Survival of *Listeria monocytogenes* During Storage of Ready-to-Eat Meat Products Processed by Drying, Fermentation, and/or Smoking

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ABSTRACT

The survival of *Listeria monocytogenes* was evaluated on 15 ready-to-eat meat products made using drying, fermentation, and/or smoking. The products were obtained from 6 processors and included summer sausage, smoked cured beef, beef jerky, snack stick, and pork rind/cracklin products. The a_w of the products ranged from 0.27 (pork rinds/cracklins) to 0.98 (smoked cured beef slices). Products were inoculated with a 5-strain cocktail of *L. monocytogenes*, re-packaged under either vacuum or air, and then stored at either room temperature (21°C) or under refrigeration (5°C) for 4- 11 weeks. Numbers of *L. monocytogenes* fell for all products during storage, ranging from a decrease of 0.8 log CFU on smoked cured beef slices during 11 weeks under vacuum at 5°C to a decrease of 3.3 log CFU on a pork rind product stored 5 weeks under air at 21°C. All of the products tested could be produced under Alternative 2 of the United States Department of Agriculture (USDA) regulations mandating control of *L. monocytogenes* on ready-to-eat meat and poultry products. For many of the products, 1 week of post-processing storage prior to shipment would act as an effective post-lethality treatment and would allow processors to operate under Alternative 1 of these regulations.

On June 6, 2003, the United States Department of Agriculture (USDA) published an interim final rule addressing the control of *Listeria monocytogenes* on ready-to-eat (RTE) meat and poultry products (*2*). This rule went into effect October 6, 2003 and has already had a major effect on processors of these products. The rule is intended to encourage processors of RTE products to take one or more specific steps to ensure the absence of *L. monocytogenes* from their products. These steps range from focused sanitation steps to adding formulation or processing steps designed to kill or inhibit *L. monocytogenes* growth. The processor is also required to perform testing for *L. monocytogenes* or *Listeria* spp. on food contact surfaces in the area of the plant in which RTE products are handled after cooking. The amount of testing is related to the types of RTE products made, product ingredients, and how the products are processed and handled. In particular, the rule requires processors of RTE meat and poultry products to adopt one of three designated "Alternatives" to control *L. monocytogenes* on their products. The Alternatives involve varying levels of control and microbiological testing. In Alternative 1, the processor uses a post-lethality treatment that reduces or eliminates *L. monocytogenes* AND an antimicrobial agent or process that suppresses or limits *L.m.* growth throughout product shelf-life. In Alternative 2, the processor uses either a post-lethality treatment that reduces or eliminates *L. monocytogenes* OR an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout product shelf-life. Under Alternative 3, only sanitation measures are relied upon to control *L. monocytogenes*.

Most small-scale meat processors in Wisconsin have chosen Alternative 3 for controlling *L. monocytogenes*. This alternative provides the least assurance of safety and requires the most frequent testing of food contact surfaces among the three alternatives. For many RTE meat products though, the reduction of water activity, accomplished through the addition of salt and cooking or drying, could serve as an antimicrobial process by making the finished product unsuitable for *L. monocytogenes* growth. Similarly, the reduction of pH via fermentation or addition of an acidulant, or surface-deposition of inhibitory compounds via smoking could also be effective antimicrobial processes. Items produced in these ways could thus fall under Alternative 2. Compliance guidance from USDA (*3*) has stated that an effective antimicrobial process will allow no more than a 1.0 log increase in *L. monocytogenes* on an RTE product throughout its shelf-life. This guidance also summarized scientific studies indicating that *L. monocytogenes* will not multiply at a water activity of < 0.92 or a pH of <4.39.

It is also possible that some RTE products could fall under Alternative 1, when the above-mentioned antimicrobial processing techniques are combined with short-term storage prior to distribution that effectively serves as a post-lethality treatment. The compliance guidance from USDA states that an effective post-lethality treatment must reduce numbers of *L. monocytogenes* by at least 1 log.

