1	Lethality of Commercial Whole-Muscle Beef Jerky Manufacturing Processes
2	Against Salmonella serovars and Escherichia coli O157:H7
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1 ABSTRACT

2 Thermal processing used in making whole-muscle beef jerky also involves drying. This 3 drying may cause enhanced pathogen thermotolerance and evaporative cooling that 4 reduce process lethality. Several salmonellosis outbreaks have been associated with beef 5 jerky. In this study, a standardized process was used to inoculate beef strips with 5-strain 6 cocktails of either Salmonella serovars or Escherichia coli O157:H7, marinade the strips 7 in pH 5.3 marinade for 22-24 h at 5°C, and then convert the strips to jerky using various 8 heating/drying regimes. Numbers of surviving organisms were determined during and 9 after the heating/drying. In some trials, a commercial lactic acid starter culture was also 10 evaluated as a potential surrogate for the pathogens. The 5-log Salmonella reduction 11 mandated by the United States Department of Agriculture (USDA), along with a 5-log 12 reduction in E. coli O157:H7, was best achieved by ensuring that high wet-bulb 13 temperatures were reached and maintained early in the process (51.7°C or 54.4°C for 60 14 minutes, 57.2°C for 30 minutes, or 60°C for 10 minutes) followed by drying at 76.7°C 15 (dry-bulb temperature). Processes that met the USDA guideline with smaller safety 16 margins were 1) heating and drying at 76.7°C (dry-bulb) within 90 minutes of beginning 17 the process, 2) heating for successive hourly intervals at 48.9, 54.4, 60, and 76.7°C (dry-18 bulb), or 3) heating at 51.7°C (dry-bulb), followed by 76.7°C (dry-bulb) drying started 19 before product a_w was < 0.86. Achieving a > 3.0 log reduction in the starter culture is a 20 possible standard for validating process lethality.

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2 Beef jerky processing, using whole muscle or restructured ground meat, is unique compared to the processing of other ready-to-eat meat products because heat processing 3 4 is intended to attain considerable drying and desired texture and shelf-stability. This 5 drying may reduce the process lethality against pathogenic bacteria in beef, and outbreaks 6 of salmonellosis have been linked to the consumption of beef jerky (6). Previous 7 research has suggested that sub-lethal drying conditions may lead to increased heat-8 resistance in pathogens such as Salmonella serovars (7). Furthermore, evaporative 9 cooling during drying may lessen the effective temperature to which pathogens are 10 exposed. A possible decrease in lethality related to evaporative cooling on the surface of 11 cooked beef was noted previously in studies of Salmonella spp. survival during cooking 12 of beef (2, 8). In fact, Blankenship et al., (1) recommended introduction of steam into the 13 oven when cooking beef roasts to ensure that adequate lethality against salmonellae was 14 attained on the roast surface. Evaporative cooling was also noted as a factor contributing 15 to insufficient thermal lethality in making jerky associated with an outbreak of 16 salmonellosis in New Mexico in 2003 (11). All of these factors have resulted in heightened scrutiny of the lethality of the heating and drying steps typically used during 17 18 jerky processing.

19 United States Department of Agriculture (USDA) officials have issued a 20 compliance guideline for jerky processors (*14*) that stresses the importance of 21 maintaining high humidity during thermal processing in order to ensure sufficient 22 destruction of *Salmonella* spp. and *Escherichia coli* O157:H7. However, processors have 23 had difficulty either complying with USDA guidance or developing and validating

1 adequate alternative processes while still obtaining desired finished product 2 characteristics. Previously suggested techniques for ensuring adequate lethality included boiling beef strips in marinade prior to cooking and drying, and oven-heating beef jerky 3 4 strips after drying (9, 10). Commercial processors have not widely adopted these 5 methods, either because of perceived adverse effects on product sensory characteristics or 6 because of economic or efficiency concerns. Development of validated heating/drying 7 guidelines for processors of whole-muscle jerky has been further complicated by 8 variables such as thickness of jerky strips, whether the strips have been marinated and, if 9 so, the composition and conditions of marination, the type of smokehouse or oven used 10 for heating/drying, and the weather and altitude at the processing plant.

11 Presently, the USDA has indicated that a jerky-making process has sufficient 12 lethality if it results in a 5-log reduction of *Salmonella* spp. (written communication from 13 Dr. Paul Uhler, USDA – FSIS, 2005). Furthermore, USDA officials have stated that the 14 lethality of non-thermal steps in jerky-making, such as marination, can be counted toward 15 meeting the overall process lethality requirement (Dan Englejohn, USDA - FSIS, personal communication, 2005). Pre-existing USDA guidance for certain other beef 16 17 products specified a 6.5 log reduction in Salmonella spp. (15). The newer 5-log 18 reduction standard resulted from a USDA draft risk assessment of the impact of different 19 lethality standards for ready-to-eat meat and poultry products on the incidence of 20 salmonellosis (13). This risk assessment determined that decreasing the pathogen 21 reduction standard from 6.5 logs to 5 logs for products such as jerky that do not support 22 the growth of salmonellae, would have little effect on the incidence of salmonellosis.

1 The first objective of this study was to develop and validate sufficiently lethal 2 processes for use by commercial jerky manufacturers in heating and drying whole-muscle 3 beef jerky. In working towards this objective, marination and beef strip thickness were 4 standardized based on typical industry practice. A standardized smokehouse loading 5 procedure was used, and ambient weather conditions were noted.

A second objective of this work was to develop a simple method for commercial processors of whole-muscle beef jerky to evaluate the lethality of new processes. Because in-plant challenge studies involving pathogenic bacteria are not recommended for commercial meat processing establishments for safety reasons, and because laboratory-based challenge studies are neither practical nor affordable for most processors, we investigated the use of a commercially available lactic acid bacterial starter culture as a surrogate for *Salmonella* serovars and *E. coli* O157:H7.

