

Validation of a Commercial Process for Inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on the Surface of Whole Muscle Beef Jerky[†]

ANNA C. S. PORTO-FETT, JEFFREY E. CALL, AND JOHN B. LUCHANSKY*

U.S. Department of Agriculture, Agricultural Research Service, Eastern Research Regional Center, Microbial Food Safety Research Unit, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

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ABSTRACT

We validated the lethality of three time and temperature regimens for commercial processing of whole muscle beef jerky. A total of ca. 8.9 log CFU per strip of multiple-strain cocktails of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, or *Listeria monocytogenes* were separately applied onto the surface of beef strips that were treated as follows: (i) inoculated but not marinated or (ii) inoculated and then marinated. A total of three beef strips for each treatment in each of three trials were separately inoculated with a cocktail of one of the three pathogens and placed on the top, middle, and bottom racks of a loading truck. The strips on the rack were loaded into a commercial smokehouse and cooked and dried for 1.5, 2.5, or 3.5 h at a target temperature of 180°F (82.2°C) with constant (natural hickory) smoking, but without the addition of humidity. Regardless of how the strips were treated or where the strips were placed on the loading rack, drying for 1.5, 2.5, or 3.5 h to a target temperature of 180°F (average of 177.2 ± 5.6°F [80.7 ± 3.1°C]), with constant smoke at an initial average relative humidity of 63.1% to a final average relative humidity of 20.9% resulted in a decrease of ≥7.3 log CFU per strip (≥6.9 log CFU/g) for each of the three pathogen cocktails. Of note, marinated strips that were cooked and dried for 2.5 and 3.5 h or nonmarinated strips cooked or dried for 3.5 h also satisfied the U.S. Food Safety and Inspection Service standard of identity (moisture-to-protein ratio ≤ 0.75:1) and/or shelf-stability (water activity ≤ 0.8) requirements for jerky.

Jerky is a very popular, highly nutritious, and shelf-stable ready-to-eat (RTE) dried meat product that is typically stored at ambient temperature (5, 6, 14, 35). Beef and poultry jerky account for \$305 million of the \$2 billion U.S. meat snack industry (<http://www.meatnews.com>). American households purchase some type of meat snack at least 3.5 times per year, with a yearly consumption of one pound per capita (<http://www.meatnews.com>). According to the U.S. Department of Agriculture, the standard of identity for jerky requires that it have a moisture-to-protein ratio (M:Pr) value of ≤0.75:1, whereas to be shelf stable, jerky must have a water activity (a_w) value of ≤0.8 (35, 36). If cooked or dried to an a_w of ≤0.8, jerky has an estimated shelf life of approximately 2 years when vacuum packaged and stored at ambient temperature. Both the safety and shelf stability of jerky-type products are achieved through the collective interaction of a_w , pH, chemical composition of the marinade, type and delivery method of smoke, and/or the time, temperature, and relative humidity (RH) maintained during the cooking and drying steps.

Although it is a shelf-stable RTE meat product, due to improper processing and/or postprocess contamination hu-

man illnesses caused by both *Salmonella* (8, 10, 31) and *Escherichia coli* O157:H7 (21) have been linked to commercial whole muscle beef and homemade venison jerky, respectively. There have also been at least five recalls of beef jerky over the past 7 years due to contamination with *Salmonella* and/or *Listeria monocytogenes* (<http://www.fsis.usda.gov/OA/recalls>). In response to these illnesses and recalls, the U.S. Food Safety and Inspection Service (FSIS) has questioned whether commercial establishments are using cooking and drying processes that are sufficient to destroy foodborne pathogens (35). The FSIS has also published a proposed rule that would require manufacturers to validate that their processes achieve a 5-log reduction for *E. coli* O157:H7 and a 6.5-log reduction for *Salmonella*, as well as achieve zero tolerance for *L. monocytogenes* in RTE red meat products (34, 35).

Although several studies have evaluated the lethality of various jerky processes toward foodborne pathogens, the majority of these experiments evaluated home-type dehydrators that rely on relatively lower temperatures (120 to 155°F [48.9 to 68°C]) and longer processing times (from 4 to 20 h) compared with commercial jerky processing, which typically relies on relatively higher temperatures (≥170°F [≥76.7°C]) and shorter processing times (for up to 3 h) (3, 5, 6, 11, 14). Therefore, the objective of this study was to evaluate and validate commercially relevant time and temperature parameters for lethality toward cells of *E. coli*

* Author for correspondence. Tel: 215-233-6620; Fax: 215-233-6581; E-mail: john.luchansky@ars.usda.gov.

[†] Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

TABLE 1. Strains of *L. monocytogenes*, *Salmonella Typhimurium*, and *E. coli* O157:H7 used in this study

Bacterial strain	Strain	Other designation	Source	Type ^a	Reference
<i>L. monocytogenes</i>	MFS2		Environmental isolate from a pork processing plant	1/2a	27
	MFS102	H7776	Frankfurter isolate, 1998 Bil Mar outbreak	4b	9
			Clinical isolate, 1983 Massachusetts pasteurized-milk outbreak	4b	23
	MFS104	ScottA	Beef and pork sausage isolate	4b	12
	MFS110	F6854	Turkey frankfurter isolate	1/2a	29
<i>Salmonella Typhimurium</i>	JBL3266	H3278	Clinical isolate, CDC	DT104b	20
	JBL3267	G7601	Clinical isolate, CDC	U302	20
	JBL3268	H3402	Clinical isolate, CDC	DT104	20
	JBL3269	H2662	Clinical isolate, CDC	DT104b	20
	JBL3270	H3380	Clinical isolate, CDC	DT104	20
	JBL3271	G8430	Clinical isolate, CDC	U302	20
	<i>E. coli</i>	JBL1411	EC505B	Beef isolate, FRI University of Wisconsin Clinical isolate, 1991 Massachusetts apple cider out- break	O157:H7
JBL2139		C7927	Clinical isolate, 1994 Wisconsin daycare outbreak	O157:H7	1
JBL2347		SLH21788	Meat outbreak	O157:H7	28
		USDA-011-82	Salami isolate, 1994 fermented salami outbreak	O157:H7	33
		USDA-380-94		O157:H7	33

^a Serotypes for *L. monocytogenes* and *E. coli* strains, phage types for *Salmonella Typhimurium* strains.

