

# Validation of Ground-and-Formed Beef Jerky Processes Using Commercial Lactic Acid Bacteria Starter Cultures as Pathogen Surrogates

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## ABSTRACT

Beef jerky has been linked to multiple outbreaks of salmonellosis and *Escherichia coli* O157:H7 infection over the past 40 years. With increasing government scrutiny of jerky-making process lethality, a simple method by which processors can easily validate the lethality of their ground-and-formed beef jerky process against *Salmonella* and *E. coli* O157:H7 is greatly needed. Previous research with whole-muscle beef jerky indicated that commercial lactic acid bacteria (LAB) may be more heat resistant than *Salmonella* and *E. coli* O157:H7, suggesting the potential use of LAB as pathogen surrogates. Of six commercial LAB-containing cultures evaluated for heat resistance in ground-and-formed beef jerky, Saga 200 (*Pediococcus* spp.) and Biosource (*Pediococcus acidilactici*) were identified as consistently more heat resistant than *Salmonella* and *E. coli* O157:H7. Six representative ground-and-formed beef jerky commercial processes, differing widely in lethality, were used to identify an appropriate level of LAB reduction that would consistently indicate a process sufficiently lethal ( $\geq 5.0$ -log reduction) for *Salmonella* and *E. coli* O157:H7. Both Saga 200 and Biosource consistently predicted adequate process lethality with a criterion of  $\geq 5.0$ -log reduction of LAB. When either LAB decreased by  $\geq 5.0$  log CFU, processes were sufficiently lethal against *Salmonella* and *E. coli* O157:H7 in 100% of samples ( $n = 39$  and  $40$ , respectively). Use of LAB as pathogen surrogates for ground-and-formed beef jerky process validation was field tested by three small meat processors, who found this technique easy to use for process validation.

Beef jerky is a popular shelf-stable, protein-rich snack that is enjoyed across the United States. The popularity of jerky increased during the low-carbohydrate diet fad, with meat snacks doubling in market share from 1997 to 2003 and sales increasing by 147% during the same time (24). Although some of the more common forms of jerky are made from whole muscle, jerky also can be constructed from ground meat. Whole-muscle jerky is typically seasoned by soaking intact beef strips in a liquid marinade, whereas ground-and-formed beef jerky is seasoned by combining ground meat with dried spices and then shaping the batter into strips. For both types of jerky, the seasoned meat is dehydrated to eventually become a shelf-stable jerky product.

Multiple outbreaks of illness involving *Escherichia coli* O157:H7 or *Salmonella* have been linked to beef jerky (7, 9, 19, 23). After the outbreak of salmonellosis linked to jerky in fall 2003, the U.S. Department of Agriculture (USDA) began to question the lethality of jerky-making processes (29). To provide better information and guidance to small and very small meat processors, the USDA Food Safety and Inspection Service (FSIS) published the “Compliance Guideline for Meat and Poultry Jerky Produced by

Small and Very Small Plants” (29). Within this guideline, the FSIS identified two areas where jerky processors needed to improve: ensuring adequate lethality and utilizing water activity ( $a_w$ ) rather than moisture:protein ratio as an indicator of shelf stability (29).

According to the USDA (25, 28), to have sufficient lethality in a jerky-making process a 5.0-log reduction in *Salmonella* must be achieved. Although there is no USDA regulation concerning lethality treatments against *E. coli* O157:H7 in beef jerky, the industry standard is also a 5.0-log reduction (20). The FSIS 2007 Compliance Guideline recommended several methods to help achieve adequate thermal lethality (29). These recommended methods, which result in greater pathogen reduction beyond that achieved by heating alone, included (i) preheating jerky strips in a marinade to a minimum internal temperature of 71.1°C (160°F) before drying (13, 16), (ii) dipping the product in 5% acetic acid for 10 min before marination and drying (4, 6), or (iii) dipping the product in either calcium sulfate or acidified sodium chlorite before drying (17).

The methods recommended by the USDA to help meet pathogen lethality standards are more appropriate for whole-muscle beef jerky than for ground-and-formed product. Ground-and-formed jerky is seasoned with a dry spice-cure mix and is not marinated; dipping of strips in acid solution is not feasible because of the nonintact nature of the product. Pathogen contamination in whole-muscle jerky is likely to occur on the surface of the strip, whereas in

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ground-and-formed jerky contamination could easily be distributed throughout the strip. Therefore, meeting the performance standard with a ground-and-formed product can be more challenging, and yet few studies have evaluated the lethality of processes used for ground-and-formed jerky. Harrison et al. (14) noted that during the drying of spiced-cured inoculated ground-and-formed beef jerky at 60°C (140°F), populations of *Salmonella* and *Listeria monocytogenes* decreased by only 4.2 and 4.0 log CFU/g, respectively, after 8 h of drying in a home-style dehydrator. Work in our laboratory indicated that only two of eight processes for drying *E. coli* O157:H7–inoculated ground-and-formed beef jerky in a home-style dehydrator achieved sufficient lethality when judged to be dry (11).

Unfortunately, none of the studies that laid the groundwork for the recommendations made in the FSIS Compliance Guideline (29) were performed using a commercial oven-smokehouse, making it difficult to evaluate the applicability of these guidelines to a commercial plant setting. The FSIS Compliance Guideline also acknowledged that recommended treatments can result in undesirable textures and flavors in the finished product. In our laboratory, marinated whole-muscle beef strips dried at 62.5°C (145°F) for 10 h were hard and crumbly and judged unacceptable by a group of semitrained panelists (data not shown).

Recently, validated whole-muscle jerky-making processes have been established that ensure the achievement of the 5.0-log pathogen reduction goal under commercial conditions (2, 21), but no research exists to date for validating the commercial processing of ground-and-formed jerky. In addition to the need for scientifically validated commercial processes for ground-and-formed jerky, processors would gain an economic advantage if unique jerky-making processes could be easily validated. The Compliance Guideline indicates that unique processes can be validated and that challenge studies are an excellent means to achieve process validation (29). Unfortunately, it is not feasible for small and very small meat processors to perform scientific challenge studies for their processes, and in-plant challenge studies involving pathogens are not recommended for commercial meat processors because of potential safety concerns. An alternative approach to process validation would be to utilize nonpathogenic bacteria as pathogen surrogates during in-plant validation studies.

The effective use of nonpathogenic surrogate organisms for in-plant validation of lethality processes has a long history within the food industry. One instance in particular is the use of *Clostridium sporogenes* strain PA 3976 endospores as surrogates for the endospores of the less heat tolerant but very dangerous *Clostridium botulinum* for establishing safe thermal processes for low-acid canned foods (22). The meat industry routinely uses commercial lactic acid bacteria (LAB) starter cultures in the preparation of summer sausages and other similar products. As such, the presence of LAB within a meat processing plant and potentially in the finished product would be acceptable to regulators.

The objectives of this research were (i) to identify suitable commercial LAB starter cultures that could be used as

surrogates for *Salmonella* serovars and *E. coli* O157:H7 for evaluating the safety of processes for the manufacture of ground-and-formed beef jerky, (ii) to demonstrate the effectiveness of LAB surrogates in process validation for ground-and-formed beef jerky produced under a wide variety of commercial conditions, and (iii) to develop a method whereby LAB surrogates can be easily and successfully used by small meat processors for in-plant validation of unique processes.

## MATERIALS AND METHODS

**Preparation of jerky batter.** Lean ground beef (93 or 96% lean) was used to prepare the jerky batter. Meat was purchased in 2.3-kg packages from local grocery stores, held at -20°C until needed for experiments (<2 months), and thawed at 4°C before use. A sample (25 g) was taken prior to preparation of each batch of jerky batter, diluted in Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI), and plated on Petrifilm aerobic count plates (3M, St. Paul, MN) to determine aerobic plate count (APC). Each jerky strip prepared from the ground beef weighed roughly 25 g (see below), so APC results for raw product were reported on a per-strip basis.

Ground-and-formed beef jerky is traditionally prepared by mixing ground beef with dry spice and then extruding the mixture into strips for drying. Two popular spice mixes were used in experiments, Colorado and Barbeque (BBQ) flavor (Excalibur Seasoning, Pekin, IL). The Colorado and BBQ dry spice mixes were chosen because they represent ranges of pH (6.13 and 3.35, respectively) and final  $a_w$  (0.64 and 0.77, respectively) in trial experiments. To prepare jerky batter, the dry spice mixture (13.05 g of Colorado or 55.6 g of BBQ) and cure (1.15 g; Sure Cure, Excalibur Seasoning) were added to 453 g of ground beef in a 3.78-liter (1-gal) Ziploc bag. The spice/cure/meat ratios were based on the manufacturer's recommendations. After addition of the spice-cure mixture, inoculum was added (8 ml, see below), the Ziploc bag was sealed, and the seasoning, meat, and inoculum were massaged by hand for 2 min until evenly mixed.

**Strain selection.** Five strains each of *Salmonella* serovars and *E. coli* O157:H7 were combined in a cocktail used to inoculate lean ground beef before jerky strip formation and processing. The *Salmonella* serovars were initially obtained from Dr. Eric Johnson (Food Research Institute, University of Wisconsin–Madison) and are now available from this laboratory. The five strains used were *Salmonella* Enteritidis E40 (chicken ovary isolate, New York State Department of Health, Albany), *Salmonella* Typhi S9 and *Salmonella* Heidelberg S13 (clinical isolates, Wisconsin Laboratory of Hygiene, Madison), and *Salmonella* Infantis S20 and *Salmonella* Hadar S21 (origins unknown). These *Salmonella* isolates have routinely been used in our laboratory for thermal processing studies (2).

