; Billy, 1997).

Studies on the survival of *E. coli* O157:H7 cells in dried and fermented meat products (Hinkens et al., 1996; Faith, Parniere, Larson, Lorang, & Luchansky, 1997, 1998b; Faith et al., 1998c; Riordan et al., 1998) have indicated that traditional fermentation processes, which do not include a thermal processing step, have not achieved a 5-log reduction of inoculated *E. coli* O157:H7 cells. Contaminated dried meats, such as jerky, may pose a potentially greater threat to consumers than fermented meats since jerky can easily be prepared at home with limited equipment and precision in methods used (Faith et al., 1998a).

Studies on the survival of *E. coli* O157:H7 in dried jerky products (Harrison & Harrison, 1996; Keene et al., 1997; Faith et al., 1998a; Harrison, Harrison, & Rose, 1998; Harrison, Harrison, Rose-Morrow, & Shewfelt, 2001) have produced variable results, depending in part on the type (beef vs. venison), form (whole muscle vs. ground) and shape (thick strips vs. thin slices) of meat used in jerky formulation, dehydrator (air) temperature

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(51.7–68°C), drying time (6–20 h) and the agar medium used (tryptic soy agar, sorbitol MacConkey, sorbitol MacConkey supplemented with 4-methyl-umberlliferyl-D-glucoronide, or modified eosin methylene blue) for recovery of surviving bacteria. Harrison and Harrison (1996) and Harrison et al. (2001) reported >5 log cfu/g reductions in beef jerky marinated using a traditional method (Reynolds & Williams, 1993) and dried at 62.5°C for 10 h. In comparison Keene et al. (1997) concluded that low-temperature (⩽62.8°C) dehydration was an unreliable means of eradicating *E. coli* O157:H7 from contaminated meat and Faith et al. (1998a) reported that only two of eight jerky preparation procedures evaluated achieved a 5-log reduction by the time the product was judged visually dry.

Results of a study in our laboratory (Albright, Kendall, Avens, & Sofos, 2002) indicated that bacterial reductions in marinated whole muscle beef slices dried at 62.5°C and 68.5°C from the unheated state were 2.1–2.3 and 3.5–4.1 log cfu/cm^2, respectively, after 4 h of drying, but only 2.2 and 3.0–4.6 log cfu/cm^2, respectively, by the end of 10 h of drying. The authors suggested that casehardening on the surface of the jerky (McWilliams, 1993) might have interfered with further bacterial destruction between 4 and 10 h of drying. Based on the conditions and results of this study, it was concluded that drying beef jerky for 10 h at 62.5°C or 68.3°C resulted in significant ($P<0.05$) reductions of inoculated *E. coli* O157:H7 cells, but a 5-log reduction of bacteria was not obtained. With conflicting results reported concerning dried jerky, it appears that there is a need for additional studies to evaluate procedures for achieving adequate (⩾5 log cfu/g) reductions of inoculated *E. coli* O157:H7 during jerky processing.

The multiple hurdle concept of food preservation employs combinations of barriers with the objective of ensuring that microorganisms are controlled (Leistner, 1987, 1995). With multi-hurdles, lower intensities of individual factors can be used than would be needed if the factors were used alone (Leistner, 1987; Marth, 1998). Leistner (1987, 1995) recommended the use of a multi-hurdle strategy by food processing industries that prepare fermented and dried meat, since a combination of hurdles can lead to a more stable product. The most important hurdles commonly used in food processing include temperature (high or low), water activity (a_w), acidity (pH), redox potential (E_h) and preservatives (organic acids, spices) (Leistner, 1995).

The objective of this study was to evaluate survival of inoculated *E. coli* O157:H7 populations in beef jerky prepared using four multi-hurdle pre-drying treatments and dried in home-type dehydrators (air RH 19–24%) for up to 10 h at 62.5°C (145°F), then stored (21°C) for up to 90 d. Since the drying process may result in injured cells, recovery of surviving bacterial cells was evaluated with two agar media.

2. Materials and methods

2.1. Preparation of bacterial inoculum

Four strains of *E. coli* O157:H7 (EO139, a venison jerky isolate provided by Dr. Michael Doyle, Center for Food Safety, University of Georgia, Griffin, Georgia; ATCC 43895, ATCC 43890 and ATCC 43894) were used to prepare a composite inoculum for inoculation of the meat slices before processing to make jerky. Cultures were maintained frozen at −30°C in tryptic soy broth (TSB) (Difco Laboratories, Sparks, MD, USA) with 20% (v) added glycerol (Sigma Chemical Co., St. Louis, MO, USA). Each strain was propagated individually on tryptic soy agar (TSA; pH 7.0) (Difco) slants at 35°C before use in experiments. The experimental inocula were prepared by transferring a loopful of each activated culture into 9-mL tubes of TSB (pH 7.0) and incubating at 35°C for 18–24 h on the day prior to each experiment. The cultures used in the experiments averaged 1.7×10^9 cfu/mL. Immediately before use, the individual culture inocula were combined and diluted in Butterfield's phosphate buffer for inoculation of the meat slices.