O157:H7, *Salmonella Typhimurium*, and *L. monocytogenes* surface inoculated onto marinated and nonmarinated strips of whole muscle beef jerky that were subsequently cooked or dried at a target temperature of 180°F (82.2°C) for 1.5 to 3.5 h.

MATERIALS AND METHODS

Bacterial strains and preparation of inoculum. The five isolates of *L. monocytogenes*, six isolates of *Salmonella Typhimurium*, and five isolates of *E. coli* O157:H7 used in this study to inoculate whole muscle beef strips are listed in Table 1. The individual strains of each pathogen were maintained at -80°C in brain heart infusion broth (BHI; Difco, Becton Dickinson, Sparks, Md.) plus 10% glycerol (Sigma-Aldrich, St. Louis, Mo.). A portion ($\leq 100 \mu\text{l}$) of a semifrozen suspension of each strain of each pathogen cocktail was separately streak plated onto BHI agar and incubated overnight at 37°C. A single colony of each strain was then individually transferred into 50 ml of BHI and incubated at 37°C with shaking (100 rpm) for 24 h. A 100- μl volume was transferred into 50 ml of fresh BHI and incubated at 37°C for 18 h with shaking to attain a population of primarily stationary-phase cells. The cells were harvested by centrifugation at $4,000 \times g$ for 5 min at 4°C, and then washed and resuspended in 10 ml of sterile peptone water (0.1%; Difco, Becton Dickinson). To prepare the cocktail, approximately equal volumes of cell suspensions of each strain for a given pathogen were combined, and the final volume was adjusted to 30 ml with sterile peptone water to yield ca. 9.5 log CFU/ml. Prior to each experimental trial, inoculum levels were quantified by spread plating a 100- μl portion of the freshly prepared cocktails onto duplicate sorbitol MacConkey (SMAC; Difco, Becton Dickinson), xylose lysine Tergitol 4 (XLT4; Difco, Becton Dickinson), or modified Oxford (MOX; Difco, Becton Dickinson) agar plates for *E. coli* O157:H7, *Salmonella Typhimurium*, or *L. monocytogenes*, respectively. The SMAC and XLT4 agar plates were incubated at 37°C for 24 h, and the MOX agar plates were incubated at 37°C for 48 h.

Inoculation of beef strips and processing of whole muscle beef jerky. Between January and February of 2007, three batches

(one batch per trial) of frozen, raw, whole muscle, top eye rounds of beef were sliced by our collaborator (Wild Bill's Foods, Inc., Leola, Pa.) into strips (25 cm long, 2.75 cm wide, and 5.5 mm high) weighing on average ca. 37.2 ± 4.4 g and stored at -18°C for up to 5 days. Prior to inoculation, the beef strips were thawed at 4°C for ca. 24 h. The beef strips were then subjected to one of the following treatments: (i) inoculated but not marinated or (ii) inoculated and then marinated in bulk in a sealed bag that was tumbled manually for 2 min at ambient temperature ($73.4 \pm 3.6^\circ\text{F}$ [$23 \pm 2^\circ\text{C}$]) and held for 13 min at 4°C in a nonacidic ($\sim\text{pH}$ 5.5) marinade solution applied at a ratio of 18% (vol/wt) relative to the weight of the meat. The commercial marinade solution was formulated with soy sauce (water, salt, hydrolyzed soy protein, corn syrup, caramel color, and potassium sorbate), and seasoning powder (black pepper, red pepper, and garlic powder) that was supplied and prepared as recommended by Wild Bill's Foods.

Prior to marinating, individual beef strips were aseptically weighed and placed on sterile polystyrene foam packing trays (Koch Supplies, Kansas City, Mo.) and separately inoculated with 0.5 ml of the multistrain cocktails of *E. coli* O157:H7, *Salmonella Typhimurium*, or *L. monocytogenes* on one side of each strip. The inoculum was distributed across the entire surface area of each strip with the aid of a sterile, L-shaped plastic cell spreader (MID-SCI, St. Louis, Mo.). The uncovered trays were placed into a laminar flow hood for ca. 15 min at ambient temperature ($73.4 \pm 3.6^\circ\text{F}$) to allow the bacteria to better attach to the meat. The beef strips were then flipped over, and the inoculation procedure was repeated on the opposite side. As such, each strip was separately inoculated with each pathogen to a target level on average of ca. 8.9 log CFU per strip, that being equal to ca. 7.3 log CFU/g.

Next, the inoculated and noninoculated beef strips, with and without marinating, as well as additional filler strips of meat, were strung on stainless steel skewers that were hung vertically on the top, middle, and bottom levels of a three-level loading truck to fill the smokehouse to capacity. The loading truck was then wheeled into a commercial, one-truck smokehouse (volume = ca. 5,088 ft³ [472.7 m³]; Vortron Smokehouse model TR2-1700, Kusel Equipment Co., Beloit, Wis.) for cooking and drying. Smoke was supplied using hickory wood chips (Gregory General Farms,

