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DEATH OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP. DURING PROCESSING OF WHOLE-MUSCLE BEEF JERKY

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Introduction

Beef jerky processing, using whole muscle or restructured ground meat, is unique compared to the processing of other ready-to-eat meat products because during heating considerable drying is done to attain the desired texture and shelf-stability. This drying may reduce the process lethality against pathogenic bacteria in beef, and outbreaks of salmonellosis have indeed been linked to the consumption of beef jerky. Previous research has suggested that sub-lethal drying conditions may lead to increased heat-resistance in pathogens such as *Salmonella* spp. Furthermore, evaporative cooling during drying may lessen the effective temperature to which pathogens are exposed. All of these factors have resulted in heightened scrutiny of the lethality of the heating and drying steps typically used during jerky processing. United States Department of Agriculture (USDA) officials have issued several compliance guidelines for jerky processors that stress the importance of maintaining high humidity during thermal processing in order to ensure sufficient destruction of *Salmonella* spp. and *Escherichia coli* O157:H7. Processors have had difficulty either complying with USDA guidance or developing and validating adequate alternative processes. Development of validated heating/drying guidelines for processors of whole-muscle jerky has been complicated by variables such as thickness of jerky strips, whether the strips have been marinated and, if so, the composition and conditions of marination, and the type of smokehouse or oven used for heating/drying.

Objectives

The objective of this study was to develop and validate sufficiently lethal processes for heating and drying whole-muscle beef jerky.

Methodology

Five-strain cocktails of *Salmonella* spp. and *E. coli* O157:H7 were used to inoculate beef strips prior to jerky processing. Inoculation cultures were prepared for each strain by transferring a loopful of growth from working culture plates (maintained at 4°C on Brain Heart Infusion agar (BHIA; Difco, Becton-Dickinson, Sparks, MD) and prepared monthly from frozen stock cultures) to 9 ml of Brain Heart Infusion Broth (BHIB; Difco) and incubating at 35°C for 20-24 h. To prepare a 5-strain inoculum cocktail of each organism, the BHIB cultures of each organism were combined into one 50-ml sterile plastic centrifuge tube, and centrifuged for 12 minutes at 5,000 x g. The supernatant in each tube was decanted and the pellets were

resuspended with approximately 20 ml of Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI). Beef strips (5 – 7 mm thick) were placed in a biosafety hood on aluminum foil that had been previously sanitized with 70% (v/v) ethanol. To inoculate each strip, 0.4 ml of the undiluted cocktail was pipetted onto the product surface and distributed as evenly as possible using a sterile plastic spreader. Aluminum foil was placed over the strips in a tented manner to minimize the amount of drying during microbial attachment (30 min). After the initial attachment phase, strips were turned over and the inoculation/attachment process was repeated on the other side. For each trial, one group of beef strips was inoculated with *Salmonella* spp., another group was inoculated with *E. coli* O157:H7, and a third group of uninoculated strips was used to monitor yield and water activity throughout thermal processing. Initial pathogen levels on inoculated beef strips were approximately 10^8 CFU per beef strip. Each group of beef strips was then tumbled manually for 5 minutes in a non-acidic (pH 5.3) spice-containing marinade applied at a level of 15% (w/w) intended to result in a pre-processing level of 2% (w/w) sodium chloride, 2% sucrose, 156 ppm sodium nitrite (w/w) in the meat. Following marination, strips were held for 22-24 h at 5°C. The next day, strips were arranged on racks placed in the center of a commercial one-truck smokehouse for processing. Pans of water were placed on the lower level of the rack and a low fan speed was used to simulate as much as possible a drying rate consistent with a smokehouse full of beef strips. The smokehouse dry-bulb and wet-bulb temperatures were monitored using thermocouples. Smoke was not introduced to the beef strips during processing and the dry-bulb temperature controller was set at 62.8°C (145°F) in the first 15 minutes and then at 76.7°C (170°F) during the next 15 minutes. Next, humidity (steam or water) was introduced into the smokehouse via the wet-bulb temperature controller to obtain targeted increases in wet-bulb temperatures, referred to as "wet-bulb spikes". The process lethality was determined for a series of trials conducted using early-process wet-bulb spikes of 51.7°C (125°F) for 60 min, 54.4°C (130°F) for 60 min., 57.2°C (135°F) for 30 min., and 60°C (140°F) for 10 min. Following completion of wet-bulb spikes, no further humidity was introduced into the smokehouse chamber as the product was further dried at 76.7°C (170°F).

The numbers of *Salmonella* spp. and *E. coli* O157:H7 on beef strips were determined prior to marination, after the early-process wet-bulb spike in the smokehouse, and following drying when the beef strips had reached an average water activity of $\leq$ 0.90. One beef strip comprised a sample and three samples were analyzed at each sampling time. Each sample was placed in a whirl pak filter bag, BPD (99 ml) was added, and the bag contents were stomached for 2 minutes at medium speed (Stomacher 400 Circulator lab blender; Seward) or samples were manually massaged for 1 minute and shaken for 1 minute. This initial dilution was arbitrarily defined as 10^{-1}. Serial decimal dilutions were made in BPD as needed. For the initial dilution, 1.0 ml was distributed for spread-plating among three plates of BHIA. From the original dilution and each subsequent dilution 0.1 ml was spread on one BHIA plate per dilution. Plates were incubated at 35°C for 1 h to allow for repair of injured cells, and then overlaid with MacConkey Sorbitol agar (SMAC; Difco) or XLD agar (Difco) for selective/differential enumeration of *E. coli* O157:H7 and *Salmonella* spp., respectively. After 20-24 h incubation at 35°C, plates were examined for typical *E. coli* O157:H7 or *Salmonella* spp. colonies. Selected presumptive *Salmonella* spp. and *E. coli* O157:H7 colonies were transferred to BHIA, incubated at 35°C for 20-24 h, and then tested to confirm colony identity. The log CFU for a given pathogen was calculated for each sample with a mean log CFU calculated for each

sampling time. A value of 9 CFU (0.95 log CFU) was assigned when no colonies were present for the least dilute plating.