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14 MATERIALS AND METHODS

15 **Preparation of inoculum.** Five-strain cocktails of *Salmonella* serovars and *E*. 16 *coli* O157:H7 were used to inoculate beef strips prior to jerky processing. E. coli 17 O157:H7 strains ATCC 43894, 51657, and 51658 were clinical isolates and strain ATCC 18 43895 was originally from ground beef implicated in an outbreak of food-borne illness; 19 each of these strains was obtained from the American Type Culture Collection 20 (Manassas, VA). Strain USDA-FSIS-380-94 was originally from salami implicated in an 21 illness outbreak and was obtained from the laboratory of Dr. John Luchansky at the Food 22 Research Institute, University of Wisconsin-Madison. The Salmonella serovars were all 23 obtained from the laboratory of Dr. Eric Johnson at the Food Research Institute,

1	University of Wisconsin-Madison. To obtain a working culture, each strain was cultured
2	twice successively (from a previously frozen culture) at 35°C for 18-24 hours in Brain
3	Heart Infusion Broth (BHIB; Difco, Becton-Dickenson, Sparks, MD), streaked to Brain
4	Heart Infusion Agar (BHIA; Difco), incubated at 35°C for 18-24 hours, examined for
5	purity, and then stored at 5°C. According to the work of Calicioglu et al. $(3 - 5)$, acid-
6	adaptation is unlikely to increase pathogen resistance to hurdles involved in beef jerky
7	processing. Therefore, cultures for inoculation were grown in Brain Heart Infusion broth
8	(BHIB; Difco, Becton Dickinson, Sparks, MD), a medium containing only a small
9	amount of glucose that could be metabolized to produce organic acids. To achieve
10	stationary-phase inoculum cultures, an isolated colony of each strain was transferred from
11	its working culture plate to 9 mL of BHIB, and incubated at 35°C for 24 hours. To
12	prepare a 5-strain inoculum cocktail of Salmonella serovars or E. coli O157:H7, the
13	BHIB culture of each strain was combined into one 50-ml sterile plastic centrifuge tube,
14	and centrifuged for 12 minutes at 5,000 x g. The supernatant in each tube was decanted
15	and the pellets were re-suspended with approximately 20 ml of Butterfield's phosphate
16	diluent (BPD; Nelson Jameson, Marshfield, WI). A commercial lactic acid bacteria
17	starter culture, intended for making fermented meat products, was evaluated in several
18	trials as a surrogate for the pathogens. The starter culture (Formula 100; Trumark,
19	Linden, NJ) was stored at -20°C. Three different lots of this starter culture were tested
20	during the study. Preliminary experiments showed that the starter culture was
21	considerably more thermotolerant than the Salmonella and E. coli O157:H7 strains used,
22	so a lower inoculum level was used for it. To prepare the starter culture for inoculation

of beef strips, 0.5 g of culture was added to 99 ml BPD, mixed well, and then diluted
 another 100-fold in BPD.

3 **Inoculation of beef strips.** Frozen vacuum-packaged beef strips were obtained 4 from a commercial jerky processor and thawed at 4° C or under running tap water prior to 5 inoculation. The individual beef strips (5 - 7 mm thick) were placed in a biosafety hood 6 on aluminum foil that had been previously sanitized with 70% (v/v) ethanol. To 7 inoculate each strip, 0.4 ml of the undiluted pathogen cocktail or 0.4 ml of the diluted 8 starter culture was pipetted onto the product surface and distributed as evenly as possible 9 using a sterile plastic spreader. Aluminum foil was placed over the strips in a tented 10 manner to minimize the amount of drying during microbial attachment (30 min), after 11 which strips were turned over and the inoculation/attachment process was repeated on the 12 other side. For each trial, one group of 9 - 12 beef strips was inoculated with Salmonella 13 spp., another group of 9 - 12 beef strips was inoculated with E. coli O157:H7, and a third 14 group of 4 - 6 uninoculated beef strips was used to monitor yield and water activity 15 throughout thermal processing. In many trials, four additional strips were inoculated with 16 the starter culture. Initial pathogen levels on inoculated beef strips were approximately 10^8 CFU per beef strip and initial starter culture levels were about 10^4 CFU per beef strip. 17 18 Jerky processing. Each group of beef strips was tumbled manually in a closed zip-lock plastic bag for approximately 5 minutes in a non-acidic (pH 5.3) spice-19 20 containing marinade applied at a level of 15% (w/w; 9.7% water and 5.3% dry 21 ingredients) intended to result in a pre-processing level of 2% (w/w) sodium chloride, 2% 22 (w/w) sucrose, and 156 ppm sodium nitrite (w/w) in the meat. Following marination, 23 strips were held for 22-24 h at 5°C. The next day, strips were arranged on racks placed in

1	the center of a commercial one-truck smokehouse (Model TR2, Vortron, Beloit, WI) for
2	processing. Pans of water were placed on the lowest rack in the smokehouse and a low
3	fan speed was used to simulate as much as possible a drying rate consistent with a
4	smokehouse containing several racks filled with product. The smokehouse dry-bulb and
5	wet-bulb temperatures were monitored using thermocouples (L#113-1055 P/M,
6	ThermoWorks, Alpine, UT) and a data logger (Model 92000-00, Barnant Co.,
7	Barrington, IL). Percent relative humidity (%RH) was calculated from the wet-bulb and
8	dry-bulb temperatures using a slide rule (Alkar, Lodi, WI). In all trials, the product-
9	internal temperature was measured by inserting a thermocouple probe into the geometric
10	center of a beef strip. Because insertion of the probe in this location is relatively difficult,
11	a surrogate product-internal temperature was also obtained by tightly folding a beef strip
12	once over a thermocouple probe in the majority of the trials. The latter temperature
13	measurement method is considerably easier, but it was not known at first whether it could
14	be considered an accurate surrogate for internal beef strip temperature. Smoke was not
15	applied to the beef strips during processing. Several types of heating/drying processes
16	were tested (summarized in Table 1). In Type 1-A processes, the dry-bulb temperature
17	controller was set at 62.8°C (145°F) in the first 15 minutes and then at 76.7°C (170°F)
18	during the next 15 minutes, with no added humidity. This two-step increase in dry-bulb
19	temperature was done to simulate the beginning stages of heating a full smokehouse of
20	moist, ambient-temperature beef strips. Next, humidity (steam or water) was introduced
21	into the smokehouse via the wet-bulb temperature controller to obtain targeted increases
22	in wet-bulb temperatures, referred to as "wet-bulb spikes". The process lethality was
23	determined for a series of trials conducted using early-process wet-bulb spikes of 51.7°C