Java, Va.) and an external smoke generator (model 32 03 22, Koch Supplies). The entire volume of air inside the smokehouse was exchanged ca. 40 to 50 times per min. When the smokehouse chamber achieved the target dry-bulb temperature of 180°F, the beef strips were dried for 1.5, 2.5, or 3.5 h, with constant smoke and with the dampers completely open. The come-up-time (CUT) temperature of the air within the smokehouse was monitored and recorded at 60-s intervals, using a stainless steel, type K thermocouple placed on the middle level of the loading truck (temperature range: -328 to 2,282°F [-200 to 1,250°C], accuracy: $\pm 2^\circ\text{F}$ [$\pm 1.1^\circ\text{C}$]; Omega Engineering Inc., Stamford, Conn.). This thermocouple was separately connected to a six-channel digital panel temperature indicator (model 500T, Doric Instruments, VAS Engineering, Inc., San Diego, Calif.). Data were recorded manually until the target temperature (180°F) of the air within the smokehouse was achieved before the cooking and drying period was timed. However, during cooking and drying, the temperature of the air and the relative humidity (RH) within the smokehouse were recorded using two temperature-RH combination probes (temperature range: -40 to 185°F [-40 to 85°C], accuracy: $\pm 0.8^\circ\text{F}$ [$\pm 0.44^\circ\text{C}$]; RH range: 0 to 100%, accuracy: $\pm 2\%$) connected to data loggers (model TM325, Dickson, Addison, Ill.) that were placed on both the high and low levels of the loading truck. The air temperature and the RH were measured and recorded every 30 s during CUT and throughout cooking and drying by each of the two temperature-RH probes for each of three trials for the following three cooking and drying regimens: 1.5 h (326 measurements during CUT and 1,080 measurements during cooking and drying, for a total of 1,406 measurements), 2.5 h (262 measurements during CUT and 1,800 measurements during cooking and drying, for a total of 2,062 measurements), and 3.5 h (276 measurements during CUT and 2,520 measurements during cooking and drying, for a total of 2,796 measurements). The internal temperature of the beef strips was also monitored continuously using a 12-bit temperature probe (range: -40 to 212°F [-40 to 100°C], accuracy: $\pm 0.36^\circ\text{F}$ [$\pm 0.2^\circ\text{C}$]; HOBO S-TMB-M0XX, Onset Computer, Bourne, Mass.) inserted at the center of one strip that was placed toward the middle level of the loading truck and connected to a data logger (HOBO FlexSmart H22, Onset Computer). The temperature of the meat was measured and recorded every 30 s during the CUT and during cooking and drying for each of three trials over the course of 1.5 h (163 measurements during CUT and 540 measurements during cooking and drying, for a total of 703 measurements), 2.5 h (131 measurements during CUT and 900 measurements during cooking and drying, for a total of 1,031 measurements), and 3.5 h (138 measurements during CUT and 1,260 measurements during cooking and drying, for a total of 1,398 measurements). After cooking and drying, the strips on the loading truck were cooled (10 to 15 min) to room temperature (ca. 72°F [22.2°C]) in the smokehouse by opening the door. The strips were then aseptically weighed and transferred into sterile bags (B00736WA, Nasco, Modesto, Calif.) and held on ice until further processed.

Microbiological analyses. For enumeration of the initial populations of total aerobic bacteria and total lactic acid bacteria (LAB) on marinated and nonmarinated, uncooked whole muscle beef strips, a total of three beef strips for each of the three batches of jerky were analyzed ($n = 3$ batches, $n = 3$ strips per batch; 9 strips total). Total aerobic bacteria and total LAB were recovered by transferring the marinated or nonmarinated uncooked strips (ca. 37.2 g each) to a filtered stomacher bag, adding 45 ml of sterile peptone water, and macerating by stomaching for 2 min (Stomacher 400, Seward, Cincinnati, Ohio). The resulting fluid was

transferred to a sterile screw-cap conical centrifuge tube, with the aid of a sterile pipette. Total aerobic bacteria were enumerated by spread plating the resulting fluid, with or without prior dilution in sterile peptone water, onto BHI agar plates and incubating for 72 h at 30°C. For enumeration of total LAB, the fluid was spread plated, with or without prior dilution in sterile peptone water, onto deMan Rogosa Sharp (Difco, Becton Dickinson) agar plates and incubated anaerobically (10.1% carbon dioxide, 4.38% hydrogen and balance nitrogen; Bactron IV anaerobic/environmental chamber, Sheldon Manufacturing, Inc., Cornelius, Oreg.) for 48 h at 37°C.

E. coli O157:H7, *Salmonella* Typhimurium, or *L. monocytogenes* were recovered by adding sterile peptone water to both uncooked (45 ml) and cooked (15 ml) whole muscle beef strips and massaging by hand for 2 min. The resulting rinsate was transferred to a sterile screw-cap conical centrifuge tube. Pathogen levels were determined by serial diluting the rinsate in sterile peptone water as needed and surface plating onto SMAC, XLT4, or MOX agar plates, as described above. After incubation, typical colonies of each pathogen on representative plates were counted manually. When levels of the three pathogens decreased to below detection (≤ 1.2 log CFU per strip or ≤ 0.4 log CFU/g) by direct plating, their presence or absence was determined by enrichment as previously described (18, 20, 26). For each of three trials, a total of nine noninoculated beef strips and nine beef strips that were inoculated with *E. coli* O157:H7, *Salmonella* Typhimurium, or *L. monocytogenes* (three strips placed on each level of the loading truck for each pathogen type) for each treatment and for each cooking and drying regimen were sampled. Bacterial numbers were expressed as log CFU per strip.

Proximate composition of whole muscle beef jerky. The fat, carbohydrate, ash, moisture, protein, salt, pH, and a_w values of whole muscle beef jerky were determined in two of the three trials using a 60-g composite sample comprising one noninoculated strip obtained from each of the three levels of the loading truck ($n = 2$ trials, $n = 3$ strips per trial) for both marinated and nonmarinated strips. The analyses were performed according to methods approved and described by the AOAC International (formerly the Association of Official Analytical Chemists (25)), as conducted by a commercial testing laboratory.