The regulation requires that post-lethality treatments must be scientifically validated and that evidence must be provided to substantiate the effectiveness of

antimicrobial processes. Unfortunately, most small-scale processors are unable to provide this evidence.

The objective of the present study was to evaluate the survival, and perhaps death, of inoculated *L. monocytogenes* during storage (after re-packaging) on a variety of RTE meat products made using drying, fermentation and/or smoking techniques. Information obtained in the study could thereby provide evidence needed by processors to implement Alternatives 1 or 2 in their RTE meat product operations.

MATERIALS AND METHODS

Packages of RTE meat products were submitted by six processors. Product ingredients are summarized in Table 1. Depending on the product, 1 – 3 lots of product were tested. A representative sample from each lot of product was vacuum packaged and sent to a commercial testing laboratory to be analyzed for water activity, pH, and % water-phase salt (forced air oven determination of moisture – AOAC method 950.46Bb and potentiometric method for salt – AOAC method 980.25; *1*). For each product, the lowest % water-phase salt value, and the highest water activity and pH values, *i.e.* conditions least restrictive to microbial growth, are reported in Table 2. Because post-lethality contamination of RTE products by *L. monocytogenes* will occur only on the product surface, 0.6 cm thick sections of product, with surface dimensions of 3.7 x 3.7 cm, were cut from each product using a knife [previously sanitized using 70% (v/v) ethanol] and placed in a biosafety hood on aluminum foil that

had previously been sanitized with ethanol and ultraviolet light. For beef jerky, the 3.7 x 3.7 cm pieces were cut from individual jerky slices. For the pork rind/cracklin products, existing individual pieces, each about 2.5 x 2.5 cm, were individually inoculated.

The *L. monocytogenes* strains used in this study were obtained from the laboratory of Dr. Eric Johnson at the Food Research Institute, University of Wisconsin-Madison, and are listed in Table 3 . Stock cultures were maintained at -20°C in Brain Heart Infusion broth (BHIB; Difco, Becton-Dickinson, Sparks, MD) with 10% (w/v) added glycerol (Fisher Scientific, Itasca, IL). Working cultures, maintained at 4°C on Brain Heart Infusion agar (BHIA; Difco) were prepared monthly from frozen stock cultures. To obtain a working culture, a strain was cultured twice successively at 35°C for 18-24 h in BHIB, streaked to a BHIA plate, incubated at 35°C for 18-24 h and examined for purity, and then stored at 4°C. Inoculation cultures were prepared for each strain by transferring a loopful of growth from the working culture plate to 9 ml of BHIB and incubating at 35°C for 20-24 h. To prepare the 5-strain inoculum cocktail, the BHIB cultures were combined and distributed evenly into two 50-ml sterile plastic centrifuge tubes, and centrifuged for 10 minutes at 5,000 x g. The supernatant in both tubes was decanted and the pellets were resuspended to the original volume in Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI). The resulting cocktail was serially diluted in BPD and plated to determine cell concentration. To inoculate the meat or pork rind/cracklin pieces, a 0.025 ml

volume of the undiluted cocktail was pipetted onto the product surface and distributed as evenly as possible using a sterile plastic spreader. The product pieces were then allowed to dry for at least 15 minutes and then vacuum packaged (0.8 atm.; Food Saver bags and packaging machine; Tilia, Inc.; San Francisco, CA) and stored at either 5 or 21°C. Pork rind/cracklin pieces were allowed to dry and then stored aerobically in zip-lock plastic bags at 21°C.