2.2. Preparation and inoculation of beef slices

Vacuum-packaged beef inside rounds (approximately 30 kg) were purchased frozen from local supermarket warehouses and stored at −18°C until use (i.e., within 1 week). The frozen meat was cut into slices ($8.7\times4.0\times0.6$ cm^3) using a food slicer (model 610, Hobart Corp., Troy, OH, USA). Slices were randomly placed in 1-kg portions in 20×25 cm^2 plastic bags (Kapak Heat Sealable, Fisher Scientific, Springfield, NJ, USA). The bags of meat were evacuated (29 mm/Hg), heat sealed (Multivac, Sepp Haggenmüller KG, Allgau, Germany), and then frozen (−18°C) to minimize potential changes until use within 2 weeks.

For each treatment, frozen beef slices were thawed at 4°C for 24 h and placed on aluminum foil covered trays. Under a biohazard hood, 0.5 mL of the composite *E. coli* O157:H7 inoculum was placed on the top surface (8.7×4.0 cm^2) of each slice and spread across the entire surface area using a sterile bent glass rod. Meat pieces were then turned using sterile forceps and the opposite surface area was inoculated with 0.5 mL of the composite culture. Trays of inoculated slices were covered with aluminum foil and refrigerated (4°C, 24 h) to enhance adherence of pathogen cells to meat slices. Following inoculation and storage, inoculum levels on meat slices ranged between 5.3 and 7.6 log cfu/cm^2.

2.3. Pre-drying treatments

Four pre-drying treatments, each involving two multi-hurdle steps, were evaluated. These included: immersing

in boiling water (94°C, 15 s), followed by marinating (4°C, 24 h) (BW-M); seasoning with pickling spices (4°C, 24 h), followed by immersing in hot pickling brine (78°C, 90 s) (S-HP); immersing in warm vinegar/water (750/750 mL) solution (57.5°C, 20 s), followed by marinating (4°C, 24 h) (VW-M); and marinating (4°C, 24 h), followed by immersing in warm vinegar/water (750/750 mL) solution (57.5°C, 20 s) (M-VW).

The marinade (pH = 4.3) used in the BW-M, VW-M and M-VW treatments was selected from a commonly used food preservation book (Reynolds & Williams, 1993) and consisted (per kg of meat slices marinated; 65 slices of 15 g each) of 60 mL soy sauce (Kikkoman Foods, Walworth, WI, USA), 15 mL Worcestershire sauce (Heinz, Pittsburgh, PA, USA), 0.6 g Shilling black pepper (McCormick and Co., Hunt Valley, MD, USA), 1.25 g garlic powder (Kroger Co., Cincinnati, OH, USA), 1.5 g onion powder (Kroger Co., Cincinnati, OH, USA), and 4.35 g Spice Islands hickory smoke-flavored salt (Specialty Brands Inc., San Francisco, CA, USA). The S-HP treatment was selected from a wild game bulletin (Field & Raab, 1983). The pickling spices used consisted of 27 g Morton iodized salt (Morton International, Inc., Chicago, IL, USA), 12 g Food Club granulated sugar (Topco Associates, Inc., Skokie, IL, USA) and 1.9 g Shilling black pepper per kg of meat slices seasoned. The pickling brine consisted of 108 g Morton iodized salt, 48 g Food Club granulated sugar and 5.8 g Schilling black pepper per gallon of water used.

2.4. Pre-drying treatment application

For the BW-M treatment, inoculated beef slices (11 slices, 165 g) were placed in a single layer on the bottom of a 2-qt steamer basket, then immersed for 15 s in a 2-qt stainless-steel pot of boiling water (94°C), removed rapidly, drained, and laid flat in aluminum pans using heat sterilized forceps. This process was repeated six times with the water replaced after two repetitions. One mL of marinade was then applied to each meat slice using a sterile bent glass rod and sterilized forceps. Marinated pieces were placed in aluminum pans, covered with aluminum foil, and stored (4°C, 24 h) before drying. For the S-HP treatment, inoculated slices were placed on aluminum foil covered trays, evenly sprinkled (top and bottom sides) with pickling spices (0.6 g per inoculated meat slice) and pounded with a rubber mallet. Trays were then covered with aluminum foil and stored (4°C, 24 h). Following refrigerated storage, seasoned beef slices were placed in a single layer on the bottom of a 2-qt steamer basket (11 slices, 165 g at a time), immersed for 90 s in a 2-qt stainless-steel pot containing the pickling brine (78°C), removed rapidly and drained. The process was repeated six times per replication and the pickling brine replaced with fresh brine after two uses. The meat slices were dried immediately following dipping.