1	(125°F) for 60 min, 54.4°C (130°F) for 60 min., 57.2°C (135°F) for 30 min., and 60°C
2	(140°F) for 10 min. Following completion of wet-bulb spikes, no further humidity was
3	introduced into the smokehouse chamber as the product was further dried at a dry-bulb
4	temperature of 76.7°C (170°F). To investigate the possible protective or lethal effects of
5	marinade ingredients, a selected Type 1 process (wet bulb spike of 54.4°C for 60
6	minutes) was done to products that were marinated either only in water (9.7% initial
7	product weight gain) or only in the dry ingredients (5.3% initial product weight gain). In
8	four trials, a Type 1-B process was used (wet-bulb spike of 54.4°C for 60 minutes) in
9	which the dry-bulb temperature was held at either 65.5°C (150°F) or 87.8°C (190°F)
10	throughout a 15-minute equilibration period before the wet-bulb spike, the wet-bulb spike
11	itself, and final drying. Type 2 and Type 3 processes involved rapid (15 minutes) and
12	slow (90 minutes) increases in dry-bulb temperature to 76.7 °C (170°F) followed by final
13	drying at a dry-bulb temperature of 76.7°C (170°F). In Type 4 processes, the dry-bulb
14	temperature was held constant at 51.7°C (125°F) until a desired approximate jerky water
15	activity was attained, whereupon the dry-bulb temperature was increased to 76.7°C
16	(170°F) for final drying (no humidity added during the process). In Type 5 processes, the
17	dry-bulb temperature was held constant at 60, 71.1, or 82.2°C (140, 160, or 180°F) and
18	no attempt was made to control wet-bulb temperature. Type 6 and 7 processes both
19	involved sequential 1 hour exposures to dry-bulb temperatures of 48.9, 54.4, and 60°C
20	(120, 130, and 140°F); in the Type 7 process beef strips were exposed an additional hour
21	to a dry-bulb temperature of 76.7°C (170°F). In addition to trials in the commercial-scale
22	smokehouse, a consumer-scale smokehouse (Pragotrade Model TS160, Cabela's, Sidney,
23	NE) was used for additional trials testing the relationship between thermally induced

1	death of the starter culture surrogate and inoculated pathogens ("consumer-type
2	process"). Inoculated and uninoculated beef strips were placed on two racks in this
3	smokehouse, two large (15 cm diameter) Petri dishes containing water were placed on the
4	floor of the smokehouse, and the dry-bulb temperature was set at either 60 or 70.1°C (140
5	or 160°F). These trials were done to compare pathogen and starter culture surrogate
6	survival over a broad range of conditions. In all trials, uninoculated strips were evaluated
7	at the intermediate sampling point for water activity (measured on-site using an AquaLab
8	Series 3TE water activity meter, Decagon Devices, Inc., Pullman, WA). In all trials,
9	additional uninoculated finished beef jerky strips were sent to a commercial testing
10	laboratory for pH, water activity, % water (forced air oven method, AOAC method
11	950.46Bb), % protein (Kjeldahl method, AOAC method 991.20.I), and % salt
12	(potentiometric method, AOAC method 980.25). From these analyses, Moisture: Protein
13	ratio (MPR), and % water-phase salt values were calculated.

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15 Enumeration of inoculum organisms. The numbers of *Salmonella* spp. and *E*. 16 coli O157:H7 on beef strips were determined prior to marination, after marination (in 21 17 trials), after the early-process wet-bulb spike in the smokehouse (Type 1 process) or some 18 other intermediate time (other process types), and following drying when the beef strips 19 had reached a yield level predetermined to correlate with an average water activity of \leq 20 0.90. One beef strip comprised a sample and three samples were analyzed for pathogen 21 numbers at each sampling time. In several trials, two strips each were analyzed for 22 starter culture numbers after inoculation and at the end of drying. Each sample was 23 placed in a whirl pak filter bag (Nasco, Fort Atkinson, WI), BPD (99 ml) was added, and

1	the bag contents were stomached for 2 minutes at medium speed (Stomacher 400
2	Circulator lab blender; Seward) or samples were manually massaged for 1 minute and
3	shaken for 1 minute. This initial dilution was arbitrarily defined as 10^{-1} . Serial decimal
4	dilutions were made in BPD as needed. From the initial dilution, 1.0 ml was distributed
5	for spread-plating among three plates of BHIA. From the original dilution and each
6	subsequent dilution 0.1 ml was spread on one BHIA plate per dilution. Plates were
7	incubated at 35°C for 1 h to allow for repair of injured cells, and then overlaid with
8	MacConkey Sorbitol agar (SMAC; Difco), XLD agar (Difco), or mEnterococcus agar
9	(mE; Difco) for selective/differential enumeration of E. coli O157:H7, Salmonella
10	serovars, and starter culture surrogate, respectively. After 20-24 h (E. coli O157:H7,
11	Salmonella serovars) or 72 h (starter culture surrogate) incubation at 35°C, plates were
12	examined for typical colonies. For each sampling time, one presumptive colony each of
13	E. coli O157:H7 and Salmonella was transferred to BHIA, incubated at 35°C for 20-24 h,
14	and then tested to confirm colony identity. A single plate containing presumptive starter
15	culture colonies was retained for confirmation tests at each sampling time. Confirmation
16	tests for the presumptive pathogens were Gram reaction, cellular morphology, oxidase
17	activity, and biochemical characteristics (API 20E kit, bioMerieux, Hazelwood, MO) for
18	the pathogens, with an additional O157 latex agglutination test (Oxoid, Ogdensburg, NY)
19	done to confirm E. coli O157:H7 isolates. Presumptive starter culture surrogate colonies
20	were evaluated for Gram reaction, cellular morphology, and catalase activity. The log
21	CFU for a given inoculum organism was calculated for each sample with a mean log
22	CFU calculated for each sampling time. A value of 9 CFU (0.95 log CFU) was assigned
23	when no colonies were present for the least dilute plating.