At the start of the study and after 1and 4, 5, or 11 weeks of storage, samples were analyzed for the number of *L. monocytogenes* cells per sample. The sample bag was aseptically opened, BPD (99 ml) was added, and the sample was stomached for 2 minutes at medium speed (Stomacher 400 lab blender; Fisher). Serial dilutions were made in BPD as needed. For the initial dilution, 1.0 ml was distributed for spread-plating among three plates (0.3, 0.3, and 0.4 ml) of Listeria Selective Agar (LSA; Oxoid, Ogdensburg, NY) with Listeria Selective Supplements (Oxford formulation; Oxoid). From the original dilution and each subsequent dilution, 0.1 ml was spread on one LSA plate per dilution. Plates were incubated at 35°C for 48 h and then examined for typical *L. monocytogenes* colonies (small-medium, grey-brown-to-black; raised, flat, or sunken colonies surrounded by a black precipitate zone). Replica plating from Nutrient agar (Difco) to LSA was performed for some summer sausage samples but there was little evidence of cell injury. Furthermore, injured cells would be unlikely to survive the low-pH stress of human gastric juice if products were ingested. Therefore, direct plating on LSA was used throughout the study. For each product lot

analyzed, one presumptive *L. monocytogenes* colony was selected at each sampling time for confirmation testing. The colony was transferred to Nutrient Agar and after incubation was tested for Gram stain reaction, cellular morphology, oxidase activity, and biochemical characteristics (API Listeria kit, bioMerieux, Hazelwood, MO). Throughout the study, all presumptive isolates were confirmed as *L. monocytogenes*.

With the exception of the pork rind/cracklin products, where only a small amount of sample was available, three pieces were analyzed at each sampling time for each lot of a particular product. For the pork rind/cracklin products, only one sample was analyzed per sampling time for each lot. The log CFU was calculated for each piece. Then the mean log CFU per piece was calculated for each lot and the mean of all lots tested was determined for the product at that sampling time. A value of 0.9 log CFU/g was assigned when no colonies were present for the least dilute plating.

RESULTS AND DISCUSSION

The RTE meat products varied widely in water activity, pH and % water-phase salt (Table 2). Given that higher water activity and pH and lower % water-phase salt would increase the likelihood of *L. monocytogenes* growth, the product judged most likely to allow growth of this pathogen was the smoked cured beef slices and the products judged least likely to support growth were the pork rind/cracklin products and beef jerky. Numbers of *L. monocytogenes* recovered from stored products were consistent with these

compositional factors. Levels of *L. monocytogenes* fell rapidly on the pork rind/cracklin products during room temperature storage (Table 4), with decreases of 1.2 – 2.1 log CFU in the first week of storage, and subsequent decreases to levels at or near the detection limit for samples stored 5 weeks. Similar results were obtained for the beef jerky (Table 4). Processing RTE products to yield water activity of $\leq$ 0.75, combined with 1 week of 21°C storage, appears to effectively allow the processor to operate under Alternative 1, with the processing technique as the antimicrobial process and the one-week storage as the post-lethality treatment. It is not known whether the decrease in *L. monocytogenes* numbers during storage occurred at a uniform rate or was early in the one-week period. The latter situation would result in a shorter pre-shipment storage period serving as the post-lethality treatment.

The sausage products (summer, elk, buffalo) had maximum water activity of 0.94 – 0.96, considerably higher than for beef jerky. However, fermentation of the summer sausage products during processing resulted in a lower finished product pH than for the pork rind/cracklin products and jerky. (Table 2). However, neither the water activity nor the pH of these products was low enough to predict, based on USDA compliance guidance, that *L. monocytogenes* would not grow. The combination of somewhat reduced water activity and pH seemed to effectively inhibit growth and cause death of *L. monocytogenes*. *L. monocytogenes* decreased in numbers by $\geq$1.0 log at room temperature (Table 4) or during refrigeration (Table 5). These results

strongly suggest that sausage products, with a mandatory one-week pre-distribution storage period, could also be produced under Alternative 1. To do so, processors would be required to either ensure that their summer sausage had water activity and pH at least as low as those used in the present study, or have a challenge study conducted to validate the post-lethality treatment for their products.

Somewhat less death of *L. monocytogenes* occurred during storage of snack stick products (Table 5). It is clear that the snack stick products could be produced under Alternative 2; case-by-case studies would be necessary to determine if Alternative 1 could be chosen for these products. Room-temperature storage of snack stick products was not evaluated, although many products of this type are stored at ambient temperatures.