For the VW-M treatment, equal parts (750 mL) of distilled white household vinegar (Kroger Co., Cincinnati, OH, USA) (5% acetic acid) and distilled water were combined and heated in a 2-qt stainless-steel pot to 57.5°C. Inoculated beef slices (11 slices, 165 g) were placed in a single layer on the bottom of a 2-qt steamer basket, immersed (20 s) in the vinegar/water solution, removed rapidly, drained and laid flat in aluminum pans using heat sterilized forceps. The process was repeated six times per replication with the vinegar/water solution replaced after two uses. Following the dipping process, each inoculated meat slice was marinated using the procedure described above for the BW-M treatment, stored at 4°C for 24 h and dried. The M-VW treatment was similar to the VW-M treatment, except that the pre-treatment steps were reversed. In the M-VW treatment, inoculated meat slices were marinated as described for the BW-M treatment, covered with aluminum foil and stored at 4°C for 24 h, then dipped (20 s) in warm (57.5°C) vinegar/water (750/750 mL) solution as described above for the VW-M treatment. The vinegar/water solution was replaced after two uses and the meat slices were dried immediately following the dipping process.

2.5. Drying process and sample preparation

Two cylindrical-shaped home food dehydrators (Gardenmaster® Dehydrator FD-1000, Nesco®/American Harvest®, Chaska, MN, USA), with three dehydrator trays each, were used for the drying process with one dehydrator used per replicate. Dehydrators with empty trays were preheated to 62.5°C/145°F (air) for at least 15 min, then replaced with trays loaded with meat slices laid flat on dehydrator trays, without touching other slices. Measurement of drying time began immediately upon replacement of the empty trays. Circulating air temperature within the dehydrators was monitored during the drying process with thermocouples (Type K Beaded Probes, MM Micromeasurements, Raleigh, NC, USA) placed through the open circular top of each dehydrator. Temperatures were recorded on a Speedomax strip chart recorder (Leeds and Northup, Northwales, PA, USA). The temperatures of meat surfaces were monitored using an infrared heat gun (Oakton Infrared Temperature Tester, Gainesville, FL, USA). At 30 min intervals throughout drying, dehydrator trays were raised slightly and the surface temperature of meat on each tray was measured randomly in three locations using the heat gun.

At each sampling interval, three sets of two meat slices per treatment were aseptically transferred into sterile plastic bags (Nasco, Modesto, CA, USA) for analysis. After drying, the remaining jerky slices were

held in dehydrators overnight, then placed (25–30 pieces/bag) in 1-qt Ziploc® freezer bags, closed and stored in the dark, at ambient temperature (21 ± 2°C) and a room relative humidity of 19–24%, for 30–90 d.

2.6. Microbiological analysis

Samples taken after inoculation (4°C, 24 h) and after each pre-drying step were analysed immediately. Samples taken at 4, 6, 8, and 10 h of drying were analysed after storage at 21°C for 24 h. Stored samples were analysed on 30, 60 and 90 d post-drying. Bags containing two slices of product per sample were weighed. Appropriate amounts of Butterfield's phosphate buffer were added to obtain a 1:10 sample dilution and bags were pummeled (IUL Instruments Masticator, Barcelona, Spain) for 120 s. Serial decimal dilutions were made and spread plated in duplicate on tryptic soy agar (TSA) and sorbitol MacConkey (SMAC) agar (Difco Laboratories). Following incubation at 35°C for 24 h, colonies were counted. The minimum detection limit was 10 cfu/cm^2. All counts were converted to log cfu/cm^2. Six colonies per treatment and drying time were confirmed as *E. coli* serotype O157:H7 using the *E. coli* O157:H7 latex agglutination assay (OXOID Diagnostic Reagents, Hampshire, England).

2.7. Physical analyses

Meat pieces (10–15 g) were analysed for a_w and pH at each sampling time. Water activity was determined with a Rotronic Hygroskop DT water activity meter (Model D2100, Rotronic, Huntington, NY, USA) calibrated with standard saturated salt solutions. A Hanna Instruments, HI8424, (Woonsocket, RI, USA) pH meter was used to determine the pH of the inoculated meat pieces. Distilled water was added to obtain a dilution of 1:10 and bags were pummeled (IUL Instruments Masticator, Barcelona, Spain) for 120 s.