Statistical analyses. Data were analyzed with version 9.1.3 of the SAS statistical package (SAS Institute, Inc., Cary, N.C.). Analysis of variance was performed to evaluate the effects and interactions of marinating on proximate composition of whole muscle beef jerky dried for 1.5, 2.5, or 3.5 h at a target temperature of 180°F. Mean separations were performed using the Bonferroni least significant difference method.

RESULTS

Initial levels of total aerobic bacteria and total LAB in raw, whole muscle beef strips. Direct plating and/or enrichment of three batches of raw whole muscle beef strips that were not inoculated and/or marinated revealed the absence of any indigenous *E. coli* O157:H7, *Salmonella* Typhimurium, or *L. monocytogenes* (data not shown). In addition, the initial populations of total aerobic bacteria or total LAB were relatively similar among batches or treatments ($n = 3$ batches, $n = 3$ raw beef strips per batch); the average total aerobic and LAB levels on marinated raw, whole muscle beef strips were 3.93 and 3.73 log CFU per strip (2.36 and 2.16 log CFU/g), respectively, whereas for

nonmarinated raw whole muscle beef, the total aerobic and LAB initial populations were 4.16 and 3.62 log CFU per strip (2.59 and 2.05 log CFU/g), respectively.

Proximate composition of raw meat and whole muscle beef jerky cooked and dried at 180°F for 1.5, 2.5, or 3.5 h. The proximate composition of marinated and nonmarinated whole muscle beef jerky strips that were dried at a target temperature of 180°F for 1.5, 2.5, or 3.5 h are presented in Table 2. Neither marinating nor cooking and drying significantly ($P > 0.05$) affected the pH of the final product. The average final pH values for marinated and nonmarinated beef jerky were 5.42 ± 0.12 and 5.45 ± 0.12 , respectively. As expected, the salt and ash content were significantly ($P < 0.05$) higher in marinated beef jerky than in beef jerky strips that were not marinated. Regardless of whether strips were marinated or nonmarinated, proximate composition per se had no significant effect on lethality given that a reduction of ≥ 7.3 log CFU per strip (≥ 6.9 log CFU/g) was achieved for all treatments (see below).

The results showed a significant ($P < 0.05$) effect for both marinating and cooking time on total protein levels. The M:Pr ratio values did not differ significantly ($P > 0.05$) for marinated and nonmarinated jerky. However, cooking time did have a significant effect ($P < 0.05$) on the M:Pr ratio; the longer the cooking time, the lower the M:Pr ratio. More importantly, M:Pr values of $\leq 0.75:1$ ratio were achieved for all treatments, except for marinated beef strips that were cooked and dried for 1.5 h. A significant ($P < 0.05$) interaction between marinating and cooking time was observed for salt, ash, and a_w . Marinating did not significantly ($P > 0.05$) affect the a_w of the final product; however, a significant effect ($P < 0.05$) of cooking was observed of a_w values for the marinated beef strips. For marinated beef strips, the a_w decreased from 0.98 to ≤ 0.67 after cooking and drying for 2.5 or 3.5 h, whereas for marinated beef strips cooked and dried for 1.5 h, the a_w decreased from 0.98 to 0.82. However, for nonmarinated beef strips that were cooked and dried at 180°F for 1.5, 2.5, or 3.5 h, the a_w decreased from 0.98 to 0.93, 0.86, and 0.72, respectively. These data show that only marinated beef strips cooked and dried for 2.5 or 3.5 h and nonmarinated beef strips cooked and dried for 3.5 h to a target temperature of 180°F achieved the FSIS recommended M:Pr ($\leq 0.75:1$) and a_w (≤ 0.8) values to be designated as shelf-stable beef jerky (35).

CUT, internal meat temperature, smokehouse temperature, and RH during cooking and drying of whole muscle beef jerky. The average CUT to achieve the target air and smokehouse temperature of 180°F was 24 ± 5.4 min ($n = 3$ trials, $n = 1$ measurement of 180°F per trial) (Table 3). For each of the three trials, measurements were taken at 30-s intervals for the internal temperature of the jerky, the air temperature, and the RH, and the data were averaged (Table 3). During cooking and drying, the average temperature of the air inside the smokehouse was $177.2 \pm 5.6^\circ\text{F}$ ($80.7 \pm 3.1^\circ\text{C}$). This temperature is the average of 5,400 total temperature measurements and represents all

TABLE 2. Proximate composition of marinated and nonmarinated whole muscle beef jerky strips^a