1	Weather data. Because outdoor weather conditions, particularly temperature and
2	relative humidity, were believed to have a potentially important effect on jerky
3	processing lethality, the dewpoint at noon in Madison, WI, on the day of each trial was
4	obtained from meteorological archives
5	(http://www.channel3000.com/weather/index.html).
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8	RESULTS AND DISCUSSION
9	Finished jerky made in the present study had a pH of $5.6 - 6.1$, with 22 of 30 samples
10	having pH of $5.7 - 5.9$. The water activity, MPR, and % water-phase salt varied widely,
11	as expected, given the range of heating and drying conditions evaluated. When the
12	normal marination and a Type 1 process were used, the ranges of finished product water
13	activity, MPR, and % water-phase salt were $0.78 - 0.93$, $0.52 - 0.95$, and $7.9 - 16.6$,
14	respectively. In contrast, the Type 1 product made with no spices had water activity,
15	MPR, and % water-phase salt of $0.94 - 0.96$, $0.63 - 0.65$, and $0.60 - 0.70$, respectively.
16	Type 1 product marinated only with spices had a water activity, MPR, and % water-phase
17	salt of $0.86 - 0.87$, 0.67 - 0.68 , and $11.1 - 11.5$, respectively. Products made using the
18	normal marination procedure and any of the Type 2 - 7 processes had compositional
19	values similar to those for Type 1 processes with the exception of product from one Type
20	2 and three Type 3 processes that had water activity, MPR, and % water-phase salt ranges
21	of $0.64 - 0.75$, $0.41 - 0.66$, and $11.2 - 17.1$, respectively. It should be noted that in many
22	trials achieving sufficient lethality, the finished product water activity and MPR were
23	higher (and the % water-phase salt was lower) than desired for optimum consumer

1	acceptance and required in USDA labeling standards (12). However, processors using
2	the same heating/drying processes could simply extend the drying period to obtain
3	desired product characteristics. This extended drying would have no adverse effect on
4	lethality and might, in some situations, increase it.
5	In 21 trials, pathogen numbers were determined following the 22-24-hour post-
6	marination refrigerated storage step. Numbers of Salmonella serovars and E. coli
7	O157:H7 fell by $0.04 - 0.43$ and $0.04 - 0.34 \log CFU$, respectively. We concluded that
8	this marination step had little lethality, and discontinued post-marination sampling.
9	Throughout the study, consistent trends in dry-bulb, wet-bulb, product-internal,
10	and surrogate product-internal (beef strip folded over probe) temperatures were observed.
11	As shown for a typical Type 1 process (Figure 1), wet-bulb temperature was initially well
12	below dry-bulb temperature. Product-internal temperature was always similar to the wet-
13	bulb temperature early in the process and could effectively serve as an estimate of wet-
14	bulb temperature until later in the process. At some time, though, evaporative cooling of
15	the strips diminished and the product-internal temperature rose toward the dry-bulb
16	temperature. It is important to note that throughout the jerky heating/drying process,
17	product-internal temperature was always close to (within 1°C) or higher than the chamber
18	wet-bulb temperature. Thus, maintaining the chamber wet-bulb temperature (and thereby
19	the product temperature) high enough to cause pathogen destruction (ca. $51.7^{\circ}C/125^{\circ}F$
20	and higher) can strongly influence process lethality. Early in the heating/drying process,
21	the surrogate product-internal temperature, measured with a jerky strip folded over the
22	thermocouple, was often lower than the product-internal temperature because the applied
23	heat had to pass through twice the thickness of meat in the folded strip. Later in the

1 process, the surrogate product-internal temperature rose above the product-internal 2 temperature, presumably because the greater meat thickness with the folded strip 3 diminished evaporative cooling near the thermocouple. By the end of the process, when 4 little evaporative cooling still was occurring, the two temperatures were the same. The 5 divergence of the two temperatures by a variable amount during much of the process calls 6 into question the practice of using the surrogate product-internal temperature for overall 7 process control or evaluation. However, the surrogate product-internal temperature was 8 very close to the product-internal temperature early in processing and during the wet-bulb 9 temperature spikes in Type 1 processes, and could be useful in early-process control or 10 evaluation. 11 Earlier research has established the fact that sub-lethal drying can make pathogens

12 such as *Salmonella* serovars more resistant to heat (7). This phenomenon was likewise 13 observed in several early jerky-making trials (data not shown). Therefore, several early-14 process wet-bulb temperature spikes were applied to determine the extent of elevated-15 humidity heating conditions necessary to achieve desired lethality while maintaining 16 product quality (Table 2). During the 54.4, 57.2, and 60° C (130, 135, and 140°F) wet-17 bulb spikes with concurrent 76.7°C (170° F) dry-bulb temperature, the product-internal 18 temperature was generally quite similar to the wet-bulb temperature. However, during 19 the 54.4°C (130°F) wet-bulb spike with a concurrent 87.8°C (190°F) dry-bulb 20 temperature, the product-internal temperature rose faster than it did during the wet-bulb 21 spike treatments conducted with 76.7°C (170°F) dry-bulb temperature, and the product-22 internal temperature reached at least 5°C higher (Figure 2) than in the latter process 23 (Figure 1). This more rapid increase in product temperature resulted from faster jerky

1	drying at the lower %RH under 87.8°C (190°F) dry-bulb/54.4°C (130°F) wet-bulb
2	temperature conditions (see % RH values in Table 2). Similarly, when a wet-bulb
3	temperature spike of 51.7°C (125°F) was applied concurrently with 76.7°C (170°F) dry-
4	bulb temperature, the %RH was lower than under 54.4°C (130°F) wet-bulb/76.7°C
5	(170°F) temperature conditions. This lower %RH led to a faster increase in product-
6	internal temperature and a more rapid achievement of $a \ge 5.0 \log$ reduction in
7	salmonellae numbers (see first four lines of Table 2).
8	Presently, the USDA has indicated that a jerky-making process has sufficient
9	lethality if it results in a 5-log reduction of Salmonella serovars. However, USDA
10	guidance for certain other beef products specified a 6.5 log reduction in Salmonella
11	serovars. All tested Type 1 processes (early-process wet-bulb temperature spike
12	following the standard marination process) resulted in $a \ge 6.4$ log reduction in both
13	pathogens by the end of the complete process (including final drying; Table 2). Three
14	Type 1 processes achieved a \geq 5.2 log reduction in <i>Salmonella</i> serovars but caused
15	smaller decreases in E. coli O157:H7 numbers at the end of the wet-bulb temperature
16	spike (Table 1). These treatments were 51.7°C (125°F) for 60 min, 57.2°C (135°F) for
17	30 min, and 60°C (140°F) for 10 min. However, $a \ge 6.4$ log reduction of both <i>Salmonella</i>
18	serovars and E. coli O157:H7 was achieved by the end of drying after each of these
19	processes. One Type 1 process did not cause $a > 5.0$ log reduction in either pathogen by
20	the end of the web-bulb spike but subsequent drying resulted in sufficient overall
21	lethality. This treatment, wet-bulb temperature of 54.4°C (130°F) for 60 minutes,
22	resulted in decreases of $3.2 - 3.9$ and $2.0 - 2.1 \log$ CFU for <i>Salmonella</i> serovars and <i>E</i> .
23	<i>coli</i> O157:H7, respectively. By the end of drying after these treatments, reductions of \geq