2.8. Statistical analyses

Each trial was replicated twice with three samples analysed per replication. Different dehydrators were used for the two replications. Data were evaluated using a $2 \times 4 \times 2 \times 7$ factorial design (2 trials/dehydrators × 4 pre-drying treatments × 2 agar media × 7 drying/sampling times, respectively). Each mean value reported represents six values (three samples from each of two trials/dehydrators). Bacterial counts of duplicate plates per dilution were averaged and cfu/cm^2 counts were converted into log values. Data were analysed with the Statistical Analysis System (SAS, 1996) for analysis of variance of main (fixed) effects, as well as all interactions among fixed effects. When F values were significant ($P < 0.05$), the least significant difference (LSD) procedure was used for separation of means at the $P < 0.05$ level.

3. Results and discussion

3.1. Dehydrator and product temperatures

Air and product temperatures did not vary by trial/dehydrator. The air temperature of the pre-heated dehydrators declined by 10–17°C following placement of meat strips on dehydrator trays, then increased steadily until the desired air temperature of 62.5°C (±2.5°C) was reached, approximately 3–4 h after placement of meat strips in the dehydrators; this air temperature (62.5 ± 2.5°C) was then maintained throughout the remainder of the drying time. The surface temperatures of the meat strips equaled the target air temperature of 62.5 ± 2.5°C after 4.5–5 h in the dehydrator and remained within this range for the remainder of the drying process (data not shown in tabular form).

3.2. Effects of pre-drying treatments

With the exception of trial/dehydrator, the main factors (drying time, treatment method and culture media) had significant effects ($P < 0.05$) on bacterial populations recovered (Table 1). Significant interactions ($P < 0.05$) were seen for treatment × drying time, treatment × culture media, drying time × culture media and treatment × drying time × culture media. In this study, TSA tended to yield higher counts than SMAC, but overall there was no evidence of major bacterial injury, except for the BW-M treatment at 6, 8 and 10 h of drying (Table 1).

Dipping slices in boiling water (94°C) (step 1 of the BW-M pre-drying treatment) resulted in bacterial population reductions ($P < 0.05$) of 2.4 and 3.1 log cfu/cm^2 for SMAC and TSA, respectively (Table 1). The first step of the S-HP pre-drying treatment (slices seasoned with pickling spices at 4°C for 24 h) resulted in reductions in SMAC and TSA populations of 0.8 and 0.1 log cfu/cm^2, respectively. Corresponding reductions after the first step of the VW-M pre-drying treatment (slices dipped in 57.5°C vinegar/water solution) were ($P < 0.05$) 0.8 and 0.6 log cfu/cm^2. In comparison, when the pre-drying steps were reversed (M-VW), bacterial populations increased slightly (0.1–0.2 log cfu/cm^2) following the first step (marinating slices at 4°C for 24 h).

The second step of the BW-M pre-drying treatment (slices marinated at 4°C for 24 h) resulted in significant ($P < 0.05$) increases in counts (1.1 log cfu/cm^2 with SMAC and TSA) compared to populations after pre-drying step 1 (Table 1); net reductions following both steps of the BW-M pre-drying treatment were 1.3 and

Table 1
Effect of beef jerky pre-drying treatment, drying time (62.5°C, 0–10 h) and culture media for bacterial enumeration (tryptic soy agar—TSA and sorbitol MacConkey agar—SMAC) on survival of inoculated *Escherichia coli* O157:H7 and natural bacterial flora