A total of 110 strains of *Escherichia coli* O157:H7 were isolated from raw beef trim from carcasses that had passed through a multiple-hurdle intervention treatment system at a large beef slaughter facility. The 110 beef trim isolates were subjected to pulsed-field gel electrophoresis using the *Xba*I restriction enzyme (Food Safety Net Services, San Antonio, TX), and 18 isolates were chosen for further study. The 18 strains were selected from a dendrogram of results and represented a wide range of genotypic relatedness (data not shown). Once the 18 strains were chosen, they were compared with five outbreak strains that our lab routinely uses in challenge studies (2): clinical isolates ATCC 43894, ATCC 51657, and ATCC 51658; ATCC 43895 originally isolated

from ground beef implicated in an outbreak; and USDA-FSIS-380-94 originally from salami implicated in an illness outbreak. All 23 strains (18 from beef trim and 5 from outbreaks) were tested for thermal tolerance as follows. Cultures were grown for 24 h at 35°C in 9.0 ml of beef extract broth (Becton Dickinson, Sparks, MD) with added tryptone (5 g/liter; Becton Dickinson), 0.1 ml of this culture was added to 9.0 ml beef extract broth with tryptone and incubated at 54.4°C, and survivors were serially diluted using BPD and enumerated by plating on nutrient agar (NA; Difco, Becton Dickinson). The five most heat tolerant of the 23 strains were selected for use in this study: one outbreak-associated strain (ATCC 43895) and four beef trim isolates (UWIL-BT-1, UWIL-BT-8, UWIL-BT-9, and UWIL-BT-11).

Six commercial LAB cultures intended for use in making fermented meat products were evaluated as potential pathogen surrogates: Bactoform LHP Dry (CHR Hansen, Hørsholm, Denmark), Trumark Formula 100 (Trumark, Inc., Linden, NJ), Bactoform T-SXP (CHR Hansen), Saga 200 (Kerry Bio-Science, Rochester, MN), Saga 75 (Kerry Bio-Science), and Biosource (Biosource Flavors, Inc., Muskego, WI). In preliminary experiments, each of these cultures was added to seasoned jerky batter along with the 10-strain pathogen cocktail (see below), and the inoculated batter was formed into strips (see below) and dried in either a small-scale commercial dehydrator (commercial food dehydrator 160L, Cabela's Inc., Sidney, NE) or a vacuum drying oven (model 5850, National Appliance Co., Portland, OR) set at 65.6°C (150°F). Jerky was dried for 6 h, and pathogen survivors were enumerated every 90 min. The Cabela's dehydrator created a low-humidity environment (~20% relative humidity [RH]), and the drying oven with vacuum not engaged created a high-humidity environment (~60% RH).

**Preparation of inoculum.** To obtain a working culture for pathogens, each strain was cultured twice successively (from a previously frozen culture) at 35°C for 18 to 24 h in brain heart infusion broth (Difco, Becton Dickinson), streaked on NA, incubated at 35°C for 18 to 24 h, examined for uniform colony morphology, and stored at 5°C. A plate of NA was streaked with one colony of each working culture to produce a lawn of growth after incubation at 35°C for 18 to 24 h. The lawn of growth was removed from each plate with a sterile loop and suspended by vortex mixing in 25 ml of BPD to make a two-pathogen, 10-strain mixture. Inoculum concentration for the 10-strain pathogen cocktail was approximately  $10^9$  CFU/ml.

The LAB cultures were held at -20°C until needed. To prepare an LAB solution for inoculating ground beef, 0.5 g of thawed culture was added to 9.0 ml of BPD and mixed well. One milliliter of each culture mixture (pathogen and LAB) was removed for plating to determine initial concentrations. The inoculum concentration for LAB cultures was approximately  $10^9$  CFU/ml.

**Inoculation of batter and strip formation.** Seasoned ground-and-formed jerky batter was prepared (453 g) and inoculated with 8.0 ml of a single LAB culture or 8.0 ml of the 10-strain cocktail of *Salmonella* and *E. coli* O157:H7. After the meat, spice, cure, and culture were mixed, the batter was rolled out onto sterilized baking sheets (37.5 by 25 by 1.9 cm). The sheets were marked at 2.5-cm intervals along the length and in half along the width to allow for consistent strip dimension. Eighteen jerky strips were obtained per 453 g of meat, with a standard dimension of 12.7 by 2.5 by 0.7 cm. The average raw strip weight was  $22.8 \pm 3.4$  g ( $n = 72$ ). Either the strips were removed from the baking sheets and placed inside a small-scale commercial dehydrator in the laboratory, or the baking sheets were covered with aluminum foil and placed inside a cooler with ice packs for transport to the

Alkar-RapidPak Research and Technology Center (ARPRTC, Lodi, WI; 30 mi from Madison) where a biosafety level II commercial oven-smokehouse was located. Time from batter inoculation through placement of samples in the commercial dehydrator or oven-smokehouse was no more than 3 h.

**Overnight refrigeration of batter.** Meat processors may occasionally prefer to prepare jerky batter ahead of time, so the effect of overnight refrigeration on the levels of pathogens and LAB in inoculated batter was examined (nine strips per spice mixture). One representative sample strip was taken before refrigeration for each combination of spice mixture and inoculum. After 24 h of refrigeration at 4 or 10°C, another representative sample strip was taken from each combination of spice mixture and inoculum for plating of inoculum organisms (see below).

**Jerky processing.** Ground-and-formed beef jerky was prepared based on process schedules obtained from a survey of Wisconsin processors (Table 1) and dried using either a small-scale commercial dehydrator (Cabela's) for process 1 or a large commercial oven-smokehouse at the ARPRTC (Alkar model 2000 oven) for processes 2 through 6. The Cabela's dehydrator is used by some small commercial jerky processors and represents one extreme in jerky processing. The user has the option of setting the process dry bulb temperature but no control of the wet bulb temperature, humidity, or air flow. Many jerky manufacturers use a commercial oven-smokehouse similar to the Alkar oven that was used in this study, allowing control and/or monitoring of wet bulb and dry bulb temperatures and air flow and providing the option of adding smoke. Samples selected for the oven-smokehouse were processed in two ways: without smoke (process A) and with smoke (process B). The sawdust used for smoking was a hardwood mix (Franz Company, Milwaukee, WI). During the without-smoke processes, the fan speed was set to 10, the dampers were open, and there were 19.93 air exchanges per min. When smoke was added, the fan speed was set to 5, the dampers were closed, and the oven had 9.96 air exchanges per min.

The wet bulb and dry bulb temperatures during each process were monitored using dataloggers (model SP150, The Dickson Company, Addison, IL) with K-type thermocouple probes. Within the commercial oven-smokehouse, wet bulb and dry bulb temperature, %RH, and smoke all were controlled and recorded by computer in addition to the wet bulb and dry bulb temperature readings taken by the dataloggers. In all trials, internal temperature was measured by inserting a datalogger probe into the center of one jerky strip for each run. Within the dehydrator, hot and cold zones were identified and thus internal temperature measurements were taken from a strip placed in each zone. The oven-smokehouse did not have such zones, and the internal temperature was measured in one strip during these processes. The  $a_w$  of strips was monitored during the drying process with an AquaLab Series 3TE water activity meter (Decagon Devices, Inc., Pullman, WA) as an indicator of doneness and to determine the effect the different spice mixes on water retention in the finished product. Although  $a_w$  was measured throughout each run, only the final value was reported; in most cases this represented a single reported measurement per run.

Roughly 2.7 kg of meat was dried in each run in the oven-smokehouse, and 0.5 kg was dried in each run in the dehydrator. The meat was formed into strips and placed on a rack in the dehydrator or oven-smokehouse. Pairs of strips were arranged in close proximity to allow the LAB strips and pathogen strips to be taken as paired samples for statistical analysis. The pattern within the dehydrator took into account the hot and cold zones and also allowed for samples to be taken from each zone at each sampling

TABLE 1. Representative commercial processing schedules used in the preparation of ground-and-formed beef jerky

Process <sup>a</sup>	Step time (min)	Cumulative time (min)	Smoke <sup>b</sup>	Set dry bulb temp, °C (°F)	Set wet bulb temp, °C (°F)	Actual dry bulb temp range, °C (°F)	Actual wet bulb temp range, °C (°F)
1	420	420	No	68.3 (155)	NC <sup>c</sup>	49.4–66.1 (121–151)	13.9–38.3 (57–101)
2-A	30	30	No	76.7 (170)	60.0 (140)	57.8–77.2 (136–171)	51.7–61.1 (125–142)
	120	150	No	54.5 (130)	NC	52.8–57.8 (127–136)	28.9–37.2 (84–99)
	90	240	No	76.7 (170)	NC	76.1–77.8 (169–172)	48.3–55.6 (119–132)
2-B	30	30	Yes	76.7 (170)	60.0 (140)	57.2–77.8 (135–172)	33.3–60.0 (92–140)
	120	150	Yes	54.5 (130)	NC	67.2–88.9 (153–192)	ND <sup>d</sup>
	90	240	Yes	76.7 (170)	NC	81.7–90.6 (179–195)	ND
3-A	30	30	No	76.7 (170)	NC	76.1–77.8 (169–172)	30.0–41.7 (86–107)
	15	45	No	76.7 (170)	71.7 (161)	65.6–78.3 (150–173)	33.9–56.7 (93–134)
	130	175	No	76.7 (170)	NC	76.7–80.6 (170–177)	ND
3-B	30	30	No	76.7 (170)	NC	59.5–77.2 (139–171)	28.9–34.5 (84–94)
	15	45	No	76.7 (170)	71.7 (161)	70.0–76.7 (158–170)	38.3–70.6 (101–159)
	130	175	Yes	76.7 (170)	NC	75.6–83.9 (168–183)	ND
4-A	90	90	No	57.2 (135)	51.7 (125)	55.6–58.9 (132–138)	47.8–52.2 (118–126)
	150	240	No	85.0 (185)	NC	83.9–88.9 (183–192)	30.6–55.6 (87–132)
4-B	90	90	Yes <sup>e</sup>	57.2 (135)	51.7 (125)	56.7–59.5 (134–139)	49.5–52.2 (121–126)
	150	240	Yes <sup>f</sup>	85.0 (185)	NC	74.5–91.1 (166–196)	ND
5-A	90	90	No	54.5 (130)	NC	53.3–57.8 (128–136)	31.7–37.8 (89–100)
	60	150	No	60.0 (140)	21.1 (70)	58.9–62.8 (138–145)	39.5–42.2 (103–108)
	60	210	No	65.6 (150)	26.7 (80)	64.5–67.8 (148–154)	44.5–47.2 (112–117)
	60	270	No	70.1 (160)	32.2 (90)	70.6–76.1 (159–169)	50.0–51.7 (122–125)
	60	330	No	76.7 (170)	NC	75.6–81.1 (168–178)	53.3–56.1 (128–133)
5-B	90	90	Yes <sup>e</sup>	54.5 (130)	NC	54.5–77.2 (130–171)	48.9–70.6 (120–159)
	60	150	Yes	60.0 (140)	21.1 (70)	68.9–81.7 (156–179)	58.9–74.5 (138–166)
	60	210	Yes	65.6 (150)	26.7 (80)	69.5–83.3 (157–182)	68.9–76.1 (156–169)
	60	270	Yes	70.1 (160)	32.2 (90)	76.7–84.5 (170–184)	ND
6-A	75	75	No	51.7 (125)	NC	51.1–52.2 (124–126)	ND
	30	105	No	51.7 (125)	NC	50.6–51.7 (123–125)	ND
	75	180	No	65.6 (150)	NC	64.5–66.1 (148–151)	ND
	30	210	No	85.0 (185)	NC	84.5–87.8 (184–190)	ND
6-B	75	75	No	51.7 (125)	NC	51.1–52.8 (124–127)	42.2–47.8 (108–118)
	30	105	Yes	51.7 (125)	NC	50.6–72.8 (123–163)	52.8–65.0 (127–149)
	75	180	Yes	65.6 (150)	NC	65.6–86.1 (150–187)	59.5–77.2 (139–171)
	30	210	No	85.0 (185)	NC	84.5–85.6 (184–186)	77.2–77.8 (171–172)