1	6.9 logs had occurred for both pathogen species. The overall effectiveness of these Type
2	1 processes appeared to result from the fact that product temperature was controllable via
3	control of wet-bulb temperature and was increased rapidly to levels at which pathogens
4	were killed while the beef strips were still moist enough to achieve high lethality. It is of
5	interest to note that the time for which the wet-bulb temperature was elevated, i.e. the
6	duration of the wet-bulb temperature spike, was generally shorter than the corresponding
7	times for the same wet-bulb temperatures listed in USDA guidance (15) for the cooking
8	of beef. The latter times were 112 minutes at 54.4°C (130°F), 36 minutes at 57.2°C
9	(135°F), and 12 minutes at 60°C (140°F). By comparison, times used in the present
10	study were 60, 30, and 10 minutes, respectively.
11	The dry-bulb temperature during a Type 1 process was found to have a major
12	effect on process lethality. The application of a wet-bulb temperature spike of 54.4°C
13	(130°F) for 60 minutes with a concurrent dry-bulb temperature of 65.5C (150°F; Figure
14	3) reduced <i>Salmonella</i> serovar populations by $4.9 - 5.0 \log CFU$, but only resulted in 3.2
15	– 3.6 log reductions in numbers of <i>E. coli</i> O157:7 (Table 2) without further drying.
16	Subsequent drying at 65.5°C (150°F), however, did lead to overall \geq 6.7 log CFU
17	reductions for both pathogens. These final-sampling results were not noticeably different
18	than results obtained with the same wet-bulb temperature applied with a concurrent dry-
19	bulb temperature of 76.7°C (170°F), perhaps because pathogen populations following
20	both types of process had fallen below the detection limit. When the concurrent dry-bulb
21	temperature was increased to 87.8°C (190°F), though, the wet-bulb temperature spike
22	caused a \geq 6.5 log CFU reduction of both pathogens before final drying was even begun
23	(Table 2).

1	Although USDA guidance (14) recommended 90% relative humidity (RH) during
2	the early heating period in jerky processing, it also stated that such a high humidity may
3	not be necessary if alternative procedures are validated. In the Type 1-A processes
4	conducted here using a dry-bulb temperature of 76.7°C (170°F), the calculated RH for
5	early-process wet-bulb temperature spikes was 27 - 43% RH. When followed by drying
6	at 76.7°C (170°F), these processes were sufficient to provide \geq 6.4 log reduction in
7	numbers of Salmonella serovars and E. coli O157:H7. Taking into account current USDA
8	expectations for jerky processing lethality, processors using the Type 1 process
9	conditions employed in this study [achieving 76.7°C (170°F) dry-bulb temperature within
10	30 minutes and maintaining this temperature throughout processing] could employ any of
11	the early-process wet-bulb spike treatments listed in Table 1 followed by drying at
12	76.7°C (170°F) as scientifically validated processes for making safe whole-muscle beef
13	jerky.
14	Lethality was compared for several different Type 1 processes at a
15	common wet-bulb temperature spike time of 60 minutes. The relevant processes were
16	51.7°C (125°F) or 54.4°C (130°F) wet-bulb temperature spikes with concurrent 76.7°C
17	(170°F) dry-bulb temperature, and 54.4°C (130°F) wet-bulb temperature spikes with
18	65.5°C (150°F) or 87.8°C (190°F) dry-bulb temperatures. The 60-minute lethality for
19	Salmonella serovars for these processes averaged 5.4, 3.5, 4.9, and 6.6 logs, respectively
20	(Table 2). Corresponding values for <i>E. coli</i> O157:H7 were 4.7, 2.1, 3.4, and 7.1 logs,
21	respectively. The reason for the highest lethality during the 51.7°C (125°F) wet-bulb
22	spike (76.7°C dry-bulb temperature) and the 54.4°C (130°F) wet-bulb temperature spike
22 23	spike (76.7°C dry-bulb temperature) and the 54.4°C (130°F) wet-bulb temperature spike with 87.8°C dry-bulb temperature (Figure 2) is that both these processes resulted in a

lower environmental relative humidity, and a more rapid increase in product temperature
 to lethal levels early in the process while there was still sufficient product moisture for
 enhanced lethality.

4 Separate trials were conducted with a Type 1-A process to evaluate the relative 5 importance of the marinade components in achieving desired process lethality. Results 6 showed that omission of either water or dry ingredients from the marinade led to 7 somewhat greater reductions in pathogen numbers after a 54.4°C (130°F)/60 minute wet-8 bulb temperature spike (Table 2) but comparable lethality after the subsequent drying. 9 Whole-muscle beef jerky prepared without the addition of salt, however, had a much 10 higher water activity when trials were completed. We concluded that the choice of water 11 level in the jerky marinade used in this study (and hence the amount of marinade pick-up) 12 or the addition of only dry marinade ingredients was not a critical factor in attaining 13 desired process lethality.