Results & Discussion

Earlier research has established the fact that sub-lethal drying can make pathogens such as *Salmonella* spp. more resistant to heat (Goepfert et al., 1970). This phenomenon was likewise observed in several early jerky-making trials (data not shown). Therefore, a variety of early-process wet-bulb temperature spikes were applied to determine the extent of elevated-humidity heating conditions necessary to achieve desired lethality while maintaining product quality. During the 54.4, 57.2, and 60°C (130, 135, and 140°F) wet-bulb spikes, the interior temperature of a beef strip (measured by inserting a probe into the meat or by folding the strip around the probe) was generally quite similar to the wet-bulb temperature. However, in the 51.7°C (125°F) wet-bulb spike, the product temperature rose to a temperature several degrees higher than the wet-bulb temperature. Of the two pathogens studied, *E. coli* O157:H7 was better able to survive early-process wet-bulb spike treatments employed in making whole-muscle beef jerky. However, foodborne illness outbreaks linked to beef jerky have primarily involved *Salmonella* spp.; it is therefore prudent for any validation of a jerky-making process to involve both pathogens. Presently, the USDA has indicated that a jerky-making process has sufficient lethality if it results in a 5-log reduction of *Salmonella* spp. However, USDA guidance for certain other beef products specifies a 6.5 log reduction in *Salmonella* spp. All early-process wet-bulb spike treatments tested resulted in a $\geq$ 6.4 log reduction in both pathogens by the end of the complete process (including final drying). Three wet-bulb spike treatments achieved a $\geq$ 5.2 log reduction in *Salmonella* spp. but caused smaller decreases in *E. coli* O157:H7 numbers at the end of the wet-bulb spike (Table 1). These treatments were 51.7°C (125°F) for 60 min, 57.2°C (135°F) for 30 min, and 60°C (140°F) for 10 min. However, a $\geq$ 6.4 log reduction of both *Salmonella* spp. and *E. coli* O157:H7 was achieved by the end of drying after each of these wet-bulb spike treatments. One wet-bulb spike treatment did not cause a > 5.0 log reduction in either pathogen by the end of the web-bulb spike but subsequent drying resulted in sufficient overall lethality. This treatment, 54.4°C (130°F) for 60 minutes resulted in decreases of 3.2 – 3.9 and 2.0 – 2.1 log CFU for *Salmonella* spp. and *E. coli* O157:H7, respectively. By the end of drying after these treatments, reductions of $\geq$ 6.9 log had occurred for both pathogen species. Although USDA guidance (2004) recommended 90% relative humidity (RH) during the early heating period in jerky processing, it also stated that such a high humidity may not be necessary if alternative procedures are validated. In the studies conducted here using a wet-bulb temperature of 76.7°C (170°F), early-process wet-bulb spikes resulted in 28 - 48% RH. When followed by drying at 76.7°C (170°F), these processes were sufficient to provide $\geq$ 6.4 log reduction in numbers of *Salmonella* spp. and *E. coli* O157:H7. Processors could therefore monitor wet-bulb spike treatments directly with a wet-bulb thermometer or indirectly with a hygrometer.

Conclusions. Taking into account current USDA expectations for jerky processing lethality, processors using the conditions employed in this study [non-acidic marinade, initial dry-bulb smokehouse target temperatures of 62.8°C (145°F) for the first 15 min and 76.7°C (170°F) for the next 15 min] could employ any of the early-process wet-bulb spike treatments listed in Table 1 followed by drying at 76.7°C (170°F) as scientifically validated processes for making safe whole-muscle beef jerky.

References

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Tables and Figures

Table 1. Process lethality against *Salmonella* spp. (S) and *Escherichia coli* O157:H7 (EC) during the heating and drying of whole-muscle beef jerky. Inoculated beef strips were marinated in a non-acidic marinade for 22-24 h at 5°C, and then exposed to pre-heating [smokehouse heated to 76.7C (170°F) dry-bulb temperature in 30 minutes], followed by an early-process wet-bulb spike (addition of humidity to attain desired wet-bulb temperature), and then final drying [continued exposure to 76.7°C (170°F) with no added humidity] processes. Reduction in log CFU per sample is relative to initial pathogen levels prior to beef strip marination.

Early-Process				Post-spike Drying	Lethality (reduction in log CFU) and Water Activity (a_w)					
Wet-Bulb Spike				Time at 76.7°C	For Sample After					
Temperature		Time	RH[a]	(170°F)	Wet-Bulb Spike			Drying		
(°C)	(°F)	(min)	(%)	(min)	S	EC	Mean a_w	S	EC	Mean a_w
51.7	125	60	28	60	5.6	5.6	0.87	6.5	6.7	0.81
51.7	125	60	28	60	5.2	3.8	0.89	6.4	7.1	0.78
54.4	130	60	38	120	3.2	2.0	0.93	6.9	7.1	0.88
54.4	130	60	41	120	3.9	2.1	0.92	6.9	7.0	0.87
57.2	135	30	40	120	6.4	2.7	---	7.0	7.1	0.86
57.2	135	30	40	90	5.3	3.1	0.93	7.0	7.1	0.90
60	140	10	48	120	6.2	3.8	0.96	7.0	7.2	0.90
60	140	10	48	120	6.8	2.2	0.96	6.9	7.0	0.84

[a]Mean percent relative humidity during wet-bulb spike.