Analyses	Marinated cooking and drying time (h) ^b :					Nonmarinated cooking and drying time (h) ^b :						
	0	1.5	2.5	3.5	0	1.5	2.5	3.5	0	1.5	2.5	3.5
Salt (g/100 g)	2.24 ± 0.22 C ^c	4.29 ± 1.93 AB	7.18 ± 0.01 A	5.26 ± 0.66 AB	0.01 ± 0 BC	0.04 ± 0 C	0.08 ± 0.05 C	0.09 ± 0.02 C	0.01 ± 0 BC	0.04 ± 0 C	0.08 ± 0.05 C	0.09 ± 0.02 C
pH	5.37 ± 0.14 A	5.34 ± 0.11 A	5.40 ± 0.04 A	5.53 ± 0.18 A	5.49 ± 0.30 A	5.37 ± 0.01 A	5.40 ± 0.13 A	5.60 ± 0.06 A	5.49 ± 0.30 A	5.37 ± 0.01 A	5.40 ± 0.13 A	5.60 ± 0.06 A
a_w	0.98 ± 0.01 A	0.82 ± 0.07 AB	0.67 ± 0 B	0.67 ± 0.02 B	0.96 ± 0.01 A	0.93 ± 0.03 AB	0.86 ± 0.06 AB	0.72 ± 0.11 AB	0.96 ± 0.01 A	0.93 ± 0.03 AB	0.86 ± 0.06 AB	0.72 ± 0.11 AB
Moisture (g/100 g)	62.8 ± 14.9 AB	37.4 ± 5.02 BC	22.7 ± 0.99 C	22.5 ± 1.27 C	70.9 ± 1.06 A	35.4 ± 5.37 B	24.5 ± 4.74 C	15.1 ± 4.03 C	70.9 ± 1.06 A	35.4 ± 5.37 B	24.5 ± 4.74 C	15.1 ± 4.03 C
Protein (g/100 g)	24.5 ± 1.63 CD	44.5 ± 5.73 BC	59.2 ± 2.97 AB	63.3 ± 1.13 AB	21.6 ± 1.98 CD	55.9 ± 10.5 AB	69.1 ± 4.88 A	75.7 ± 0.92 A	21.6 ± 1.98 CD	55.9 ± 10.5 AB	69.1 ± 4.88 A	75.7 ± 0.92 A
M:Pr	2.55 ± 0.44 A	0.86 ± 0.22 B	0.38 ± 0 B	0.36 ± 0.02 B	3.29 ± 0.26 A	0.66 ± 0.22 B	0.36 ± 0.09 B	0.20 ± 0.06 B	3.29 ± 0.26 A	0.66 ± 0.22 B	0.36 ± 0.09 B	0.20 ± 0.06 B
Fat (g/100 g)	0.62 ± 0.50 A	6.95 ± 3.45 A	3.25 ± 2.47 A	4.81 ± 1.03 A	1.99 ± 0.95 A	5.54 ± 2.52 A	3.32 ± 0.57 A	7.16 ± 1.11 A	1.99 ± 0.95 A	5.54 ± 2.52 A	3.32 ± 0.57 A	7.16 ± 1.11 A
Carbohydrates (g/100 g)	2.62 ± 0.31 A	1.60 ± 1.56 A	3.56 ± 1.92 A	0.67 ± 0.23 A	2.56 ± 2.91 A	1.38 ± 1.24 A	0.50 ± 0 A	0.50 ± 0 A	2.56 ± 2.91 A	1.38 ± 1.24 A	0.50 ± 0 A	0.50 ± 0 A
Ash (g/100 g)	1.06 ± 0.08 B	7.94 ± 1.88 A	11.3 ± 0.42 A	9.59 ± 0.28 A	3.58 ± 0.13 B	2.88 ± 0.01 B	3.42 ± 0.25 B	3.81 ± 0.18 B	3.58 ± 0.13 B	2.88 ± 0.01 B	3.42 ± 0.25 B	3.81 ± 0.18 B

^a Strips were cooked and dried for 1.5, 2.5, or 3.5 h at an average air temperature of $177.2 \pm 5.6^\circ\text{F}$ ($80.7 \pm 3.1^\circ\text{C}$), an average internal meat temperature of ca. 163.3°F (72.9°C), and with constant smoke at an average RH of 29.3%.

^b Values are the mean of two trials \pm standard deviation. Means represent the results from analyses performed on a composite sample (60 g) taken from one strip from each of three levels on the loading truck in each of the two trials ($n = 2$ trials, $n = 3$ strips per trial).

^c Means with different letters within the same row are significantly ($P < 0.05$) different.

TABLE 3. Relevant processing parameters, proximate composition values, and inactivation of *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* on marinated and nonmarinated whole muscle beef jerky strips^a

Time (h)	Marinated										Nonmarinated				
	<i>Salmonella</i> Typhimurium ^b					<i>L. monocytogenes</i> ^c					<i>Salmonella</i> Typhimurium		<i>L. monocytogenes</i>		
	CUT (min) ^b	Smokehouse air temp (°C) ^c	Jerky internal temp (°C) ^d	Initial RH (%) ^e	Final RH (%) ^e	<i>E. coli</i> ^f	8.55 (±0.05)	9.02 (±0.02)	0.98 (±0.01)	2.55 (±0.44)	8.95 (±0.01)	8.61 (±0.01)	9.24 (±0.02)	0.96 (±0.01)	3.29 (±0.26)
0	NA ^h	15.2 ⁱ (±2.1) [10.3–18.2]	13.2 ^j (±3.3) [8.4–19.3]	63.1 ^k (±7.9)	NA	8.77 (±0.08)	8.55 (±0.05)	9.02 (±0.02)	0.98 (±0.01)	2.55 (±0.44)	8.95 (±0.01)	8.61 (±0.01)	9.24 (±0.02)	0.96 (±0.01)	3.29 (±0.26)
1.5	27.2 (21.5, 24.5, 35.5)	81.1 (±1.2) [79.8–81.5]	67.5 (±4.8) [66.7–68.8]	63.4 (±3.5)	21.9 (±2.6)	≤1.2 [0/27] ^l	≤1.2 [2/27]	≤1.2 [0/27]	0.82 (±0.07)	0.86 (±0.22)	≤1.2 [1/27]	≤1.2 [19/27]	≤1.2 [0/27]	0.93 (±0.03)	0.66 (±0.22)
2.5	21.8 (19.0, 23.0, 23.5)	81.3 (±1.2) [80.9–81.9]	75.1 (±5.3) [72.3–79.2]	63.8 (±13.2)	21.5 (±3.8)	≤1.2 [0/27]	≤1.2 [2/27]	≤1.2 [0/27]	0.67 ^j (±0)	0.38 ^j (±0)	≤1.2 [0/27]	≤1.2 [12/27]	≤1.2 [0/27]	0.86 (±0.06)	0.36 (±0.09)
3.5	23 (19.5, 20.0, 29.5)	81.4 (±3.2) [79.8–80.4]	75.8 (±5.5) [75.2–76]	62.3 (±3.6)	19.2 (±2.7)	≤1.2 [0/27]	≤1.2 [8/27]	≤1.2 [3/27]	0.67 ^j (±0.02)	0.36 ^j (±0.02)	≤1.2 [0/27]	≤1.2 [22/27]	≤1.2 [2/27]	0.72 ^j (±0.11)	0.20 ^j (±0.06)

^a Strips were cooked and dried for 1.5, 2.5, and 3.5 h at an average air temperature of 177.2 ± 5.6°F (80.7 ± 3.1°C), an average internal meat temperature of ca. 163.3°F (72.9°C), and with constant smoke at an average RH of 29.3%.