14 The Type 2 process involving a rapid (15 min) increase in dry-bulb temperature to 15 76.7°C (170°F) achieved greater pathogen destruction than the Type 3 process in which 16 dry-bulb temperature did not increase to 76.7°C (170°F) until after 90 minutes (Table 3). 17 However, both of these processes did result in pathogen reductions exceeding 5.0 log 18 CFU after drying was completed. An alternative approach that some processors may 19 elect to use is to initially heat beef strips at the relatively moderate dry-bulb temperature 20 of 51.7°C (125°F) for a short period of time followed by heating/drying of the strips at a 21 the relatively high dry-bulb temperature of 76.7°C (170°F). The rationale for this 22 approach (Type 4 process) is that the short initial heating imparts desirable product 23 characteristics without increasing pathogen thermotolerance via sublethal stress. As seen

1	in Table 4, the success of a Type 4 process depends on beginning the high-temperature
2	drying when the product water activity is still relatively high. When drying was begun at
3	a product water activity of 0.72 or 0.81, the reduction in Salmonella serovars was 4.7 log
4	CFU. Reductions in numbers of E. coli O157:H7 in these trials were 5.4 log CFU,
5	however. When drying was begun at product water activity of 0.86, 0.87, 0.95, or 0.96,
6	the reduction in both pathogens was $4.9 - 6.7 \log CFU$. Although such a process clearly
7	provided less of a safety margin than using a Type 1 process (wet-bulb temperature
8	spike), it is likely that short-term heating at a low dry-bulb temperature such as 51.7°C
9	followed by 76.7°C (dry-bulb temperature) drying could achieve sufficient lethality if the
10	drying is begun when product water activity is ≥ 0.86 .
11	Heating whole-muscle beef strips at a constant dry-bulb temperature of either 60
12	or 71.1°C (140 or 160°F) without the addition of humidity (Type 5 processes) did not
13	achieve USDA-mandated lethality even when product was dried to water activity levels
14	typical of commercial beef jerky (Table 5). These processes resulted in decreases in
15	Salmonella serovars and E. coli O157:H7 of 3.8 – 4.7 and 3.9 – 4.0 logs, respectively.
16	Heating at a constant dry-bulb temperature of 82.2°C (180°F) did result in a reduction of
17	just over 5 log CFU for both pathogens. The cause of the lower lethality in Type 5
18	processes can be induced from Figure 4. As can be seen, the wet-bulb temperature and
19	product-internal temperature remained at sub-lethal levels for long periods of time,
20	allowing pathogen survival during the drying that took place. The surviving cells
21	apparently had enhanced thermotolerance during subsequent heating, as previously
22	described (7). Three Type 5 process trials were conducted on winter days with very low
23	noon dewpoint temperatures (-11, -12, -14°C) and one trial (60°C/140°F dry-bulb

1	temperature) was done in mid-July (noon dewpoint temperature of 20°C). Although it is
2	possible that using a constant dry-bulb temperature process could attain more lethality
3	than in the present study if it was employed in very humid weather, we observed no clear
4	relationship between outdoor dew point and process lethality. We may have mitigated
5	any weather effects, though, by adjusting process drying times to attain an acceptable
6	reduction in water activity. Furthermore, our trials were conducted in a climate-
7	controlled building. Processors with a lower degree of humidity control may need to
8	adjust process parameters to account for weather extremes.
9	The Type 6 process that had a slow increase in dry-bulb temperature to a
10	maximum of 60°C (140°F) did not cause more than a 3 log CFU reduction in pathogens.
11	In contrast, the Type 7 process which had final 1-hour exposure to dry-bulb temperature
12	of 76.7°C (170°F) caused > 5 log CFU decreases in pathogen numbers (Table 3).
13	The starter culture tested in 19 trials as a pathogen surrogate survived the jerky-
14	making process considerably better than either of the tested pathogens (Table 6). When
15	the starter culture population was reduced by at least 3.0 log CFU (nine trials), the
16	populations of both pathogens decreased by at least 5.0 log CFU in eight of the trials. In
17	the one exception, the E. coli O157:H7 population decreased by 5.0 log CFU and the
18	population of Salmonella serovars was reduced by 4.7 log CFU. In contrast, when the
19	starter culture population was reduced by $< 3.0 \log CFU$ (10 trials), pathogen populations
20	were considerably less likely to decrease by at least 5.0 log CFU. Salmonella serovar and
21	<i>E. coli</i> O157:H7 levels decreased by $< 5.0 \log CFU$ in four and three of these trials,
22	respectively. We conclude that use of this starter culture surrogate with a target lethality

1 of at least 3.0 log CFU could be a useful tool for processors validating their whole-

2 muscle beef jerky processes.

3 On the basis of our results, we conclude that of the two pathogens studied, E. coli 4 O157:H7 is better able to survive the heating and drying steps used in making whole-5 muscle beef jerky. However, foodborne illness outbreaks linked to beef jerky have 6 primarily involved Salmonella serovars, so it is prudent for any validation of a jerky-7 making process to involve both pathogens. Because our results clearly show the 8 importance of wet-bulb temperature in achieving mandated lethality, we strongly 9 recommend that processors buy or make a wet-bulb thermometer for use in processing, or 10 use a hygrometer to monitor humidity and then use a commercially available slide rule to 11 determine wet-bulb temperature from known dry-bulb temperature and %RH values. 12 Reductions in *Salmonella* serovars and *E. coli* O157:H7 of > 5.0 log CFU can be 13 achieved in the production of whole-muscle beef jerky by ensuring that high enough wet-14 bulb temperatures are reached and maintained early in the process (Type 1 processes) or 15 that high dry-bulb temperature heating and drying is done before the beef strip water 16 activity has fallen below 0.86 (Type 2, 3, 4, and 7 processes). Alternatively, guidance 17 from USDA (14) has indicated that internal temperatures listed in USDA-accepted 18 "Appendix A" time/temperature combinations (15) are effectively wet-bulb temperatures. 19 Processors could consider a process valid in which the smokehouse wet-bulb temperature 20 and, therefore the product-internal temperature, was at or above a designated level for a 21 time at least as long as specified in Appendix A. 22

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- 23

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1

2 FIGURE LEGENDS 3 1. Wet-bulb, dry-bulb, and product-internal temperatures during the manufacture of 4 whole-muscle beef jerky by a Type 1-A process with a 54.4°C / 130°F, 60-minute 5 wet-bulb temperature spike. 6 2. Wet-bulb, dry-bulb, and product-internal temperatures during the manufacture of 7 whole-muscle beef jerky by a Type 1-B process with a dry-bulb temperature 8 setting of 87.8°C / 190°F. 9 3. Wet-bulb, dry-bulb, and product-internal temperatures during the manufacture of 10 whole-muscle beef jerky by a Type 1-B process with a dry-bulb temperature 11 setting of 65.5°C / 150°F. 12 4. Wet-bulb, dry-bulb, and product-internal temperatures during the manufacture of 13 whole-muscle beef jerky by a Type 5 process with a dry-bulb temperature setting 14 of 60.0°C / 140°F. 15 16

