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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 six processors and included summer sausage, smoked cured beef, beef jerky, snack stick, and pork rind and crackling products. The water activity of the products ranged from 0.27 (pork rinds and cracklings) to 0.98 (smoked cured beef slices). Products were inoculated with a five-strain cocktail of *L. monocytogenes*, repackaged under either vacuum or air, and then stored either at room temperature (21°C) or under refrigeration (5°C) for 4 to 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 U.S. Department of Agriculture regulations mandating control of *L. monocytogenes* on ready-to-eat meat and poultry products. For many of the products, 1 week of postprocessing storage prior to shipment would act as an effective postlethality treatment and would allow processors to operate under alternative 1 of these regulations.

On 6 June 2003, the U.S. 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 6 October 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 formulation or processing steps designed to kill *L. monocytogenes* or inhibit its growth. The processor is also required to test 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 type of RTE product, 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 for control of *L. monocytogenes* on their products. The alternatives involve various levels of control and microbiological testing. Under alternative 1, the processor uses a postlethality treatment that reduces or eliminates *L. monocytogenes* and uses an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout product shelf life. Under alternative 2, the processor uses either a postlethality treatment that reduces or eliminates *L. monocytogenes* or uses 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.* Of the three alternatives, this one provides the least assurance of safety and requires the most frequent testing of food contact surfaces. For many RTE meat products, the reduction of water activity 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 by surface deposition of inhibitory compounds via smoking could also be an effective antimicrobial process. Items produced in these ways could thus fall under alternative 2. Compliance guidelines from the USDA *(3)* state 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 guideline also summarized scientific studies indicating that *L. monocytogenes* will not multiply at a water activity (a_w) of <0.92 or a pH of <4.39.

Some RTE products could fall under alternative 1, when the standard antimicrobial processing techniques are combined with short-term storage prior to distribution that effectively serves as a postlethality treatment. The compliance guidelines from the USDA state that an effective postlethality treatment must reduce numbers of *L. monocytogenes* by at least 1 log.

The regulation requires that postlethality treatments must be scientifically validated and that evidence must be provided to substantiate the effectiveness of antimicrobial

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

Product	Processor	Ingredients
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,[a] 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[b]
Pork cracklings	D	Pork cracklings (fried pork fat with attached skin), salt
Beef jerky	E	Beef, water, salt, flavoring, sodium nitrate, sodium erythorbate

[a] One lot also contained pork; another tested lot also contained pork and garlic.
[b] Seasonings differed for regular, smoke flavor, and BBQ flavor.

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

The objective of the present study was to evaluate the survival of inoculated *L. monocytogenes* during storage (after repackaging) in 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. One to three lots of each 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 a_w, pH, and percentage of water-phase salt (forced air oven determination of moisture, AOAC method 950.46Bb; potentiometric method for salt, AOAC method 980.25 *(1)*). For each product, the lowest percentage of water-phase salt and the highest a_w and pH values, i.e., conditions least restrictive to microbial growth, are reported in Table 2. Because postlethality 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 by 3.7 cm were cut from each product using a knife previously sanitized using 70% (vol/vol) ethanol, and sections were placed in a biosafety hood on aluminum foil that had previously been sani-

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

Product	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 cracklings	D	0.28	6.7	69.2
Beef jerky	F	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 repackaging and storage*

Strain	Original source
Scott A	Human outbreak
LM 101	Hard salami
LM 108	Hard salami
LM 310	Goat cheese
V7	Raw milk

tized with ethanol and UV light. For beef jerky, pieces measuring 3.7 by 3.7 cm were cut from individual jerky slices. For the pork rind and crackling products, existing individual pieces, each about 2.5 by 2.5 cm, were individually inoculated.

The *L. monocytogenes* strains used in this study were obtained from the laboratory of Dr. Eric Johnson (Food Research Institute, University of Wisconsin–Madison) (Table 3). Stock cultures were maintained at −20°C in brain heart infusion (BHI) broth (Difco, Becton Dickinson, Sparks, Md.) with 10% (wt/vol) added glycerol (Fisher Scientific, Itasca, Ill.). Working cultures, maintained at 4°C on BHI agar (Difco, Becton Dickinson) were prepared monthly from frozen stock cultures. To obtain a working culture, a strain was cultured twice successively at 35°C for 18 to 24 h in BHI broth, streaked to a BHI agar plate, incubated at 35°C for 18 to 24 h, 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 BHI broth and incubating at 35°C for 20 to 24 h. To prepare the five-strain inoculum cocktail, the BHI broth cultures were combined and distributed evenly into two 50-ml sterile plastic centrifuge tubes and centrifuged for 10 min at 5,000 × *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, Wis.). The resulting cocktail was serially diluted in BPD and plated to determine cell concentration. To inoculate the meat or pork rind and crackling 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 min, vacuum packaged at 0.8 atm (Food Saver bags and packaging machine, Tilia, Inc., San Francisco, Calif.), and stored at either 5 or 21°C. Pork rind and crackling 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 1 and 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, 99 ml of BPD was added, and the sample was stomached for 2 min at medium speed (Stomacher 400 lab blender, Fisher). Serial dilutions were made in BPD as needed. For the initial dilution, 1.0 ml of the sample dilution was distributed for spread plating among three plates (0.3, 0.3, and 0.4 ml) of *Listeria* selective agar (LSA; Oxoid, Ogdensburg, N.Y.) 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 to medium, gray-brown to black, raised, flat or sunken colonies surrounded by a black precipitate zone). Replicate plating from nutrient agar (Difco, Becton Dickinson) to LSA was performed for some summer sausage samples, but there was little evidence of cell injury. 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, bioMérieux, Hazelwood, Mo.). Throughout the study, all presumptive isolates were confirmed as *L. monocytogenes.*