^b Mean of three trials ($n = 3$ trials, $n = 1$ probe per trial). The CUT temperature of the air in the smokehouse was measured with one type K thermocouple connected to a six-channel digital panel temperature indicator. Temperature measurements were recorded every minute manually. Actual CUT values for each of three trials are listed in parentheses.

^c Mean of three trials (±standard deviation) [range of minimum and maximum temperature]. The air temperature of the oven was measured with two temperature-RH combination probes. The oven temperatures listed in this table are the average of continuous readings taken every 30 s after the CUT was attained for each of the two temperature-RH probes for each trial ($n = 3$ trials, $n = 2$ probes per trial) over 1.5 h (1,080 total measurements), 2.5 h (1,800 total measurements), and 3.5 h (2,520 total measurements).

^d Mean of three trials (±standard deviation) [range of minimum and maximum temperature]. The internal temperature of the beef strips was measured using a 12-bit temperature probe inserted at the center of one strip, which was placed toward the middle level of the loading truck and connected to a data logger. The internal temperatures of the beef strips listed in this table are the average of continuous readings taken every 30 s from when CUT was attained throughout each cooking and drying regimen, using one temperature probe for each trial ($n = 3$ trials, $n = 1$ probe per trial) over 1.5 h (540 total measurements), 2.5 h (900 total measurements), and 3.5 h (1,260 total measurements).

^e Mean of three trials (±standard deviation). The RH was measured with two temperature-RH combination probes. The initial RH values listed in this table are the average of one reading of the RH values inside the smokehouse immediately after closing the door for each of the two temperature-RH probes for each trial ($n = 3$ trials, $n = 2$ probes per trial). The final RH values listed in this table are the average of one reading of the RH inside the smokehouse immediately after cooking and drying regimens were attained for each of the two temperature-RH probes for each trial for each time ($n = 3$ trials, $n = 2$ probes per trial).

^f Mean of three trials (±standard deviation) ($n = 3$ trials, $n = 9$ beef strips per trial) for each pathogen. Detection limit: ≤1.2 log CFU per strip or ≤0.4 log CFU/g. Numbers in brackets are number of positive enrichment samples/27 total samples analyzed ($n = 3$ trials, $n = 9$ beef strips per trial per pathogen).

^g Mean of two trials ± standard deviation. Means represent the results from analyses performed on a composite sample (60 g) taken from one strip from each of three levels on the loading truck in each of the two trials ($n = 2$ trials, $n = 3$ strips per trial).

^h NA, not applicable.

ⁱ The air temperature of the oven, internal temperature of the beef strips, and initial RH at time 0 were measured with two temperature-RH combination probes. The air temperature of the oven, initial RH ($n = 3$ trials, $n = 2$ probes per trial), and internal temperature of the beef strips ($n = 3$ trials, $n = 1$ probe per trial) listed in this table are the average readings taken immediately after closing the smokehouse door for each of the two temperature-RH probes for each trial.

^j Treatments and cooking and drying regimens that meet both the standard of identity (M:Pr ≤ 0.75:1) and shelf stability (a_w of ≤0.8) for jerky (35, 36).

cooking and drying regimens, that being 1.5, 2.5, and 3.5 h, for all three trials, for each of two probes used in each trial for each treatment. The internal temperature of the meat strips ranged from 47.1 to 66.3°F, with an average of 55.7°F (8.4 to 19.1°C, average of 13.2°C) upon placement in the smokehouse to 135.1 to 161.2°F, with an average of 144.3°F (57.3 to 71.8°C, average of 62.4°C) when the CUT was attained. The average internal temperature of beef strips during CUT was 111.4 ± 29.2°F (44.1 ± 16.2°C). This temperature is the average of 432 total temperature measurements for all trials and cooking and drying regimens ($n = 3$ trials, $n = 3$ cooking and drying regimens, that being 1.5, 2.5, and 3.5 h). The average internal temperature of beef strips from when CUT was attained throughout cooking and drying was 163.3 ± 6.1°F (72.9 ± 3.4°C), with a range of 153.5 to 168.4°F (67.5 to 75.6°C). This temperature is the average of 2,700 total temperature measurements and represents all cooking and drying times, that being 1.5, 2.5, and 3.5 h, for all three trials. The average initial RH inside the smokehouse immediately after closing the door was 63.1% ± 7.9%. However, during CUT for the 1.5, 2.5, and 3.5 h cooking and drying regimens, the RH decreased on average to 35.5, 40.8, and 46.5%, respectively. The average RH inside the smokehouse from when CUT was attained throughout cooking and drying was 29.3% ± 8.3%. After cooking and drying, but prior to opening the door of the smokehouse, the final RH was on average 21.9, 21.5, and 19.2% after 1.5, 2.5, and 3.5 h, respectively. The RH data from when CUT was attained throughout cooking and drying represent the average of 5,400 measurements for each cooking and drying regimen in each of three trials, using two probes per trial per each treatment.