1	Table 1.	Summary	of heating	/drying	processes used	l to make	whole-muscle	beef	jerky	ÿ.
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3	Proces	S	Dry-Bulb (Controlled	.)	Wet-Bulb (Controlled)		Cumulative
4	Type		Temperature (°C/°F)	Time (min)	Temperature (°C/°F)	Time (min)	Time (min)
5	<u>1-A</u>		62.8 / 145	15	Not Controlled = NC		15
6			76.7 / 170	15	NC		30
7		then	76.7 / 170	60	51.7 / 125	60	90
8		OR	76.7 / 170	60	54.4 / 130	60	90
9		OR	76.7 / 170	30	57.2 / 135	30	60
10		OR	76.7 / 170	10	60 / 140	10	40
11	Alway	s followed by	76.7 / 170	varied (to targeted	NC		varied
12				final product dryness)		
13							
14	1 - B		62.8 / 145	15	NC		15
15		then	65.5 / 150	15	NC		
16			65.5 / 150	60	54.4 / 130	60	90
17			65.5 / 150	varied (to targeted	NC		varied
18				final product dryness)		
19		OR	87.8 / 190	15	NC		
20			87.8 / 190	60	54.4 / 130	60	90
21			87.8 / 190	varied (to targeted	NC		varied

1				final product dryness)	
2					
3	2		62.8 / 145	15 NC	 15
4			76.7 / 170	varied (to targeted NC	 varied
5				final product dryness)	
6					
7	3		62.8 / 145	90 NC	 15
8			76.7 / 170	varied (to targeted NC	 varied
9				final product dryness)	
10					
11	4		51.7 / 125	varied (to targeted a_w) NC	 varied
12			76.7 / 170	varied (to targeted NC	 varied
13				final product dryness)	
14					
15	5		60 / 140	varied (to targeted NC	 varied
16				final product dryness)	
17		OR	71.1 / 160	varied (to targeted NC	 varied
18				final product dryness)	
19		OR	82.2 / 180	varied (to targeted NC	 varied
20				final product dryness)	
21					

1	6	48.9 / 120	60	NC	 60
2		54.4 / 130	60	NC	 120
3		60 / 140	60	NC	 180
4					
5	7	48.9 / 120	60	NC	 60
6		54.4 / 130	60	NC	 120
7		60 / 140	60	NC	 180
8		76.7 / 170	60	NC	 240

9

1

- Table 2. Process lethality^a against *Salmonella* serovars (S) and *Escherichia coli* O157:H7 (EC) during Type 1 A and 1 B processing (wet-bulb temperature spike = WBS; followed by post-spike drying at $76.7^{\circ}C = PSD$) of whole muscle beef jerky.
- 4

5	WBS	WBS		PSD	Lethal	ity as determine	ed after		Water	activity	Product-intern	al
6	Temp.	Time	%RH ^b	time	WBS		PSD		after		temperature af	fter
7	(°C)	(min)		(min)	S	EC	S	EC	WBS	PSD	WBS	PSD
8	Type 1	- A	Strips	marinated with	water a	nd dry ingredie	ents	Dry-bulb temp	perature	at 76.7°C duri	ng wet-bulb spi	ike.
9	51.7	60	27	60	5.6	5.6	6.5	6.7	0.87	0.81	60.5	70.0
10	51.7	60	27	60	5.2	3.8	6.4	7.1	0.89	0.78	56.1	65.0
11	54.4	60	32	120	3.2	2.0	6.9	7.1	0.93	0.88	54.4	63.9
12	54.4	60	32	120	3.9	2.1	6.9	7.0	0.92	0.87	55.0	64.4
13	57.2	30	37	120	6.4	2.7	7.0	7.1	ND	0.86	58.3	63.9
14	57.2	30	37	90	5.3	3.1	7.0	7.1	0.93	0.90	57.8	66.1
15	60	10	43	120	6.2	3.8	7.0	7.2	0.96	0.90	58.9	67.8
16	60	10	43	120	6.7	2.2	6.8	7.0	0.96	0.84	60.0	62.8
17	Type 1	– B	Dry-bu	ilb temperature	at 65.5	°C during wet-	bulb spi	ike				
18	54.4	60	56	90	4.9	3.2	6.7	7.1	0.95	0.85	55.6	57.2
19	54.4	60	56	150	5.0	3.6	6.9	6.9	0.97	0.91	55.0	57.8
20	Type 1	– B	Dry-bu	ilb temperature	at 87.8	°C during wet-	bulb spi	ike				
21	54.4	60	19	30	6.7	7.3	7.0	7.4	0.93	0.89	66.7	71.1

1	54.4	60	19	45	6.5	6.9	7.2	7.1	0.95	0.89	65.0	73.9
2	Type 1	- A	Strips	marinated only	with w	ater		Dry-bulb temp	perature	at 76.7°C duri	ng wet-bulb spi	ike
3	54.4	60	32	60	5.6	4.9	6.6	6.9	0.98	0.94	55.6	63.9
4	54.4	60	32	60	5.4	4.5	6.1	7.0	0.99	0.96	55.6	64.4
5	Type 1	- A	Strips	marinated only	with dr	y ingredients		Dry-bulb temp	perature	at 76.7°C duri	ng wet-bulb spi	ike
6	54.4	60	32	90	5.9	3.9	7.1	7.0	0.95	0.87	57.2	66.7
7	<u>54.4</u>	60	32	60	5.6	6.9	6.6	7.1	0.93	0.86	58.9	68.3

⁸ ^a Reduction in log CFU per sample relative to initial pathogen load prior to marination.

^b Percent relative humidity during wet-bulb temperature spike calculated from wet-bulb and dry-bulb temperature settings using a
 slide rule.