Processing steps and drying times (h), days stored (d)	Recovered bacterial population (log cfu/cm^2)[a]							
	BW-M[b]		S-HP[c]		VW-M[d]		M-VW[e]	
	SMAC	TSA	SMAC	TSA	SMAC	TSA	SMAC	TSA
Following inoculation (4°C, 24 h)	5.3 (0.6)^{BaY}	6.0 (0.3)^{AaY}	7.1 (0.2)^{BaX}	7.6 (0.1)^{AaX}	7.3 (0.1)^{AaX}	7.5 (0.3)^{AaX}	7.1 (0.5)^{AaX}	7.5 (0.6)^{AabX}
Following pre-drying step 1	2.9 (0.4)^{AcZ}	2.9 (0.5)^{AcZ}	6.3 (0.7)^{BbY}	7.5 (0.0)^{AaX}	6.5 (0.2)^{BbY}	6.9 (0.1)^{AbY}	7.2 (0.3)^{BaX}	7.7 (0.3)^{AaX}
Following pre-drying step 2	4.0 (0.2)^{AbY}	4.0 (0.2)^{AbY}	4.0 (0.5)^{AcY}	3.5 (0.9)^{BbZ}	6.6 (0.1)^{AbX}	6.7 (0.1)^{AbX}	6.4 (0.6)^{BbX}	7.0 (0.2)^{AbX}
4 h	1.5 (0.2)^{AdY}	1.8 (0.5)^{AdeY}	1.5 (0.4)^{AdY}	1.8 (0.4)^{AcY}	2.4 (0.1)^{BcX}	3.1 (0.5)^{AcX}	2.7 (0.2)^{AcdX}	3.0 (0.3)^{AcX}
6 h	1.5 (0.1)^{BdZ}	2.2 (0.6)^{AdY}	1.7 (0.3)^{AdZ}	2.0 (0.3)^{AcY}	2.5 (0.4)^{BcY}	3.0 (0.4)^{AcX}	3.0 (0.1)^{AcX}	2.9 (0.3)^{AcX}
8 h	0.6 (0.5)^{BeZ}	2.1 (0.4)^{AdeY}	1.8 (0.8)^{AdY}	2.0 (0.6)^{AcY}	2.4 (0.2)^{AcX}	2.3 (0.3)^{AdY}	2.9 (0.3)^{AcdX}	3.0 (0.2)^{AcX}
10 h	0.8 (0.6)^{BeZ}	1.7 (0.7)^{AeY}	1.4 (0.9)^{BdY}	1.8 (0.7)^{AcY}	2.4 (0.2)^{AcX}	2.3 (0.2)^{AdX}	2.4 (0.2)^{AdX}	2.7 (0.4)^{AcX}
30 d	<1.0 (0.1)^{AfX}	<1.0 (0.0)^{AfX}	<1.0 (0.0)^{AeX}	<1.0 (0.0)^{AdX}	<1.0 (0.0)^{AdX}	<1.0 (0.0)^{AeX}	<1.0 (0.0)^{AeX}	<1.0 (0.0)^{AdX}
60 d	<1.0 (0.1)^{AfX}	<1.0 (0.1)^{AfX}	<1.0 (0.0)^{AeX}	<1.0 (0.0)^{AdX}	<1.0 (0.0)^{AdX}	<1.0 (0.0)^{AeX}	<1.0 (0.1)^{AeX}	<1.0 (0.1)^{AdX}
90 d	<1.0 (0.1)^{AfX}	<1.0 (0.1)^{AfX}	<1.0 (0.0)^{AeX}	<1.0 (0.0)^{AdX}	<1.0 (0.1)^{AdX}	<1.0 (0.1)^{AeX}	<1.0 (0.1)^{AeX}	<1.0 (0.1)^{AdX}

[A–B]Means with different superscripts within a treatment row are significantly ($P<0.05$) different.
[a–e]Means with different superscripts within each column are significantly ($P<0.05$) different.
[X–Z]Means with different superscripts within each agar medium in a row are significantly ($P<0.05$) different.
[a]Means represent three values from two trials ($n = 6$) (standard deviation) of log colony forming units (cfu) cm^2; lowest detection limit by plating, 1 log cfu/cm^2.
[b]Pre-drying step 1: dipping in boiling water (95°C, 15 s); pre-drying step 2: marinating (4°C, 24 h).
[c]Pre-drying step 1: seasoning with pickling spices (4°C, 24 h); pre-drying step 2: dipping in hot pickling brine (78°C, 90 s).
[d]Pre-drying step 1: dipping in warm vinegar/water (750/750 mL) solution (57.5°C, 20 s); pre-drying step 2: marinating (4°C, 24 h).
[e]Pre-drying step 1: marinating (4°C, 24 h); pre-drying step 2: dipping in warm vinegar/water (750/750 mL) solution (57.5°C, 20 s).

2.0 log cfu/cm^2 with SMAC and TSA, respectively. These data do not support the findings of Masuda, Hara-Kudo, and Kumagaf (1998) indicating that soy sauce (the major component in the marinade) has negative effects on *E. coli* O157:H7. Differences seen in the two studies may have been due to differences in soy sauce composition or other variables. In the present study, while dipping the slices in simmering water achieved bacterial reductions of 2.4–3.1 log cfu/cm^2, the cells appeared to be able to recover during the marinating step (4°C, 24 h). It is possible that the jerky marinade (Reynolds & Williams, 1993) supplied the environment necessary for the inoculated cells to recover and multiply.