<sup>a</sup> Processes: 1, small-scale commercial dehydrator (Cabela's); 2 through 6, commercial oven-smokehouse (ARPRTC). A, processes without smoke; B, processes with smoke.

<sup>b</sup> Smoke added during the entire step (unless noted).

<sup>c</sup> NC, not controlled.

<sup>d</sup> ND, not determined.

<sup>e</sup> Smoke added after 30 min.

<sup>f</sup> Smoke discontinued after 90 min.

time. Because hot and cold zones were not present within the oven-smokehouse, strips were laid out in a consistent pattern with duplicate samples per sampling time. Sampling times were selected based upon run parameters (Table 1). For the runs using the dehydrator, samples were taken at 0, 3.5, and 7 h (end of process). The dehydrator temperature was set to 155°F (68.3°C), but this temperature was never achieved (Table 1). From the oven-smokehouse, samples were taken at the beginning of the process, before and after a programmed temperature change, and at the end of the process. Samples also were taken just before smoke was added and when it was discontinued. Intervals for sampling jerky processed in the oven-smokehouse were selected to segregate phases of the process and to identify the contribution of each phase to the overall lethality achieved. Temperature adjustment within the oven-smokehouse was very rapid, reaching the set point

for the next phase and stabilizing within 2 to 3 min. Each of five processes using the oven-smokehouse was run twice, once with smoke and once without smoke.

In some instances, samples of dried (finished) pathogen-inoculated jerky were vacuum packaged and stored at 21°C for 4 to 6 weeks to determine the effect of storage on pathogenic organisms that survived the heating and drying processes.

**Enumeration of inoculum organisms.** The numbers of *E. coli* O157:H7 and *Salmonella* cells in the jerky strips were measured at each sampling time for each jerky formulation during each process. A sample consisted of one jerky strip, and two samples were analyzed for pathogen concentrations at each sampling time. LAB samples were taken with the same method and number as the pathogen strips.

Once removed from the dehydrator or oven-smokehouse, each sample was placed in a Whirl-Pak filter bag (20.9 by 15.3 cm; Nasco, Fort Atkinson, WI). The samples from the dehydrator were processed and plated immediately, but samples from the oven-smokehouse were kept cold ( $\leq 4^{\circ}\text{C}$ ) and analyzed within 8 h of sampling. To homogenize the sample for plating, 99 ml of BPD was added to the sample bag, which was then stomached for 2 min at medium speed (Stomacher 400 Circulator Lab Blender, Seward, Worthington, UK) according to the USDA microbiology laboratory guidebook (26).

The dilution factor for the stomached sample (initial dilution) was arbitrarily defined as  $10^{-1}$ . Serial decimal dilutions were made in BPD as necessary. From the initial dilution, 1.0 ml was divided and spread among three brain heart infusion agar (BHIA; Difco, Becton Dickinson) plates ( $10^{-1}$ ). From the initial dilution and each subsequent dilution, 0.1 ml was spread on one BHIA plate per dilution ( $10^{-2}$  up to  $10^{-10}$ ). For pathogen enumeration only, the dilutions were also directly plated on modified eosin methylene blue agar (M-EMB; Difco, Becton Dickinson). M-EMB was discussed as a superior *E. coli* O157:H7 recovery medium by Clavero and Beuchat (8) and used for enumerating *E. coli* O157:H7 from ground beef jerky by Harrison et al. (15). BHIA plates were incubated at  $35^{\circ}\text{C}$  for 1 h to allow for repair of heat-injured cells and then overlaid with xylose lysine deoxycholate agar (XLD; Difco, Becton Dickinson) or lactobacilli deMan Rogosa Sharpe agar (Difco, Becton Dickinson) for selective differentiation of either *E. coli* O157:H7 and *Salmonella* serovars or LAB, respectively. Before studying the smoke-added processes (2-B through 6-B), an analysis of variance (ANOVA) was used to determine whether there was a difference in the recovery of cells between BHIA with the XLD overlay and direct plating on M-EMB. Direct plating on M-EMB resulted in significantly greater recovery ( $P < 0.05$ ) of both *E. coli* O157:H7 and *Salmonella* serovars than did plating on BHIA with the XLD overlay, so M-EMB was used for all the subsequent cultures. After incubating at  $35^{\circ}\text{C}$  for either 24 h (*E. coli* O157:H7 and *Salmonella* serovars) or 48 h (LAB), plates were examined and typical colonies were enumerated. For each process, one plate with presumptive colony growth on each medium was retained for confirmation tests as follows. One presumptive colony of each bacterium from each plate was transferred onto BHIA and incubated at  $35^{\circ}\text{C}$  for 24 h, and a representative colony was then tested to confirm colony identity. Confirmation tests for presumptive pathogens were Gram reaction, cellular morphology, and oxidase activity. API 20E strips (bioMérieux, Hazelwood, MO) were used for biochemical characterization of *Salmonella* serovars, and an O157 latex agglutination test (Oxoid, Ogdensburg, NY) was used to confirm *E. coli* O157:H7 isolates. Presumptive LAB colonies were evaluated for Gram reaction, cellular morphology, and catalase activity. The count (log CFU) for each inoculated organism was calculated for each sample on a per sample basis, and mean counts were calculated for each sampling time. A value of 0.5 CFU (0.699 log CFU at  $10^{-1}$ ) was assigned when no colonies were present on the least dilute plate. The lethality achieved by each process was calculated by subtracting the final log CFU value from the initial (time 0) log CFU value.

**Statistical analyses.** Data were analyzed with version 9.1 of the SAS statistical package (SAS Institute, Inc., Cary, NC). The ANOVA was performed to separately evaluate the recovery of *E. coli* O157:H7 and *Salmonella* serovars directly plated on M-EMB compared with BHIA with the XLD overlay. Differences of least-squared means were adjusted using the Tukey and Tukey-Kramer methods to account for differences in sample size for the media

comparison. Differences of least-squared means computed from the ANOVA (performed for determining the effect on seasoning mixture on lethality) were adjusted using the Tukey method.

## RESULTS

### Selection of LAB cultures as pathogen surrogates.

Initial results indicated that three of the six LAB cultures (Bactoform LHP Dry, Bactoform T-SXP, and Trumark Formula 100) contained more than one genus, thus complicating colony counting, and these three cultures were not evaluated further (data not shown). The final three cultures (Biosource, Saga 200, and Saga 75) were subjected to both low- and high-humidity drying in seasoned jerky batter at  $65.6^{\circ}\text{C}$  ( $150^{\circ}\text{F}$ ), and survival was compared with that in pathogen-inoculated batter to examine the death of each LAB culture compared with the pathogens. The two LAB starter cultures identified as consistently more thermotolerant than the *Salmonella* serovars and *E. coli* O157:H7 strains under both low- and high-humidity conditions were Saga 200 (described by the manufacturer as *Pediococcus* spp.) and Biosource (described as *Pediococcus acidilactici*) (data not shown). These two LAB cultures were selected for use in experiments and were used in the same concentration as *Salmonella* serovars and *E. coli* O157:H7 strains.

**Preparation and inoculation of jerky batter.** Before seasoning and inoculation, the ground beef used in this study was analyzed for APC. The average APC was  $4.26 \pm 1.41$  log CFU per strip ( $n = 21$ ). The highest and lowest APCs were 1.40 and 6.20 log CFU per strip, respectively. The initial pH of the ground beef before seasoning and inoculation ranged from 5.56 to 6.44 ( $n = 15$ ). The two seasonings used in this study were chosen because they represent extremes of pH (6.13 and 3.35 for Colorado and BBQ, respectively). However, the pH of the seasoned jerky batter was more consistent: 6.15 for the Colorado-seasoned batter and 5.88 for the BBQ-seasoned batter. Pathogen inoculation data were based on counts obtained using M-EMB; once results indicated that BHIA with the XLD overlay was not as effective at recovering pathogens, its use was discontinued (data not shown). Initial pathogen and LAB concentrations were approximately  $10^8$  CFU per strip and varied no more than 0.5 log CFU over all experiments ( $n = 228$ ).