Thermal inactivation of *E. coli* O157:H7, *Salmonella* Typhimurium, or *L. monocytogenes* on whole muscle beef jerky. Preliminary tests were conducted to quantify the viability of *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* on beef jerky inoculated before and after marinating, and then cooked and dried at a target air temperature of 180°F for either 2.5 or 3.5 h ($n = 2$ trials, $n = 3$ strips per pathogen per trial). Apart from an immediate decrease in pathogen numbers (ca. 0.06 to 0.22 log CFU per strip) due to contact with the marinade solution (~pH 5.5), there was no significant difference in the recovery of any of the three pathogens relative to whether they were inoculated onto beef strips before or after marinating (data not shown). Therefore, we conducted all further experiments using inoculation before marinating, because the surface of the raw meat represents the most likely source of contamination.

The results showed that for both marinated and non-marinated strips, cooking and drying for 1.5, 2.5, or 3.5 h at a target temperature of 180°F, with exposure to constant hickory smoke with an average initial RH of ca. 63.1% and an average final RH of ca. 20.9%, resulted in a decrease of ≥7.3 log CFU per strip (≥6.9 log CFU/g) for all three pathogens tested (Table 3). However, depending on the duration of the cooking and drying treatment, it was possible

to recover cells of *Salmonella* Typhimurium, *L. monocytogenes*, and *E. coli* O157:H7 by enrichment from 44.4 to 81.5% (12 to 22 of 27 strips), 7.4% (2 of 27 strips), and 3.7% (1 of 27 strips), respectively, of the nonmarinated strips (Table 3). For beef strips that were marinated and cooked and dried for 1.5, 2.5, and 3.5 h at a target temperature of 180°F, cells of *Salmonella* Typhimurium (7.4 to 29.6%; 2 to 8 of 27 jerky strips) and *L. monocytogenes* (11.1%; 3 of 27) were recovered by enrichment, whereas *E. coli* O157:H7 cells were not recoverable even by enrichment (Table 3).

DISCUSSION

Over the past 40 years, jerky products have been associated with at least 276 cases of foodborne illness due to *Salmonella*, *E. coli* O157:H7, or *Staphylococcus aureus* (8, 10, 21). Between 2000 and 2006, the FSIS recalled ca. 23,000 lb of jerky products that were contaminated by *Salmonella* and *L. monocytogenes* (<http://www.fsis.usda.gov/OA/recalls>). In addition, the FSIS reported that between 1990 and 1999 the cumulative prevalence of *Salmonella* and *L. monocytogenes* for meat and poultry jerky produced in federally inspected plants was 0.31 and 0.52%, respectively (22). In 2004, the FSIS issued compliance guidelines specifically for jerky products, which recommended commercial processors to reassess and validate the efficacy of their thermal processes for eliminating foodborne pathogens such as *Salmonella*, *L. monocytogenes*, *E. coli* O157:H7, and/or *S. aureus* (35). According to the FSIS, the attainment of a relatively high RH (≥90%) during the cooking step in dehydrators or smokehouses is critical for assuring sufficient lethality of foodborne pathogens in RTE dried meats such as jerky, because conditions of higher moisture and RH typically result in greater lethality toward pathogens (35). In general, the lower the RH during cooking, the greater the evaporation of moisture from the surface of meat and, in turn, the greater the evaporative cooling at the surface of meat and the lesser the lethality toward foodborne pathogens. Several investigators (2, 13) reported that thermal inactivation of *Salmonella* on the surface of fully cooked, dry-roasted beef was greater when it was cooked to an internal temperature of ≥130°F (≥54.4°C) in a smokehouse with steam injected continuously for at least 30 min compared with otherwise similar samples that were cooked without injection of humidity and steam. Although the FSIS requires that an RH of ≥90% must be maintained continuously throughout the cooking and drying step of jerky processing (35), an RH of ≤90% can be attained and maintained during cooking and drying if establishments monitor and report the RH and validate that their process meets the recommended 5-log reduction for *E. coli* O157:H7 and/or the 6.5-log reduction for *Salmonella*, as well as achieves the zero-tolerance policy for *L. monocytogenes* (30, 34, 35).

Our results revealed that regardless of whether or not strips were marinated, cooking and drying whole muscle beef strips at an average air temperature of 177.2°F, with constant smoke at an initial average RH of 63.1% to a final average RH of 20.9%, resulted in a decrease of ≥7.3 log

CFU per strip (≥ 6.9 log CFU/g) for each of the three pathogens tested. Furthermore, the reduction of a_w to ≤ 0.8 , as well as the continuous generation of hickory smoke during processing, may impart additional lethality toward the three pathogens tested. These results are in general agreement with other studies reporting that a ≥ 6.4 -log reduction in *Salmonella* and *E. coli* O157:H7 numbers was obtained when roast beef or beef jerky were commercially processed at relatively high temperatures ($\geq 170^\circ\text{F}$) and with an RH of $\geq 27\%$. As an example, Buege et al. (3) reported that when whole muscle beef strips were cooked at 170°F in a commercial smokehouse with an RH of 27 to 43% for up to 1 h and then dried for up to 3 h at a dry-bulb temperature of 170°F , a ≥ 6.4 -log CFU reduction per sample of *Salmonella* and *E. coli* O157:H7 was achieved. In another study, Mann and Brashears (24) reported that cooking RTE roast beef in a commercial oven at a constant temperature of 180°F and at an RH of $\geq 30\%$ throughout the process to an internal temperature of 145°F (62.8°C) resulted in a decrease of ≥ 6.5 log CFU/cm² in *Salmonella* numbers. The findings of the above-mentioned studies emphasize the importance of using relatively high temperatures ($\geq 170^\circ\text{F}$) and an initial RH of $\geq 63\%$ to a final RH of $\geq 20\%$ throughout the cooking and drying process to ensure for adequate lethality of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in jerky products.