Pre-drying step 2 of S-HP treatment (slices dipped in 78°C pickling brine for 90 s) achieved additional reductions ($P<0.05$) of 2.3 and 4.0 log cfu/cm^2 with SMAC and TSA, respectively; net reductions for both pre-drying steps were 3.1 and 4.1 log cfu/cm^2, respectively. Pre-drying step 2 of VW-M treatment (slices marinated at 4°C for 24 h) resulted in no significant bacterial reduction/change (+0.1 and −0.2 log cfu/cm^2 with SMAC and TSA, respectively) and resulted in total reductions of 0.7 and 0.8 log cfu/cm^2. When the pre-drying steps were reversed (M-VW), the second step, dipping slices in 57.5°C vinegar/water, resulted in significant ($P<0.05$) bacterial reductions of 0.8 and 0.7 log cfu/cm^2 with SMAC and TSA, respectively; corresponding total pre-drying reductions were 0.7 and 0.5 log cfu/cm^2. These results do not agree with those of Entani, Asai, Tsujihata, Tsukamoto, and Ohta (1998), who observed a 3 log decrease in viable cell numbers of EHEC O157:H7 NGY-10 after 50 s of dipping in a 2.5% acetic acid solution held at 50°C. It may be that that shorter exposure time to the 2.5% acetic acid solution used in the present study (57.5°C, 20 s) limited the effect of the acetic acid. Entani et al. (1998) reported greater inactivation of *E. coli* O157:H7 at higher temperatures, higher concentrations of acetic acid and with longer exposure time. Further work is needed to determine if using a higher concentration of acetic acid, higher dipping temperature and/or longer dipping time would have increased bacterial inactivation on jerky products.

3.3. Effects of drying

After 4 h of drying, the BW-M treatment achieved additional reductions of 2.5 and 2.2 log cfu/cm^2 with SMAC and TSA, respectively; corresponding total reductions were 3.8 and 4.2 log cfu/cm^2 (Table 1). The S-HP treatment method achieved bacterial reductions (2.5 and 1.7 log cfu/cm^2 with SMAC and TSA, respectively) during the first 4 h of drying that were similar to the BW-M treatment, but corresponding total reductions (5.6 and 5.8 log cfu/cm^2) were higher given the greater effectiveness of the S-HP pre-drying treatment. The VW-M and M-VW treatments achieved additional reductions of 3.6–4.2 cfu/cm^2 during the first 4 h of drying, depending on treatment and agar media, with total reductions at 4 h of 4.4–4.9 log cfu/cm^2.

Very little bacterial destruction occurred between 4 and 10 h of drying for any of the treatments (0.0 and 0.8 log cfu/cm^2). For the BW-M treatment, total reductions after 10 h of drying were 4.5 and 4.3 log cfu/cm^2 for SMAC and TSA, respectively (Table 1). Total reductions were somewhat higher for the two vinegar/water-marinade treatments (4.7–5.2 log cfu/cm^2, respectively), depending on treatment order and culture media. The S-HP treatment resulted in the highest total bacterial reduction after 10 h of drying (5.7–5.8 log cfu/cm^2).

Data of this study are in partial agreement with results from Harrison and Harrison (1996) and Harrison et al. (1998). Harrison and Harrison (1996) found that pre-heated (71.1°C) and unheated beef strips had a 5-log reduction when dried at 60°C (140°F) for 10 h in a home-type dehydrator. In this study, meat seasoned for 24 h, then heated in a salt–sugar brine at 78°C (172°F) for 90 s and dried for 10 h at 62.5°C (145°F) achieved ⩾5-log reduction (5.7–5.8 log cfu/cm^2). However, beef slices pre-heated in boiling water at 94°C (203°F) for 15 s, then marinated before drying did not achieve a 5-log reduction (4.3–4.5 log cfu/cm^2). Differences in time and temperature between the two studies could have accounted for differences in reductions of bacterial populations. In addition, the marinating step (4°C, 24 h) following the boiling water dip could have allowed sufficient time for the heat-shocked bacteria to regain resistance.

Harrison et al. (1998) evaluated ground beef jerky prepared with a spice (ingredients not listed)/cure (salt and sodium nitrite) mix and dried at 60°C (140°F) for either 6 or 8 h. The authors indicated that reductions of 5.2 logs cfu/g were achieved in pre-heated and unheated jerky containing the spice and cure mix. In this study, a cure mix was not evaluated, but the S-HP treatment relied heavily on spices (salt, sugar and black pepper) in both steps and it was the only treatment that achieved ⩾5-log reduction following drying. It is presumed that the combination of pre-drying steps 1 and 2, seasoning with pickling spices (4°C, 24 h) and dipping in hot pickling brine (78°C, 90 s), were responsible for the significant ($P<0.05$) bacterial reductions seen. It should be noted that previous research has not been done at higher elevations (4900 ft) or lower relative humidity; thus, these geographical factors (elevation and humidity) may account for some of the differences seen.