**Overnight refrigeration of batter.** Meat processors routinely prepare ground-and-formed jerky batter immediately before drying into jerky, and we followed the same process. However, we considered the possibility that processors may occasionally prefer to prepare jerky batter ahead of time, so the effect of overnight refrigeration on the levels of the pathogens and the LAB in inoculated batter was examined. Under proper refrigeration at  $4^{\circ}\text{C}$  for 24 h, there was no change in the level of the Saga 200 LAB in either jerky batter or of the Biosource LAB in BBQ-seasoned batter. Biosource decreased slightly (0.26 log CFU) in Colorado-seasoned jerky batter. Under the same conditions, both *E. coli* O157:H7 and *Salmonella* decreased slightly (0.20 to 0.30 log CFU) in seasoned batter. When batter was stored at a moderately abusive temperature of  $10^{\circ}\text{C}$  for 24 h, the level of Saga 200 decreased slightly (0.23 to 0.36 log CFU) in Colorado- or BBQ-seasoned batter, whereas Biosource decreased by 0.37 log CFU in Colorado-

TABLE 2. *Lethality against E. coli O157:H7, Salmonella serovars, Saga 200 (Pediococcus spp.), and Biosource (Pediococcus acidilactici) in Colorado-seasoned ground-and-formed jerky for each A process (no smoke added)*

Process <sup>a</sup>	Lethality ( $\Delta$ log CFU) <sup>b</sup>				a <sub>w</sub> <sup>c</sup>
	<i>E. coli</i> O157:H7	<i>Salmonella</i>	Saga 200	Biosource	
4	8.11 (7.79–8.42)	7.80 (7.78–7.82)	7.11 (6.50–7.72)	7.66 (7.64–7.69)	0.83
2	7.35 (7.35–7.36)	7.36 (7.35–7.37)	5.28 (4.97–5.61)	7.59 (4.85–4.90)	0.68
3	6.80 (6.23–7.38)	6.05 (5.70–6.40)	4.79 (4.65–4.92)	4.62 (4.34–4.91)	0.69
6	5.31 ( $\pm$ 0.71)	5.05 ( $\pm$ 0.54)	3.59 ( $\pm$ 0.48)	4.08 ( $\pm$ 0.59)	0.83 <sup>d</sup>
5	5.77 (5.06–6.49)	4.86 (4.51–5.20)	3.75 (3.59–3.92)	3.96 (3.78–4.13)	0.67
1	3.88 ( $\pm$ 0.69)	3.27 ( $\pm$ 0.43)	2.58 ( $\pm$ 0.44)	2.59 ( $\pm$ 0.84)	0.78 <sup>e</sup>

<sup>a</sup> Processes are listed in order of decreasing lethality against *Salmonella*.

<sup>b</sup> Lethality for processes 4, 2, 3, and 5 is the mean (range) for two samples; lethality for process 6 is the mean ( $\pm$ SD) of four samples; and lethality for process 1 is the mean ( $\pm$ SD) of eight samples.

<sup>c</sup> a<sub>w</sub> of finished product;  $n = 1$  except where noted.

<sup>d</sup> Mean of two samples (range, 0.74 to 0.92).

<sup>e</sup> Mean of 20 samples (SD, 0.05).

seasoned batter and did not change in BBQ-seasoned strips. There was minimal change in pathogen levels during overnight 10°C storage, with decreases of 0.13 to 0.43 CFU per strip depending on the spice mix. The pH of the refrigerated jerky strips did not change from prerefrigeration readings or differ between the storage conditions (data not shown).

**Lethality of jerky processes.** Ground-and-formed beef jerky was prepared based on commercial processing schedules (Table 1) and dried with either a dehydrator (process 1) or an oven-smokehouse (processes 2 through 6). These processes resulted in a range of lethality (Tables 2 through 5). Process 1 in the dehydrator was by far the least lethal process because of both low heat achievable in the dehydrator and low humidity. There was no control over humidity in the dehydrator, which averaged 20% over the course of each run, and the temperature, even when set at 68.3°C (155°F), averaged only 61.6°C (143°F). It took an average of 90 min for the dehydrator to reach this drying temperature once the unit was turned on. This process cannot be recommended because it failed to produce safe jerky at any point in the process (safe defined as having a  $\geq$ 5.0-log reduction in pathogens). Average decreases in *E. coli* O157:H7 and *Salmonella* by the end of process 1 were 3.88 and 3.27 log CFU, respectively ( $n = 20$  and 20; Table 2). Lethality against the LAB cultures was also low, with de-

creases of 2.59 and 2.58 log CFU for Biosource and Saga 200, respectively ( $n = 16$  and 24, respectively; Table 2). The mean final a<sub>w</sub> for the jerky made in process 1 was 0.78 ( $n = 20$ ; Table 2). Appendix A of the 1999 FSIS Compliance Guideline (27) lists acceptable minimum internal time and temperature combinations and RH requirements for the manufacture of safe shelf-stable beef products. Although each of the trials for process 1 achieved a time and internal temperature combination published in Appendix A (27) and an acceptable final a<sub>w</sub> ( $\leq$ 0.85), the Appendix A %RH conditions ( $\geq$ 90% RH throughout the process) were not met. We used only 0.5 kg of seasoned meat strips in the dehydrator during each run. Addition of more strips, as a processor certainly would do, would further decrease the temperature because of evaporative cooling, making it difficult for jerky processors using a similar small-scale commercial dehydrator to produce a product that met either the time-temperature or %RH criteria for safety.

Lethality differed widely between the processes run in the oven-smokehouse (processes 2 through 6). Because commercial processors may introduce smoke in a jerky process to add color or flavor and to determine the effect of added smoke on the viability of both pathogens and LAB cultures, each of the processes run in the oven-smokehouse was run once without smoke (A) and once with smoke add-

TABLE 3. *Lethality against E. coli O157:H7, Salmonella serovars, Saga 200 (Pediococcus spp.), and Biosource (Pediococcus acidilactici) in Colorado-seasoned ground-and-formed jerky for each B process (smoke added)*

Process <sup>a</sup>	Lethality ( $\Delta$ log CFU) <sup>b</sup>				a <sub>w</sub> <sup>c</sup>
	<i>E. coli</i> O157:H7	<i>Salmonella</i>	Saga 200	Biosource	
4	7.46 (7.30–7.62)	7.45 (7.41–7.48)	7.03 (6.97–7.08)	7.60 (7.53–7.66)	0.87
3	7.41 (7.38–7.45)	7.34 (7.34–7.34)	6.84 (6.78–6.89)	7.38 (7.38–7.38)	0.90
2	7.42 (7.38–7.47)	7.19 (7.15–7.24)	6.63 (5.79–7.46)	7.59 (7.58–7.61)	0.78
6	5.99 (3.27–5.70)	6.07 (5.82–6.31)	4.38 (4.22–4.53)	5.33 (4.90–5.79)	0.90
5	5.07 (4.54–5.60)	5.15 (5.08–5.22)	3.70 (3.67–3.74)	3.77 (3.71–3.84)	0.75

<sup>a</sup> Processes are listed in order of decreasing lethality against *Salmonella*.

<sup>b</sup> Lethality for all processes is the mean (range) for two samples.

<sup>c</sup> a<sub>w</sub> of finished product;  $n = 1$ .

TABLE 4. Lethality against *E. coli* O157:H7, *Salmonella* serovars, *Saga* 200 (*Pediococcus* spp.), and *Biosource* (*Pediococcus acidilactici*) in BBQ-seasoned ground-and-formed jerky for each A process (no smoke added)

Process <sup>a</sup>	Lethality ( $\Delta$ log CFU) <sup>b</sup>				a <sub>w</sub> <sup>c</sup>
	<i>E. coli</i> O157:H7	<i>Salmonella</i>	<i>Saga</i> 200	<i>Biosource</i>	
4	7.68 (7.45–7.91)	7.37 (7.26–7.48)	7.71 (7.18–8.24)	7.31 (7.01–7.61)	ND
3	6.20 (6.12–6.27)	6.03 (5.16–6.90)	5.48 (4.79–6.17)	4.80 (4.56–5.05)	ND
5	5.59 (5.36–5.82)	5.08 (4.96–5.21)	4.18 (3.69–4.68)	3.75 (3.49–4.01)	ND
6	4.97 ( $\pm$ 0.37)	4.40 ( $\pm$ 0.35)	3.57 ( $\pm$ 0.28)	3.69 ( $\pm$ 0.13)	0.86 <sup>d</sup>

<sup>a</sup> Processes are listed in order of decreasing lethality against *Salmonella*.

<sup>b</sup> Lethality for processes 3 through 5 is the mean (range) for two samples; lethality for process 6 is the mean ( $\pm$ SD) of four samples.

<sup>c</sup> a<sub>w</sub> of finished product;  $n = 1$  except where noted. ND, not determined (no experiments done).

<sup>d</sup> Mean of two samples (range, 0.81 to 0.90).

ed (B). Process 2-B and both the A and B versions of processes 3 through 6 were conducted using both Colorado and BBQ spice mixes. The final lethality against both the pathogens and LAB was affected significantly ( $P < 0.004$ ) by the spice mixture used, with greater lethality achieved for BBQ-seasoned strips. Depending upon the particular process and the corresponding level of humidity, the addition of smoke in the process led to adequate lethality earlier in the run (Figs. 1 through 4). Processes that included smoke had slightly higher final reductions of pathogens compared with processes without smoke, especially for *Salmonella* serovars and when the Colorado seasoning mixture was used (Tables 2 through 5). The addition of smoke to the process had an even greater effect on the destruction of LAB. There was as much as a 2.76-log difference in the lethality for LAB between smoke and nonsmoke processes (Tables 2 through 5) in Colorado-seasoned jerky and a 3.09-log difference in BBQ-seasoned jerky. The actual temperature of the smokehouse during processes with added smoke (B) was consistently higher than the set temperature (up to 34.4°C higher), presumably because smoke enters the smokehouse at 260°C (500°F). This temperature increase rather than the presence of smoke itself may have been responsible for the increased lethality observed in smoke-added products. Within the jerky industry, an a<sub>w</sub> of  $\leq 0.85$  is an indication of shelf stability and an appropriate process endpoint. Most processes reached the shelf-stability guideline with both spice mixtures, except for the Color-

do-seasoned samples from processes 3-B, 4-B, and 6-B. The a<sub>w</sub> of BBQ-seasoned jerky was not measured during processes 3-A, 4-A, and 5-A because of a laboratory error. There was a delay of up to 8 h in the analysis of samples from runs using the oven-smokehouse at the ARPRTC. Samples were kept on ice during this delay. Such a delay is consistent with conditions that jerky makers could face when collecting in-plant samples and shipping them to a commercial laboratory for process validation testing.