When home-type dehydrators that rely on relatively lower temperatures ($\leq 140^\circ\text{F}$ [$\leq 60^\circ\text{C}$]) were used for cooking and drying beef jerky, a ≤ 6 -log reduction for selected foodborne pathogens was achieved only after extended (≥ 10 h) cooking and drying. For example, Harrison and Harrison (14) evaluated lethality of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* Typhimurium in marinated whole muscle beef jerky cooked and dried at 140°F for up to 10 h in a home-style dehydrator. After drying beef jerky for 3 h, they observed a 1.8- to 3.3-log reduction, whereas a 5.5- to 6-log reduction was achieved for all three pathogens after 10 h of drying. In similar studies, Harrison et al. (15, 16) reported that *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* populations decreased by ≥ 5.2 , 4, and 4.2 log CFU/g, respectively, in ground and formed beef jerky prepared with the addition of a cure blend after 8 h of drying at 140°F . As a final example, Harrison et al. (17) reported reductions of 3.4 to 4.6 log CFU per strip in levels of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* on both marinated and nonmarinated whole muscle beef jerky heat treated at 140°F to the desired dehydration endpoint as determined (presumably) by visual and/or tactile inspection. However, the authors reported that an additional reduction in pathogen numbers was achieved when beef strips were boiled in a marinade (pH 4.5) solution before drying at 140°F or when marinated beef strips were cooked and dried at 140°F , followed by oven heating at 275°F (135°C) for 10 min. As another example, Faith and colleagues (11) reported reductions of ≥ 5 log in levels of *E. coli* O157:H7 in ground and formed beef jerky, containing 5 or 20% fat, that was cooked and dried in a home-style dehydrator at $\geq 145.4^\circ\text{F}$ (63°C) for 8 h. These data suggested that a lower cooking and drying temperature over a longer processing

time would result in a lower reduction in the levels of foodborne pathogens when compared with those processes that rely on higher temperatures and shorter times. Thus, consumers using home-style dehydrators should heat and dry the product at $\geq 140^\circ\text{F}$ for >8 h to achieve an appreciable reduction in numbers of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*. More importantly, they should control and monitor the accuracy and stability of the temperature within the dehydrator, and if possible, within the meat, with the aid of an accurate thermometer (11, 19).

There have been several studies on the lethality of jerky processes toward *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* (3, 5–7, 11, 14, 17). However, variations in lethality observed in the present study compared with those in other studies may be attributed to differences in the strains used, or their physiological state or their prior acid adaptation, and/or in the microbiological media and incubation conditions used to recover injured cells. It may also be due to differences in the prior exposure of the meat to marinade solutions, differences in proximate composition, differences in the form, shape, and thickness of the meat tested, and/or variations in the conditions and duration of the CUT needed for the meat to reach the target temperatures.

In this study, the use of a marinade ($\sim\text{pH}$ 5.5) did not appreciably reduce pathogen numbers during cooking and drying when compared with strips that were not marinated. Our results are in general agreement with previous studies that showed the extent of inactivation of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* was not significantly different between whole muscle beef strips that were marinated and beef strips that were not marinated. More specifically, Calicioglu and colleagues (5–7) evaluated the efficacy of different predrying marinade solutions on subsequent thermal inactivation of acid-adapted or non-acid-adapted cells of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in whole muscle beef jerky during drying at 140°F for up to 10 h in a home-type dehydrator. Their results showed that prior acid adaptation notwithstanding, pathogen reductions were not significantly greater in strips that were marinated in a solution of ca. pH 5.3 (3.1 to 5.0 log CFU/cm²) compared with otherwise similar strips that were not marinated ($\sim\text{pH}$ 5.6; 3.1 to 5.3 log CFU/cm²). However, acidification of a traditional marinade (soy sauce, Worcestershire sauce, black pepper, garlic and onion powder, and smoked-flavored salt) from pH 5.3 to 4.3, using sodium lactate, acetic acid, soy sauce, and ethanol and/or Tween 20, resulted in greater lethality compared with otherwise similar marinades that were not further acidified. The addition of antimicrobials such as organic acids and/or their salts and a surfactant to a traditional marinade solution increased the extent of the subsequent thermal inactivation of all three pathogens from 3.1 to 5.0 log, to 4.9 to 6.7 log (5–7). The authors speculated that this might be attributed to the lower pH of the marinade solutions formulated with the addition of these antimicrobial compounds.

The results of the present study will be useful to beef jerky manufacturers who process their products using rel-

atively high dry-bulb temperatures ($\geq 180^{\circ}\text{F}$) with continuous smoke and relatively shorter processing times (≤ 3.5 h), but without the addition of humidity. These data also validate that cooking and drying marinated and nonmarinated whole muscle beef strips at $177.2 \pm 5.6^{\circ}\text{F}$ ($80.7 \pm 3.1^{\circ}\text{C}$), with an average internal temperature from CUT throughout cooking and drying of ca. 163.3°F (72.9°C) and exposure for 1.5, 2.5, and 3.5 h to constant hickory smoke, with an average initial RH of ca. 63.1% and an average CUT RH of 35.5, 40.8, and 46.5% (average RH from CUT throughout cooking and drying ca. 29.3%) to an average final RH of 21.9, 21.5, and 19.2%, respectively, was sufficient to achieve the required 5-log reduction of *E. coli* O157:H7 and the required 6.5-log reduction of *Salmonella*, as well as sufficient to achieve the zero-tolerance requirement and/or to meet compliance guidelines for *L. monocytogenes* established for beef jerky. In addition to achieving an M:Pr of ≤ 0.75 and an a_w of ≤ 0.8 , as well as enhancing product safety and most likely extending shelf life without compromising product quality, sensory attributes, and/or consumer acceptability, we estimate that shortening the processing time at 180°F from 3.5 to 2.5 h could also result in appreciable savings (\$3,000 to \$5,000 per day) in energy, warehousing, and/or labor costs for small- to medium-sized jerky manufacturers. Studies are ongoing to validate additional time and temperature conditions and different product formulations to control *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in both beef and poultry jerky.

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