For all treatments, populations of surviving bacteria reached a plateau after 4 h of drying. Even after 10 h of drying, a residual population of cells was not destroyed (0.8–2.7 log cfu/cm^2). Perhaps, there was no further destruction of bacteria due to casehardening (McWilliams, 1993) occurring on the surface of the jerky slices when

dried at low heat (≤62.5°C) for several hours (0–4 h). As the temperature of circulating air within the dehydrator continued to climb toward the desired temperature (62.5°C), initial drying may have created a protective coating (McWilliams, 1993), making continuous destruction unachievable. This effect may have been enhanced by the dry climate of Colorado. The relative humidity (Thermo-Hygro, VWR Scientific Products, West Chester, PA, USA) within and outside the dehydrators was 4–10% and 19–24%, respectively. The tailing effect may have also been due to a resistant subpopulation of bacteria that may have been present in the inoculum. Additional studies are needed to evaluate reasons for and find ways to eliminate surviving bacteria during drying of beef jerky.

3.4. Effect of storage

Limited work has been done on the survival of inoculated *E. coli* O157:H7 during storage of jerky. Harrison and Harrison (1996) found no detectable levels of *E. coli* O157:H7 by direct plating or enrichment following 8 weeks of storage at 25°C in sterile desiccators containing sodium chloride (NaCl) solutions at 26.4%, 19.0% and 9.3% (a_w of 0.75, 9.84 and 0.94, respectively). In this study, jerky was analysed following storage at 21°C with room relative humidity of 19–24% for 30, 60 and 90 d (Table 1). For all jerky treatments, significant ($P<0.05$) bacterial reductions were achieved and counts became undetectable (<1.0 log cfu/cm^2) by direct plating after 30 d of storage, and remained so through 90 d of storage.

3.5. Product water activity and pH

It is essential that beef jerky be dried to an a_w acceptable (<0.68) for proper shelf-life (Leistner, 1987). In this study, average a_w values ranged from 0.93 to 0.94 following inoculation and remained relatively constant through both pre-drying steps (Table 2). At 4 h of drying, mean a_w values had declined to 0.84–0.89 and continued to steadily decline throughout the remainder of the drying period. The S-HP treatment product reached the target a_w of 0.68 by 8 h of drying and the two vinegar/water-marinade treatment products by 10 h; however, the a_w of the BW-M treatment product was still 0.75 after 10 h of drying, indicating a product that was not shelf stable. The a_w of the BW-M product did continue to decline somewhat with storage (0.64a_w at 90 d). Still, there was evidence of mold growth on some of BW-M pieces during aerobic storage at ambient temperature (21°C).

In this study, application of heat and marinade (BW-M treatment) and pickling spices and heat (S-HP treatment) did not affect pH; values throughout drying and storage generally ranged from 5.7 to 6.0, which is

Table 2
Effect of beef jerky pre-drying treatment, drying (62.5°C; 0–10 h) and storage (21°C; 30–90 d) on mean water activity (a_w) values of *E. coli* O157:H7 inoculated beef slices[a]

Jerky treatment	Pre-drying		Drying time (h)					Storage (d)		
	Following inoculation (4°C, 24 h)	Following pre-drying step 1	Following pre-drying step 2	4	6	8	10	30	60	90
BW-M[b]	0.94 (0.00)	0.94 (0.00)	0.93 (0.00)	0.89 (0.04)	0.87 (0.03)	0.89 (0.02)	0.75 (0.11)	0.73	0.67	0.64
S-HP[c]	0.93 (0.01)	0.93 (0.01)	0.93 (0.00)	0.84 (0.02)	0.70 (0.05)	0.68 (0.00)	0.59 (0.21)	0.65	0.63	0.61
VW-M[d]	0.93 (0.00)	0.92 (0.00)	0.93 (0.00)	0.84 (0.02)	0.81 (0.02)	0.76 (0.04)	0.50 (0.04)	0.61	0.55	0.54
M-VW[e]	0.94 (0.01)	0.93 (0.00)	0.93 (0.01)	0.87 (0.00)	0.81 (0.02)	0.71 (0.10)	0.59 (0.02)	0.62	0.53	0.52

[a] Means represent one value from two dehydrators ($n = 2$) (standard deviation) for pre-drying and drying time (4–10 h) and one dehydrator ($n = 1$) for 30–90 d storage.
[b] Pre-drying step 1: dipping in boiling water (95°C, 15 s); pre-drying step 2: marinating (4°C, 24 h).
[c] Pre-drying step 1: seasoning with pickling spices (4°C, 24 h); pre-drying step 2: dipping in hot pickling brine (78°C, 90 s).
[d] Pre-drying step 1: dipping in warm vinegar-water (750/750 mL) solution (57.5 C, 20 s); pre-drying step 2: marinating (4°C, 24 h).
[e] Pre-drying step 1: marinating (4°C, 24 h); pre-drying step 2: dipping in warm vinegar-water (750/750 mL) solution (57.5°C, 20 s).

typical of raw whole muscle beef (McWilliams, 1993). Application of the marinade and vinegar/water dip (VW-M and M-VW treatments) did result in small decreases in pH (0.42–0.55), but the pH of jerky slices remained above 5.0 (5.1–5.6) throughout drying and storage (Table 3).