The process most likely to achieve adequate destruction of pathogens was process 4. Process 4 began with relatively low wet bulb and dry bulb temperatures but created a high-humidity environment, 74% RH, and thus the wet bulb temperature setting was only 5.5°C lower than the dry bulb temperature setting (Table 1). This step was then followed by a step with a very high dry bulb temperature (setting at 85.0°C) and no addition of humidity. The final lethality achieved with process 4-A was 8.11 log CFU for *E. coli* O157:H7 and 7.80 log CFU for *Salmonella* in Colorado-seasoned jerky (Table 2). In BBQ-seasoned jerky, the final lethality was 7.68 log CFU for *E. coli* O157:H7 and 7.37 log CFU for *Salmonella*. Most cells were killed during the 85°C (185°F) step for both pathogens and LAB (Figs. 1 and 3; panel 4-A). Process 4-B did not exhibit the increased lethality observed during other smoke-added processes; the dry bulb temperature was already high at 85°C (185°F), and the addition of smoke into the oven only increased the temperature 6.1°C beyond the set temperature

TABLE 5. Lethality against *E. coli* O157:H7, *Salmonella* serovars, *Saga* 200 (*Pediococcus* spp.), and *Biosource* (*Pediococcus acidilactici*) in BBQ-seasoned ground-and-formed jerky for each B process (smoke added)

Process <sup>a</sup>	Lethality ( $\Delta$ log CFU) <sup>b</sup>				a <sub>w</sub> <sup>c</sup>
	<i>E. coli</i> O157:H7	<i>Salmonella</i>	<i>Saga</i> 200	<i>Biosource</i>	
4	7.45 (7.42–7.47)	7.51 (7.45–7.58)	7.03 (6.90–7.16)	7.62 (7.56–7.68)	0.78
2	7.34 (7.18–7.49)	7.44 (7.42–7.46)	6.93 (6.42–7.43)	7.34 (7.06–7.62)	0.79
6	7.41 (7.09–7.72)	7.23 (6.84–7.62)	5.45 (5.43–5.48)	5.57 <sup>d</sup>	0.88
3	7.11 (7.08–7.15)	6.82 (6.30–7.34)	7.16 (7.13–7.19)	7.89 (7.86–7.93)	0.85
5	4.50 (4.38–4.61)	4.57 (4.51–4.63)	4.05 (3.89–4.21)	3.53 (3.51–3.55)	0.85

<sup>a</sup> Processes are listed in order of decreasing lethality against *Salmonella*.

<sup>b</sup> Lethality for all processes is the mean (range) for two samples.

<sup>c</sup> a<sub>w</sub> of finished product;  $n = 1$ . ND, not determined (no experiments done).

<sup>d</sup> Only one sample was analyzed.

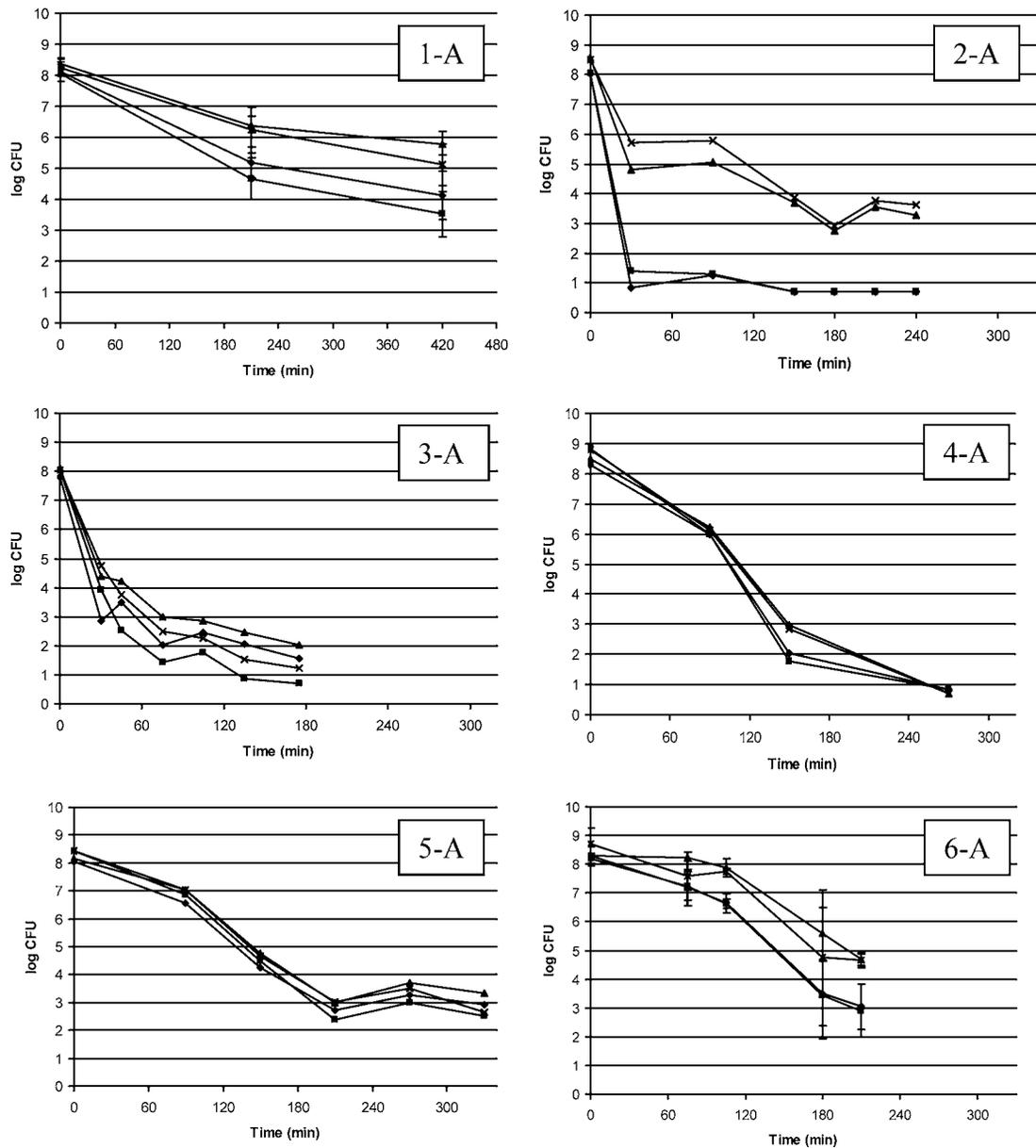


FIGURE 1. Survival of *E. coli* O157:H7, *Salmonella* serovars, and lactic acid bacteria pathogen surrogates (log CFU) in Colorado-seasoned ground-and-formed jerky during processes 1-A through 6-A (no smoke added). See Table 1 for process parameters. ◆, *Salmonella* serovars; ■, *E. coli* O157:H7; ▲, *Saga 200* (*Pedococcus* spp.); ×, *Biosource* (*Pedococcus acidilactici*).

(Table 1). During the high-heat step, the RH of the oven-smokehouse was 22%. The  $a_w$  of the finished Colorado-seasoned product was 0.83 for process 4-A and 0.87 for process 4-B (Tables 2 and 4). The BBQ-seasoned jerky had a final  $a_w$  of 0.78 for process 4-B (Table 5).

Processes 2 and 3 had adequate lethality for the pathogens. Process 2 began with a step that had both high dry bulb and wet bulb temperature settings, 30 min at 76.7°C (170°F) and 60°C (140°F), respectively (Table 1). When pathogen counts were determined immediately after this step, reductions in *E. coli* O157:H7 and *Salmonella* were 6.28 to 7.05 and 7.06 to 7.35 log CFU, respectively (Fig. 1, panel 2-A). This result is similar to the overall lethality of 7.35 log CFU for *E. coli* O157:H7 and 7.36 log CFU for *Salmonella* serovars in process 2-A (Table 2). The RH of this process was initially high (45%) and decreased

throughout the process to a low of 17% but then increased toward the end, finishing at 36%. The final  $a_w$  for the samples from this process was 0.68. The same elimination of pathogens following the initial high wet bulb and dry bulb temperature step was observed for process 2-B (smoke added; Figs. 2 and 4, panel 2-B). Process 2-B resulted in less lethality for LAB during the first step of drying compared with the same step in process 2-A (compare panel 2-B in Figs. 2 and 4 with panel 2-A in Fig. 1), but the subsequent high heat and smoke during the second step resulted in nearly complete destruction of the LAB in jerky made with either spice mixture. During the second stage of process 2-B, the actual recorded temperature was higher than the set dry bulb temperature by up to 34°C (Table 1). The final  $a_w$  of process 2-B Colorado-seasoned product was 0.78, whereas the BBQ-seasoned jerky had a final  $a_w$  of 0.79.