The intact and sliced smoked cured beef had the highest water activity and lowest % water-phase salt of the products studied (Table 2). As expected, *L. monocytogenes* survival was best on the sliced smoked cured beef product (Table 5) with decreases of only 0.8 log CFU during 11 weeks of refrigerated storage. Surprisingly, *L. monocytogenes* numbers decreased 3.0 log CFU on the surface of intact smoked beef during this same storage period. This difference in survival could reflect the somewhat lower water activity of the intact product, localized areas on the product surface possibly having even lower water activity, and perhaps the presence of anti-microbial smoke-derived compounds on the surface of the smoked beef product. The

smoked cured beef results suggest that processing of these products should be done under Alternative 2 of the regulations.

In conclusion, the smoking, cooking, and drying processes used to make the tested products can be considered effective antimicrobial processes under the USDA regulations mandating control of *L. monocytogenes* on ready-to-eat meat and poultry products. To verify that their own processing techniques allow operation under Alternative 2, processors should either verify that their products have water activity, pH, % water-phase salt levels at least as restrictive as those in the present study, or have a challenge study conducted to validate the lack of *L. monocytogenes* growth on their products. For many of the products studied, a one- week period of post-packaging room temperature storage prior to shipment could act as an effective post-lethality treatment and would allow processors to operate under Alternative 1 of the USDA *L. monocytogenes* regulations. Again, processors should verify similar product composition or conduct a challenge study to validate the post-lethality treatment lethality.

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Table 1. Ingredients of ready-to-eat meat products evaluated for survival of inoculated *Listeria monocytogenes* during storage (after re-packaging).

Product Description	Processor	Ingredient Summary
Smoked cured beef piece/slices	A	Beef, cured with water, salt, sugar, sodium erythorbate, sodium nitrite
Summer sausage	A	Beef and pork, salt, dextrose, natural spices, lactic acid starter culture, sodium erythorbate, sodium nitrite
Summer sausage	B	Beef, beef hearts[1], salt, corn syrup solids, dextrose, spices, mustard seed, sodium erythorbate, lactic acid starter culture, sodium nitrite
Summer sausage	C	Beef, pork, water, salt, corn syrup solids, dextrose, spices, ground mustard, lactic acid starter culture, sodium erythorbate, sodium nitrite
Buffalo summer sausage	C	Buffalo, pork, water, salt, sugar, pepper, mustard seed, garlic, monosodium glutamate, sodium erythorbate, sodium nitrite
Elk summer sausage	C	Elk, pork, water, salt, sugar, black pepper, mustard seed, garlic, monosodium glutamate, lactic acid starter culture, sodium erythorbate, sodium nitrite

Venison snack sticks	F	Venison, pork, water, salt, sugar, corn syrup solids, spices, dextrose, dehydrated garlic, red pepper, lactic acid starter culture, sodium nitrite
Beef snack sticks	A	Beef, salt, dextrose, natural spices, natural flavoring, lactic acid starter culture, sodium erythorbate, sodium nitrite
Pork rinds	D	Pork rinds, seasoning[2]
Pork cracklins	D	Pork cracklins (fried out pork fat with attached skin), salt
Beef jerky	E	Beef, water, salt, flavoring, sodium nitrate and sodium erythorbate

[1] One other lot also contained pork; another tested lot also contained pork and garlic.

[2] Seasonings differed for regular, smoke flavor, and BBQ flavor.

Table 2. Chemical and physical characteristics of ready-to-eat meat products evaluated for survival of inoculated *Listeria monocytogenes* during storage (after re-packaging).