4. Conclusions

Because of mandates from the USDA-FSIS (Reed, 1995; Billy, 1997), manufacturers of dried and semi-dried fermented meats are required to document a 5-log reduction in bacteria during preparation and processing to ensure the safety of the meat products. Using that mandate as a guideline, four jerky processing techniques, each using the "multi-hurdle" concept of two pre-drying treatment steps were evaluated for their ability to enhance inactivation of bacteria. After 4 h of drying, significant ($P<0.05$) destruction was achieved for all treatments. Although, bacterial counts continued to decline slowly during the 10 h of drying, a $\geqslant$5-log reduction (5.7–5.8 log cfu/cm^2) was only achieved for the S-HP treatment (seasoned, then dipped in 78°C pickling brine). After 10 h of drying, the BW-M, VW-M, and M-VW treatments had log cfu/cm^2 reductions of 4.3–4.5, 4.9–5.2, and 4.7–4.8, respectively.

Thus, based on the conditions and results of this study, the S-HP treatment is recommended for preparation of home-dried jerky, providing the consumer finds the product acceptable. The boiling water-marinade treatment is not recommended as it did not achieve a 5-log reduction and resulted in a product with a relatively high a_w value (0.75) at the end of 10 h drying. Dipping in a vinegar/water (750/750 mL) solution, proceeded or followed by marinating showed promise as an acceptable treatment as log reductions of 4.7–5.2 cfu/cm^2 were achieved. Future work should further investigate these treatments, possibly to improve destruction by modifying exposure, temperature, and/or levels of acetic acid (vinegar) used. Acidulates, such as ascorbic or citric acid, could be investigated as supplements or substitutions. After 30 d of storage, counts in all jerky treatments (<1.0 log cfu/cm^2) were significantly ($P<0.05$) lower than those at 0 d (0.8–2.7 log cfu/cm^2). This observation also needs further confirmation.

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Table 3
Effect of beef jerky pre-drying treatment, drying (62.5°C; 0–10 h) and storage (21°C; 30–90 d) on mean pH values of *E. coli* O157:H7 inoculated beef slices[a]

Jerky treatment	Pre-drying		Drying time (h)					Storage (d)		
	Following inoculation (4°C, 24 h)	Following pre-drying step 1	Following pre-drying step 2	4	6	8	10	30	60	90
BW-M[b]	5.56 (0.00)	5.78 (0.00)	5.46 (0.18)	5.73 (0.04)	5.74 (0.00)	6.04 (0.10)	5.92 (0.14)	6.22	5.86	5.66
S-HP[c]	5.83 (0.10)	5.76 (0.07)	5.91 (0.00)	6.09 (0.06)	6.04 (0.01)	6.23 (0.06)	6.00 (0.13)	6.05	6.02	5.97
VW-M[d]	5.75 (0.04)	5.38 (0.11)	5.20 (0.00)	5.52 (0.06)	5.54 (0.01)	5.60 (0.05)	5.48 (0.05)	5.46	5.22	5.48
M-VW[e]	5.68 (0.19)	5.46 (0.12)	5.26 (0.07)	5.43 (0.05)	5.48 (0.20)	5.45 (0.20)	5.39 (0.01)	5.45	5.14	5.56

[a] Means represent triplicate readings from one sample each from two dehydrators ($n = 2$) (standard deviation) for pre-drying and drying time (4–10 h) and one dehydrator ($n = 1$) for 30–90 d storage.
[b] Pre-drying step 1: dipping in boiling water (95°C, 15 s); pre-drying step 2: marinating (4°C, 24 h).
[c] Pre-drying step 1: seasoning with pickling spices (4°C, 24 h); pre-drying step 2: dipping in hot pickling brine (78°C, 90 s).
[d] Pre-drying step 1: dipping in warm vinegar-water (750/750 mL) solution (57.5°C, 20 s); pre-drying step 2: marinating (4°C, 24 h).
[e] Pre-drying step 1: marinating (4°C, 24 h); pre-drying step 2: dipping in warm vinegar-water (750/750 mL) solution (57.5°C, 20 s).
All raw pre-inoculated meat ranged in pH from 5.60 to 5.90.

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