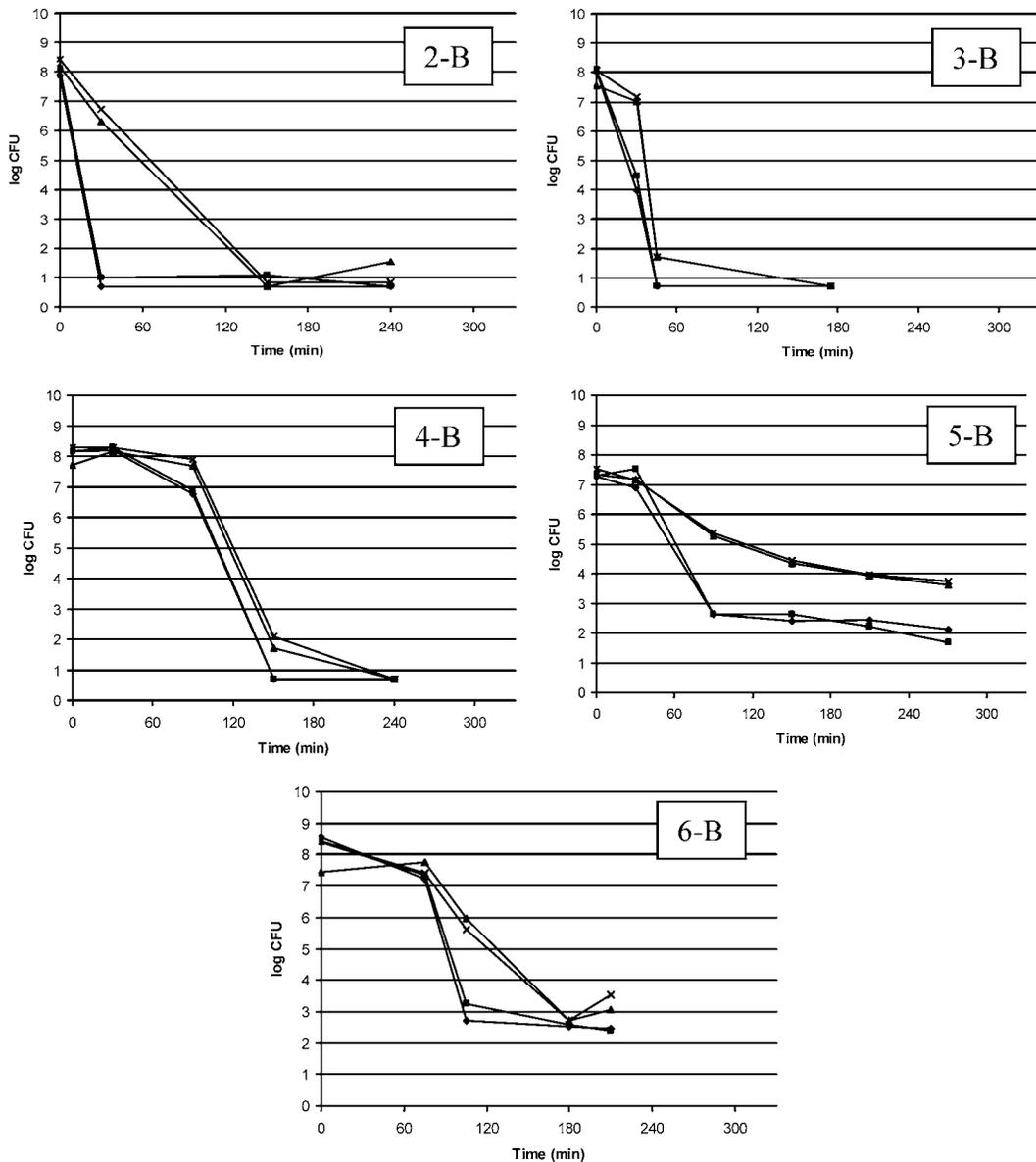


FIGURE 2. Survival of *E. coli* O157:H7, *Salmonella* serovars, and lactic acid bacteria pathogen surrogates (log CFU) in Colorado-seasoned ground-and-formed jerky during processes 2-B through 6-B (smoke added). See Table 1 for process parameters. ◆, *Salmonella* serovars; ■, *E. coli* O157:H7; ▲, *Saga 200* (*Pediococcus* spp.); ×, *Biosource* (*Pediococcus acidilactici*).

Process 3 was intended to begin with and maintain high dry bulb temperatures and to include a step with a high wet bulb temperature. A problem with the oven-smokehouse during process 3-A made it impossible to attain the 71.7°C (161°F) wet bulb temperature. The maximum wet bulb temperature and RH achieved during process 3-A were 56.7°C and 38%, respectively. The final  $a_w$  of jerky from process 3-A was 0.69 for Colorado-seasoned jerky. The wet-bulb temperature spiked during process 3-B. In reaching the set wet bulb temperature, the RH rose to 80%. Process 3-B ran warmer than the set dry bulb temperature (Table 1) by up to 7.2°C. The wet environment created by the high RH in this process resulted in an unacceptably high final  $a_w$  of 0.90 for Colorado-seasoned jerky; the BBQ-seasoned product had a final  $a_w$  of 0.85. Overall, there was a 0.61-log and a 1.29-log increase in lethality for *E. coli* O157:H7 and *Salmonella*, respectively, in Col-

orado-seasoned strips with process 3-B compared with process 3-A (Tables 2 and 3). This increase in pathogen lethality was accompanied by an increase in lethality for the LAB: 2.05 log CFU for *Saga 200* and 2.76 log CFU for *Biosource* (Tables 2 and 3). The lethality for *E. coli* O157:H7 and *Salmonella* in BBQ-seasoned jerky also increased by 0.91 and 0.79 log CFU, respectively, during process 3-B relative to the lethality achieved during process 3-A (Tables 4 and 5). Process 3-B was more lethal for LAB in BBQ-seasoned jerky strips by 1.68 and 3.09 log CFU for *Saga 200* and *Biosource*, respectively, than was process 3-A (Tables 4 and 5).

Processes 6 and 5 were the least lethal of the processes tested in the oven-smokehouse and without the temperature increase associated with the smoke; some samples barely reached the minimum pathogen lethality threshold of  $\geq 5.0$ -log reduction. Process 6 began with low dry bulb temper-

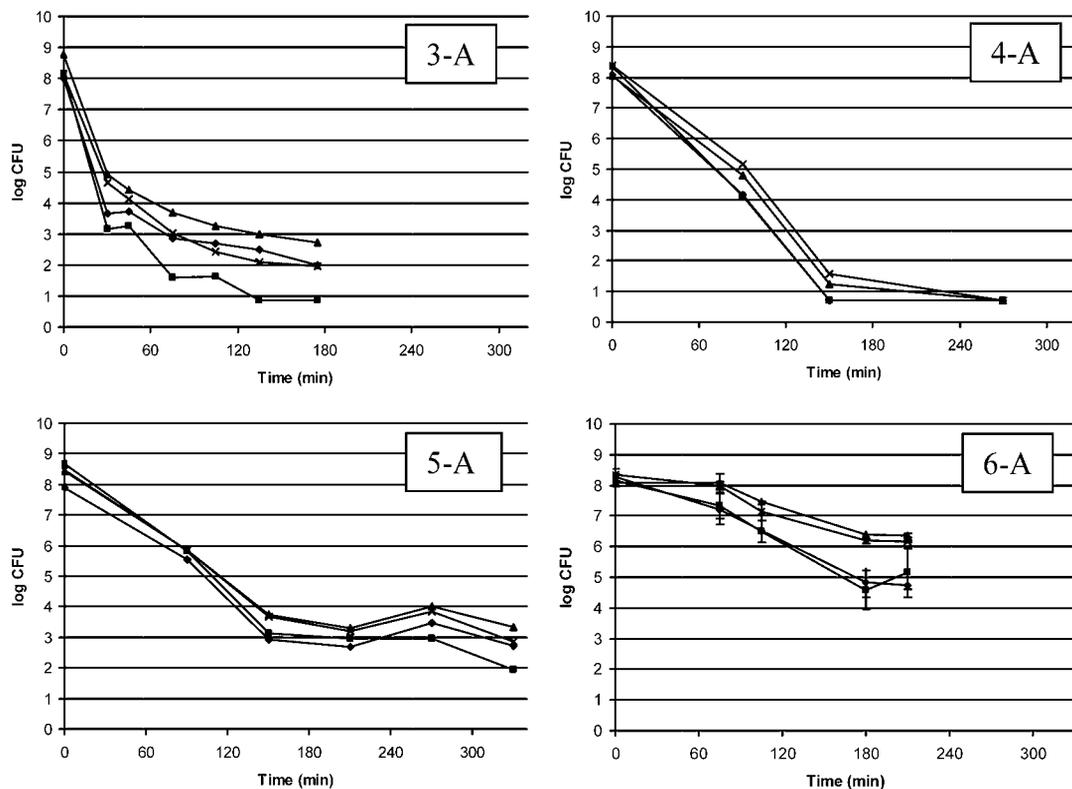


FIGURE 3. Survival of *E. coli* O157:H7, *Salmonella serovars*, and lactic acid bacteria pathogen surrogates (log CFU) in BBQ-seasoned ground-and-formed jerky during processes 3-A through 6-A (no smoke added). See Table 1 for process parameters. ◆, *Salmonella serovars*; ■, *E. coli* O157:H7; ▲, *Saga 200* (*Pediococcus spp.*); ×, *Biosource* (*Pediococcus acidilactici*).

ature steps at 51.7°C (125°F) and increased to a high dry bulb temperature of 85°C (185°F) near the end of the process. This process did not include any steps with controlled a wet bulb temperature. Based on mean values, process 6-A achieved adequate pathogen lethality for both pathogens in Colorado-seasoned jerky (Table 2) but not in BBQ-seasoned strips (Table 4). However, 1 SD (standard deviation) below the mean value for each of these products was well below the 5.0-log reduction threshold. Process 6-B did achieve adequate lethality for both pathogens (Tables 3 and 5). Lethality for both LAB cultures increased, by 0.79 to 1.88 log CFU, from process 6-A to process 6-B depending on the culture and the seasoning mixture. The final mean  $a_w$  of the product from process 6-A was 0.83 in Colorado-seasoned jerky and 0.86 in BBQ-seasoned jerky made during the same process. Dry bulb temperatures were well above the set dry bulb temperatures in process 6-B when smoke was added, by as much as 21.1°C. The final  $a_w$  achieved by process 6-B was 0.90 for strips seasoned with Colorado and 0.88 for the strips seasoned with BBQ.