11 ND = Not Determined

12

13

14

1	Table 3. Process lethality against <i>Salmonella</i> serovars (S) and <i>Escherichia coli</i> O157:H7 (EC) during the type 4, 5, 6, and 7 processes
2	for heating and drying of whole-muscle beef jerky. Processes were 2 = fast come-up [15 minutes to reach dry-bulb temperature of
3	76.7°C (170°F), followed by drying at 76.7°C], $3 =$ slow come-up [90 minutes to reach dry-bulb temperature of 76.7°C (170°F),
4	followed by drying at 76.7°C], $6 = 1$ hour each at dry-bulb temperatures of 48.9, 54.4, and 60°C (120, 130, and 140°F), or $7 = 1$ hour
5	each at dry-bulb temperatures of 48.9, 54.4, 60, and 76.7°C (120, 130, 140, and 170°F). No humidity was added to the smokehouse
6	chamber during processing.

7

8	Process Lethality (reduction in log CFU) ^a and product characteristics at												
9				Interm	Intermediate Time					End			
10					– internal – intern								
11	Treatm	nent	RH ^b	(min)	S	EC	a_{w}^{c}	Temperature (°C)	S	EC	a _w	Temperature (°C)	
12	2		- 17	60	3.3	2.7	0.95	48.9	6.1	5.6	0.86	62.8	
13	2		- 21	60	2.6	1.8	0.96	52.8	6.4	6.4	0.91	67.8	
14	3	31	- 21	90	2.0	1.5	0.97	46.7	5.5	5.6	0.87	67.8	
15	6	27	- 24	120	1.6	1.7	0.92	40.5	Produ	1ct ^{2.7}	0.84	51.1	
16	7	41	- 15	180	3.5	2.5	0.94	49.4	6.0	5.6	0.89	67.2	
17		41											

43
18 ^a Reduction in log CFU per sample relative to initial pathogen load prior to marination.

- 1 ^b Percent relative humidity, change during process (initial value final value). Values calculated from wet-bulb and dry-bulb
- 2 temperatures using a slide rule.
- ^c Water activity.

1

Table 4. Process lethality against Salmonella serovars (S) and Escherichia coli O157:H7 (EC) during the Type 4 process of heating of 2 whole-muscle beef jerky at a constant dry-bulb temperature of 51.7°C (125°F) to attain a desired water activity followed by drying at 3 76.7°C (170°F). No humidity was added to the smokehouse chamber during processing. 4

5

6	Heating Lethality (reduction in log CFU) ^a and product characteristics at												
7	Time End of heating					End of drying							
8	- internal					Cumul. Time - inte			- internal				
9	<u>(min)</u>	RH ^b	$a_{\underline{w}}^{c}$	Temperature (°C)	S	EC	(min)	<u>a_w</u>	Temperature (°C)	S	EC		
10	240	ND	0.72	47.2	3.3	3.1	300	Produc 0.65	^{et} 74.4	4.7	5.4		
11	240	32 - 33	3 0.81	42.8	3.3	2.7	300	0.75	70.0	4.7	5.4		
12	180	30 - 36	0.86	42.8	3.2	2.2	240	0.67	68.3	5.8	5.0		
13	₽79dua	_{ct} ND	0.87	45.6	4.3	2.9	330	0.82	71.7	5.8	6.7		
14	120	33 - 38	0.95	37.8	1.8	1.7	240	0.83	67.8	4.9	5.7		
15	120	35 - 38	0.96	38.9	2.1	1.9	225	0.82	70.0	5.6	5.9		
16													

Lethality (reduction in log CFU)^a and product characteristics at Heating

^a Reduction in log CFU per sample relative to initial pathogen load prior to marination. 17

^b Percent relative humidity, change during process (initial value – final value). Values calculated from wet-bulb and dry-bulb 18

19 temperatures using a slide rule.

^c Water activity. 20

1 ND = Not Determined.

Table 5. Process lethality against Salmonella serovars (S) and Escherichia coli O157:H7 (EC) during the Type 5 process of heating 1 and drying of whole-muscle beef jerky at a constant dry-bulb temperature of 60, 65.5, or 82.2°C (140, 160, or 180°F). No humidity 2 was added to the chamber during processing. 3

4

5	Dry-B	ulb	Lethality (reduction in log CFU) [*] and product characteristics at											
6	Tempe	erature	Intermediate	Гime	Final Time									
7	,					– internal						- internal		
8	<u>(°C)</u>	RH^b	<u>(min)</u>	S	EC	a _w ^c	temperature (°C)	(min)	S	EC	a _w	temperature (°C)		
9	60	32 - 24	90	4.3	3.8	0.73	46.7	120	4.2	3.9	0.64	53.9		
10	60	28 - 26	90	1.9	1.6	0.96	42.2	120	3.8	3.9	0.79	52.8		
11	71.1	34 - 18	60	4.0	3.3	0.87	50.6	75	4.7	4.0	0.80	59.4		
12	82.2	29 - 15	60	5.2	4.6	0.72	64.4	75	5.1	5.6	0.65	72.2		
13														

^a Reduction in log CFU per sample relative to initial pathogen load prior to marination. 14

^b Percent relative humidity, change during process (initial value – final value). Values calculated from wet-bulb and dry-bulb 15

16 temperatures using a slide rule.

17 PWdtent activity.

1 Table 6. Comparison of Salmonella serovar (S), Escherichia coli O157:H7 (EC), and starter culture surrogate (SC) death during

2 various processes for making whole-muscle beef jerky.

3

4

Process Lethality (reduction in log CFU)^a for Finished Sample

5	Treatment	S	EC	SC	
6	Consumer	4.4	2.8	2.5	
7	Consumer	4.2	3.0	1.8	
8	1-A	7.0	7.2	3.5	
9	1-B;87.8°C dry-bulb	7.0	7.4	3.2	
10	1-B; 87.8°C dry-bulb	7.2	7.1	3.2	
11	1-B; 65.5°C dry-bulb	6.7	7.1	3.2	
12	1-B; 65.5°C dry-bulb	6.9	6.9	2.8	
13	1-A; spices only	7.1	7.1	3.3	
14	1-A; spices only	6.6	7.1	2.8	
15	1-A; water only	6.6	6.9	2.7	
16	1-A; water only	6.1	7.0	3.0	
17	2	6.4	6.4	3.4	
18	2	6.1	5.6	2.3	
19	4	4.7	5.4	2.9	
20	4	5.8	5.0	3.2	
21	4	5.8	6.7	2.8	

1	4	4.7	5.4	3.3
2	5	3.8	3.9	1.9
3	6	6.0	5.6	2.6

4

⁵ ^a Reduction in log CFU per sample relative to initial pathogen load prior to marination.







2.

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