Product Description	Processor	a_w	pH	water-phase salt (%)[a]
Smoked cured beef piece	A	0.96	5.5	2.9
Smoked cured beef slices	A	0.98	5.6	2.5
Summer sausage	A	0.96	4.7	3.9
Summer sausage	B	0.95	4.9	5.2
Summer sausage	C	0.96	4.8	5.0
Buffalo sausage	C	0.95	5.2	6.5
Elk sausage	C	0.96	5.3	4.5
Venison snack sticks	F	0.91	4.8	7.6
Beef snack sticks, small	A	0.95	5.0	5.6
Beef snack sticks, large	A	0.93	5.0	5.9
Pork rinds	D	0.29	6.0	56.9
Pork rinds, smoke flavor	D	0.27	6.1	60.7
Pork rinds, BBQ flavor	D	0.27	6.1	69.3
Pork cracklins	D	0.28	6.7	69.2
Beef jerky	E	0.75	5.6	14.4

[a] Also referred to as “brine content”.

Table 3. Strains of *Listeria monocytogenes* used to inoculate ready-to-eat meat products prior to re-packaging and storage.

Genus and species	Strain Designation	Original Source
Listeria monocytogenes	Scott A	Human outbreak isolate
Listeria monocytogenes	LM 101	Hard salami
Listeria monocytogenes	LM 108	Hard salami
Listeria monocytogenes	LM 310	Goat cheese
Listeria monocytogenes	V7	Raw milk

Table 4. Survival of inoculated *L. monocytogenes* on ready-to-eat meat products stored after re-packaging under air or vacuum at 21°C.

Product Description (processor)	Log CFU/sample (mean) with n and range in parentheses		
	Start	1 week	5 weeks
Summer sausage (B)	4.2 (1,0)	1.0 (1,0)	0.9 (1,0)[ab]
Summer sausage (C)	3.4 (1,0)	1.5 (1,0)	0.9 (1, 0)
Elk sausage (C)	4.0 (1,0)	2.4 (1,0)	0.9 (1,0)
Buffalo sausage (C)	3.1 (1,0)	0.9 (1,0)	0.9 (1,0)
Pork rinds (D)	4.3 (2, 0.1)	2.2 (2, 0.6)	1.1 (2,0.4)
Pork rinds, smoke flavor (D)	4.4 (1, 0)	2.7 (1, 0)	1.7 (1,0)
Pork rinds, BBQ flavor (D)	4.2 (1,0)	3.0 (1,0)	0.9 (1,0)
Pork cracklins (D)	4.2 (2,0.4)	2.6 (2,1.6)	1.1 (2, 0.4)
Beef jerky (E)	3.6 (3, 1.2)	1.2 (3, 0.2)	0.9 (3,0)

[a] 0.9 is value assigned when no cells were detected.

[b]Value obtained after 4 weeks of 21°C storage.

Table 5. Survival of inoculated *L. monocytogenes* on ready-to-eat meat products stored after re-packaging under vacuum at 5°C.

Product Description (processor)	Log CFU/sample (mean) with number of lots and range in parentheses				
	Start	1 week	4 weeks	5 weeks	11 weeks
Smoked beef piece (A)	4.5 (2, 0.2)	3.7 (2, 0.9)	NT[a]	NT	1.5 (2,1.1)
Smoked beef slices (A)	4.3 (2, 0.1)	4.2 (2, 0.2)	NT	NT	3.5 (2,0.5)
Summer sausage (A)	3.6 (3, 0.6)	2.3 (3, 0.4)	0.9 (3, 0)	NT	NT
Summer sausage (B)	3.9 (3, 0.3)	1.5 (3, 1.8)	NT	NT	0.9 (3, 0)
Summer sausage (C)	3.4 (1,0)	1.5 (1,0)	NT	NT	0.9 (1,0)
Buffalo sausage (C)	3.7 (1,0)	2.6 (1,0)	NT	NT	1.5 (1,0)
Elk sausage (C)	3.8 (1,0)	3.2 (1,0)	NT	NT	2.4 (1,0)
Venison snack sticks (F)	4.0 (1, 0)	3.6 (1, 0)	NT	1.1 (1, 0)	NT
Beef snack sticks, small (A)	3.5 (2, 0.2)	2.5 (2, 0.3)	NT	2.1 (2, 1.0)	NT
Beef snack sticks, large (A)	3.7 (1, 0)	3.0 (1,0)	NT	2.0 (1, 0)	NT

[a]Not tested.