The least lethal of the processes run in the oven-smokehouse was process 5. Process 5 was a multistep process that began at a low dry bulb temperature, slowly increased to a high dry bulb temperature, and did not include any steps with high wet bulb temperatures (or high %RH). This approach did not achieve adequate lethality for *Salmonella* in process 5-A and just barely achieved the 5.0-log lethality standard for both pathogens during process 5-B for Colorado-seasoned jerky (Tables 2 and 3). For both versions of process 5, the decrease in both LAB cultures was not  $\geq 5.0$

log CFU in Colorado-seasoned jerky; similar results were obtained for BBQ-seasoned jerky. During process 5-A, the required 5.0-log decrease in levels of both *E. coli* O157:H7 and *Salmonella* was barely achieved, although lethality against either LAB did not reach 5.0 log CFU. The required 5.0-log kill of *Salmonella* and *E. coli* O157:H7 was not reached during process 5-B in BBQ-seasoned strips. The wet bulb temperature during process 5-A did not surpass 37.8°C (100°F) until 90 min into the process, and the wet bulb temperature at the end of the process was only 56.1°C (133°F). In process 5-B, the dry bulb temperature increased beyond the set point because of the addition of smoke, increasing to as much as 21.7°C above the set point near the end of the first smoking step (Table 1). Process 5-B was scheduled to run for 330 min, but the run was ended after 270 min because the jerky became very dry and crumbly. When process 5-B was stopped, the  $a_w$  of the product was 0.75 for Colorado-seasoned jerky and 0.85 for BBQ-seasoned jerky. The ending  $a_w$  for Colorado-seasoned jerky from process 5-A was 0.67.

To assess the effectiveness of LAB as pathogen surrogates for evaluating the safety of jerky processes, process lethality for both pathogens and the LAB was compared across all processes and both seasoning types. For each sampling time throughout every process, two pathogen and two LAB strips from each seasoning mix were removed from the oven-smokehouse. These samples were paired, and the lethality achieved in each LAB strip was compared with that achieved for its pathogen partner strip. The final lethality achieved by each process was calculated by sub-

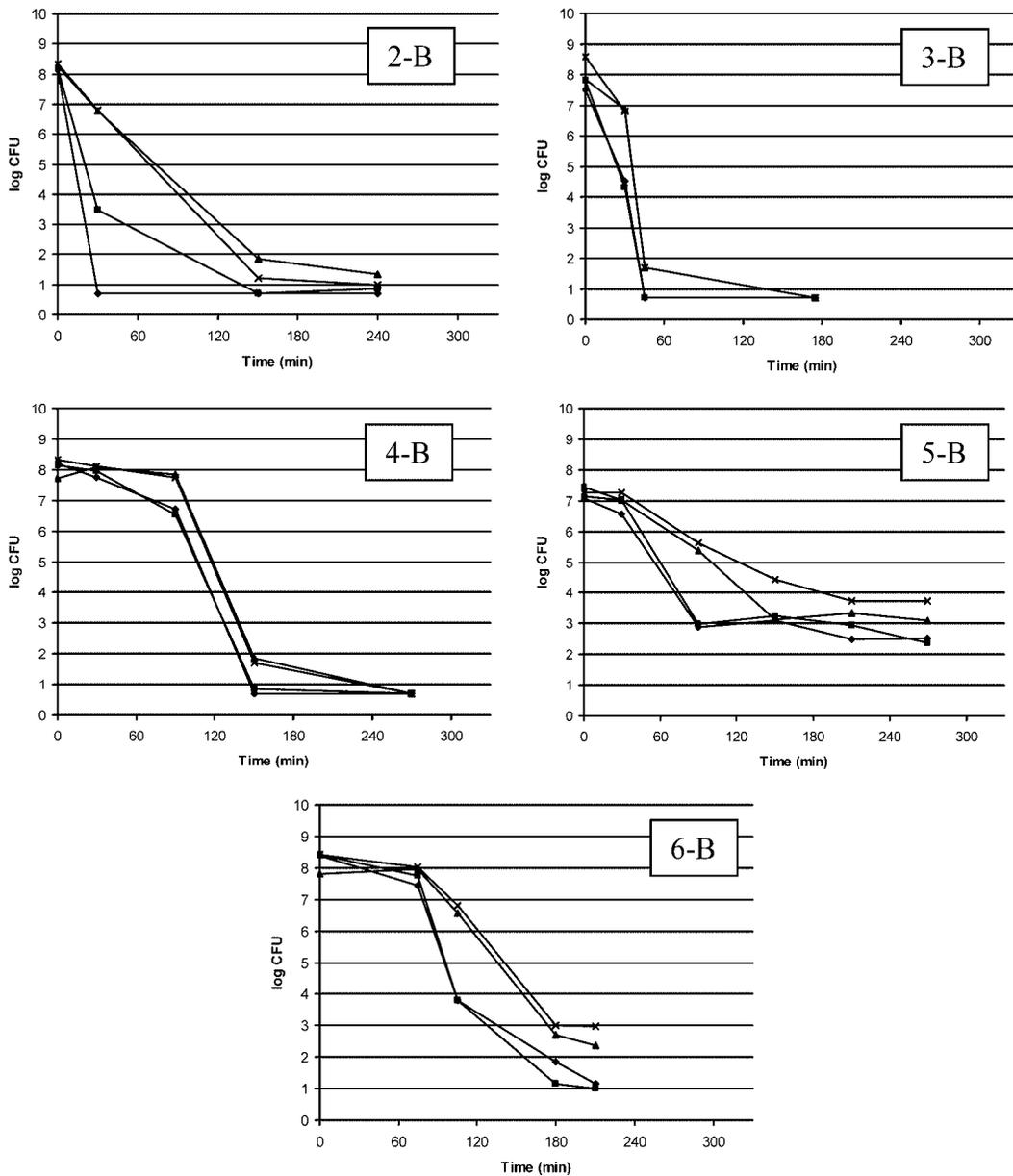


FIGURE 4. Survival of *E. coli* O157:H7, *Salmonella* serovars, and lactic acid bacteria pathogen surrogates (log CFU) in BBQ-seasoned ground-and-formed jerky during processes 2-B through 6-B (smoke added). See Table 1 for process parameters. ◆, *Salmonella* serovars; ■, *E. coli* O157:H7; ▲, *Saga 200* (*Pedococcus spp.*); ×, *Biosource* (*Pedococcus acidilactici*).

tracting the log CFU at the end of each process from the log CFU at the beginning of that process. These data (from all processes and both seasoning mixes) were combined to form lethality matrices (Table 6). Each pair of lethality values was assigned to one of the four quadrants in the lethality matrix. For ease in educating processors and providing a margin of error in predicting a safe process, the target lethality for LAB was set at  $\geq 5.0$  log CFU, the same as the target lethality for *E. coli* O157:H7 and *Salmonella* in jerky making.

The four quadrants within the lethality matrices were defined based on these target lethality levels:  $< 5.0$  log CFU lethality for both pathogens and LAB (unsafe; LAB successfully indicated that insufficient pathogen lethality was achieved);  $\geq 5.0$  log CFU lethality for pathogens but  $< 5.0$  log CFU lethality for LAB (falsely unsafe; LAB lethality

results indicated that the process did not achieve the target pathogen lethality when in fact it was achieved);  $< 5.0$  log CFU lethality for pathogens and  $\geq 5.0$  log CFU lethality for LAB (falsely safe; LAB lethality results falsely indicated that enough pathogen lethality was achieved); and  $\geq 5.0$  log CFU lethality for both pathogens and both LAB cultures (safe; LAB lethality results successfully indicated adequate pathogen lethality). The percentage of accurate predictions of sufficient lethality for either pathogen was calculated by dividing the number of samples that indicated a safe process ( $\geq 5.0$  log CFU pathogen death and  $\geq 5.0$  log CFU LAB death) by the total number of samples that had  $\geq 5.0$  log CFU LAB death and multiplying by 100.

When either *Saga 200* or *Biosource* LAB was used as a surrogate for *E. coli* O157:H7, adequate pathogen lethality was accurately predicted in 100% of samples ( $n = 20$

TABLE 6. Matrices comparing process lethality against *E. coli* O157:H7 or *Salmonella* serovars and Saga 200 (*Pediococcus* spp.) or Biosource (*Pediococcus acidilactici*) across all processes and seasoning mixes<sup>a</sup>

A. <i>E. coli</i> O157:H7 death (n = 54)	Saga 200 death		C. <i>Salmonella</i> death (n = 54)	Saga 200 death	
	<5 log CFU	≥5 log CFU		<5 log CFU	≥5 log CFU
<5 log CFU	16 (unsafe)	0 (falsely safe)	<5 log CFU	21 (unsafe)	0 (falsely safe)
≥5 log CFU	18 (falsely unsafe)	20 (safe)	≥5 log CFU	11 (falsely unsafe)	22 (safe)
B. <i>E. coli</i> O157:H7 death (n = 48)	Biosource death		D. <i>Salmonella</i> death (n = 48)	Biosource death	
	<5 log CFU	≥5 log CFU		<5 log CFU	≥5 log CFU
<5 log CFU	12 (unsafe)	0 (falsely safe)	<5 log CFU	17 (unsafe)	0 (falsely safe)
≥5 log CFU	16 (falsely unsafe)	20 (safe)	≥5 log CFU	14 (falsely unsafe)	17 (safe)

<sup>a</sup>Lethality indicated by reductions of <5 or ≥5 log CFU. Each number in the matrix represents the total number of tested samples yielding that lethality result. See Table 1 for processing parameters.

and 20) (Table 6A and 6B). The surrogates were just as accurate in predicting sufficient process lethality for *Salmonella* (Table 6C and 6D). There were no samples for which LAB lethality results falsely indicated adequate lethality against either pathogen.

For selected processes, dried pathogen-inoculated jerky samples were vacuum packaged and stored at 21°C for 4 to 6 weeks to determine the effect of hold time on pathogen survival. When pathogens survived the drying process, levels continued to decline during storage, falling below the detection limit for most processes (data not shown). No more than three colonies of *Salmonella* (1.48 log CFU) were detected on the lowest dilution plate (10<sup>-1</sup>) and no more than one *E. coli* O157:H7 colony (1.0 log CFU) was detected at the same dilution in any stored sample seasoned with Colorado spice mixture. In BBQ-seasoned samples, *Salmonella* was not recovered from any stored samples, and there were no more than two *E. coli* O157:H7 colonies (1.30 log CFU) recovered from any stored sample. Although there were still recoverable colonies of both *Salmonella* and *E. coli* O157:H7 on some stored jerky samples, these levels represent a decrease of more than 50% in the number of colonies compared with the end-of-process samples. For *Salmonella*, the final mean level was 3.32 log CFU in Colorado-seasoned jerky made with process 5-A; this level declined to 1.09 log CFU after 4 weeks of storage. Similarly, at the end of process 5-A, the *E. coli* O157:H7 level was 2.66 log CFU on Colorado-seasoned jerky, and after 4 weeks of storage the level decreased to 0.85 log CFU. BBQ-seasoned jerky made with process 5-A also exhibited reductions of recoverable *Salmonella* and *E. coli* O157:H7 as a result of storage. The levels of *Salmonella* and *E. coli* O157:H7 remaining on BBQ-seasoned jerky at the end of process 5-A were 3.35 and 2.87 log CFU, respectively. After 4 weeks of storage, pathogen recovery fell to 0.69 log CFU (no colonies on the 10<sup>-1</sup> dilution plate) for *Salmonella* and 1.00 log CFU for *E. coli* O157:H7. Similar reductions were seen for every process and both A and B versions.

## DISCUSSION

The processes that consistently achieved adequate lethality were those that included both a high-humidity step

and a high dry bulb temperature step, i.e., both A and B versions of processes 2, 3, and 4. Lethality was even greater when the high-humidity step was coupled with a high dry bulb temperature as in step 2 of processes 3-A and 3-B. Goepfert et al. (12) found that the heat resistance of *Salmonella* and *E. coli* increased as the a<sub>w</sub> of the heated environment decreased after the addition of sucrose, glycerol, fructose, or sorbitol. Goepfert et al. documented that at an a<sub>w</sub> of 0.90, the heat resistance of *Salmonella* was two- to threefold greater than it was at an a<sub>w</sub> of 0.99. *E. coli* was seven to eight times more heat resistant at an a<sub>w</sub> of 0.90 than at an a<sub>w</sub> of 0.99. Similarly, Calhoun and Frazier (3) noted that the heat resistance of *E. coli* increased in the presence of glucose and increased to a lesser extent in the presence of NaCl. This increased heat resistance as water activity decreases during drying makes it critically important for the first stages of the jerky process to include sufficient pathogen lethality. Without these early lethal steps, sufficient overall lethality is not achieved or is barely achieved, as seen in processes 1, 5-A, and 5-B. This finding is important because the seasoning and the early sublethal heating steps may increase the heat resistance of pathogenic organisms and ultimately make the high dry bulb temperature drying step at the end of most processes less effective for eliminating *E. coli* O157:H7 and *Salmonella* serovars.

The methods currently recommended to processors to ensure the production of a safe ground-and-formed jerky product either result in a poor quality jerky or do not allow for any process variability (11, 14, 29). The USDA Compliance Guideline (29) recommends processing jerky at 90% RH, an environment that is difficult to achieve even with a commercial oven-smokehouse and impossible to achieve with small-scale equipment. Other methods for producing safe jerky include preheating beef strips in marinade to 71.1°C (160°F) before drying or dipping the product in 5% acetic acid, calcium sulfate, or acidified sodium chlorite before drying, methods that can lead to objectionable product quality (29) and may not be suitable for ground-and-formed jerky. Various researchers have attempted to evaluate more traditional jerky-processing schedules. However, with a few exceptions, these studies were conducted in home-style dehydrators that do not mimic industry processes.

Validated processes for safe manufacture of whole-muscle beef jerky under commercial conditions exist (2, 21), but these processes do not allow for unique variations in the jerky-making processes and were created using whole-muscle beef jerky and thus cannot necessarily be applied to a ground-and-formed product. One important difference between whole-muscle and ground-and-formed jerky, in terms of food safety, is the likely site of bacterial contamination. For whole-muscle beef jerky, contaminating bacteria would be on the surface of the product, whereas bacterial contamination could be distributed throughout a ground-and-formed jerky product. Because the currently validated processes of Buege et al. (2) and Porto-Fett et al. (21) did not address lethality for pathogens within the meat, processors of ground-and-formed jerky would have to ensure that the product's internal temperature and time profile exceeds an Appendix A (27) combination and that an adequate %RH is achieved (29). In the present study, each of the 10 trials of process 1 reached the time and internal temperature guidelines published in Appendix A; however, none of the products reached the necessary level of lethality to ensure a safe product. This finding underscores the importance of adequate %RH (high wet bulb temperature in relation to dry bulb temperature) in process lethality.

Although the main goal of this study was not to develop a particular process, the current research provides examples of valid processes for safe manufacture of ground-and-formed beef jerky under commercial conditions. The six commercial processes evaluated were successful to varying degrees. Jerky produced in Cabela's small-scale commercial dehydrator set at 68.3°C (155°F) did not produce a safe product over the 7-h drying time. Research in other labs suggests that further drying would not have led to significant increases in lethality (1, 13, 19), and product quality would certainly have been compromised. Processes 2 through 4 that utilized the commercial oven-smokehouse achieved adequate pathogen lethality and would be considered validated and safe for commercial processors to use.

The few ground-and-formed jerky manufacturing processes validated in this study do not begin to cover the wide range of jerky manufacturing conditions in use by meat processors across the nation. Therefore, an easy method is needed that will allow jerky manufacturers to validate their own processes under in-plant conditions. LAB cultures were evaluated as surrogates for *Salmonella* and *E. coli* O157:H7 for use during in-plant validation of ground-and-formed beef jerky processes under commercial conditions. For a surrogate organism to be useful it must have thermal resistance equal to or greater than that of the target organism, have similar physiological characteristics, and be carefully evaluated in the system in which it is going to be used (10). It would have been ideal in our situation to have had access to a nonpathogenic gram-negative organism as a surrogate, but these organisms are not commercially available nor are they commonly used in meat-product formulations. LAB are commonly used in meat processing as starter cultures in the preparation of fermented products and are readily available for purchase; these features were the basis for evaluating LAB as pathogen surrogates. Of the six LAB

cultures initially screened, three contained more than one genus of bacteria, which complicated colony counting and led us to discard these three cultures as surrogate candidates. Of the remaining three LAB cultures, Saga 200 (described by the manufacturer as *Pediococcus* spp.) and Biosource (described as *P. acidilactici*), were consistently more thermotolerant than the 10-strain cocktail of *E. coli* O157:H7 and *Salmonella* serovars (Figs. 1 through 4). These two LAB starter cultures could serve as effective surrogates for *Salmonella* and *E. coli* O157:H7 for evaluating the safety of processes used in the manufacture of ground-and-formed beef jerky.

A 5.0-log reduction in either LAB (Saga 200 or Biosource) was appropriate to indicate a  $\geq 5.0$ -log reduction of either *Salmonella* or *E. coli* O157:H7. Under commercial processing conditions, there was remarkable success in predicting process lethality, with LAB death of  $\geq 5.0$  log CFU associated with sufficient pathogen lethality 100% of the time (Table 6). The overall successful use of Saga 200 for identifying appropriate levels of pathogen death and the heat resistance of this culture, which was continually greater than that of both pathogens, makes Saga 200 the more appropriate of the two studied LAB cultures for validating ground-and-formed beef jerky process lethality.

The benefits of utilizing LAB cultures as pathogen surrogates during in-plant validation trials cannot be overstated. LAB have a generally recognized as safe status with the U.S. Food and Drug Administration (30) and are used regularly for making fermented meat products such as summer sausage and snack sticks; thus, the presence of these organisms in a meat production facility would not be of concern to regulators. In-plant validation can be simple and successfully used by small meat processing facilities. We contacted four small facilities in Wisconsin and worked with them to implement our method of in-plant process validation. Our lab provided the LAB culture (Saga 200), and we tested the initial (time 0) and finished product LAB levels. In each case, the processor was able to evaluate the safety of their unique manufacturing process.

Although LAB can be useful as pathogen surrogates to measure the ability of a particular heating or drying process to eliminate both *E. coli* O157:H7 and *Salmonella* serovars, the overall lethality associated with making a finished product may be increased by storing the jerky before distribution and sale. In two separate studies, Calicioglu and colleagues (4, 5) found that both *Salmonella* and *E. coli* O157:H7 levels in beef jerky were reduced after 60 days of storage, and in some cases pathogen levels dropped below detection limits. Similarly, Ingham et al. (18) found that *Staphylococcus aureus* levels decreased by 0.6 to 5.3 log CFU and *L. monocytogenes* decreased by 2.5 to 5.6 log CFU in beef jerky stored for 4 weeks at 21°C.

We identified two commercially available LAB cultures, Saga 200 (identified by Kerry Biosciences as *Pediococcus* spp.) and Biosource (identified by Biosource Flavors as *P. acidilactici*) as excellent surrogates for *Salmonella* serovars and *E. coli* O157:H7 strains in ground-and-formed jerky processing. Under commercial processing conditions, a process lethality of  $>5.0$  log CFU for Saga

200 (*Pediococcus* spp.) achieved adequate lethality for *E. coli* O157:H7 and *Salmonella* serovars in 100% of the samples. This study is the first to validate ground-and-formed beef jerky processes using commercial equipment standard in the meat industry. By using LAB, notably Saga 200, as pathogen surrogates in validation of ground-and-formed beef jerky processes, processors are given new freedom in choosing process time and temperature schedules, and regulators are provided with a simple method for evaluating the safety of these unique processes. The ease with which this method can be adopted and the consistent success of this method for identifying adequate pathogen elimination makes using LAB as pathogen surrogates in ground-and-formed beef jerky process validation an invaluable tool for food safety assessment by processors, regulators, and, ultimately consumers.

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