With the exception of the pork rind and crackling 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 and crackling products, only one sample was analyzed per sampling time for each lot. The log CFU was calculated for each piece, and the mean log CFU per piece was calculated for each lot and the mean of all lots tested was determined for the product at each 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 a_w, pH, and percentage of water-phase salt (Table 2). Given that higher a_w and pH and lower percentage of 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

TABLE 4. *Survival of inoculated* Listeria monocytogenes *on ready-to-eat meat products stored after repackaging under air or vacuum at 21°C*

Product (processor)	Mean log CFU/sample (*n*, range [difference between highest and lowest values])		
	Start	1 week	5 weeks
Summer sausage (B)	4.2 (1)	1.0 (1)	0.9[a] (1)[b]
Summer sausage (C)	3.4 (1)	1.5 (1)	0.9 (1)
Elk sausage (C)	4.0 (1)	2.4 (1)	0.9 (1)
Buffalo sausage (C)	3.1 (1)	0.9 (1)	0.9 (1)
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)	2.7 (1)	1.7 (1)
Pork rinds, BBQ flavor (D)	4.2 (1)	3.0 (1)	0.9 (1)
Pork cracklings (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] A value of 0.9 was assigned when no cells were detected.
[b] This value was obtained after 4 weeks of storage at 21°C.

TABLE 5. *Survival of inoculated* Listeria monocytogenes *on ready-to-eat meat products stored after repackaging under vacuum at 5°C*

Product (processor)	Mean log CFU/sample (no. of lots, range [difference between highest and lowest values])				
	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)	1.5 (1)	NT	NT	0.9 (1)
Buffalo sausage (C)	3.7 (1)	2.6 (1)	NT	NT	1.5 (1)
Elk sausage (C)	3.8 (1)	3.2 (1)	NT	NT	2.4 (1)
Venison snack sticks (F)	4.0 (1)	3.6 (1)	NT	1.1 (1)	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)	3.0 (1)	NT	2.0 (1)	NT

[a] NT, not tested.

least likely to support growth were the pork rind and crackling products and beef jerky. Numbers of *L. monocytogenes* recovered from stored products were consistent with these predictions. Levels of *L. monocytogenes* fell rapidly on the pork rind and crackling pieces during room-temperature storage (Table 4), with decreases of 1.2 to 2.1 log CFU per piece in the first week of storage and subsequent decreases to or near to the detection limit for samples stored for 5 weeks. Similar results were obtained for the beef jerky (Table 4). Processing of RTE products to yield an a_w of ≤ 0.75 combined with 1 week of storage at 21°C appears to effectively allow the processor to operate under alternative 1, with the processing technique as the antimicrobial process and the 1 week of storage as the postlethality treatment. It is not known whether the decrease in *L. monocytogenes* numbers during storage occurred at a uniform rate or occurred primarily early in the 1-week storage period. If the effect occurred early, a shorter preshipment storage period could be used as the postlethality treatment.

The sausage products (summer, elk, buffalo) had a maximum a_w of 0.94 to 0.96, considerably higher than that for beef jerky. However, fermentation of the summer sausage products during processing resulted in a lower finished product pH than that for the pork rind and crackling products and jerky (Table 2). However, neither the a_w nor the pH of these products was low enough to predict, based on USDA compliance guidelines, that *L. monocytogenes* would not grow. The combination of somewhat reduced a_w and pH seemed to effectively inhibit growth and cause death of *L. monocytogenes*, whose numbers decreased by ≥ 1.0 log at room temperature (Table 4) or during refrigeration (Table 5). These results strongly suggest that sausage products with a mandatory 1-week predistribution storage period could also be produced under alternative 1. To do so, processors would be required either to ensure that their summer sausage had a_w and pH at least as low as those used in the present study or to conduct a challenge study to validate the postlethality treatment for their products.

A somewhat smaller reduction in numbers of *L. monocytogenes* occurred during storage of snack stick products (Table 5). The snack stick products could be produced under alternative 2, but case-by-case studies would be necessary to determine whether 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 a_w and lowest percentage of water-phase salt of all the products studied (Table 2). As expected, *L. monocytogenes* survival was highest on the sliced smoked cured beef product (Table 5), with decreases of only 0.8 log CFU during 11 weeks of refrigerated storage. *L. monocytogenes* numbers decreased by 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 a_w of the intact product, localized areas on the product surface possibly having even lower a_w, and the presence of antimicrobial 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.

The smoking, cooking, and drying processes used to make the products tested in this study can be considered effective antimicrobial processes under the USDA regulations mandating control of *L. monocytogenes* on RTE meat and poultry products. To verify that certain processing techniques allow operation under alternative 2, processors either should verify that their products have a_w, pH, percentage of water-phase salt at least as restrictive as those in the present study or should conduct challenge studies to validate the lack of *L. monocytogenes* growth on their products. For many of the products studied, a 1-week period of postpackaging room-temperature storage prior to shipment could act as an effective postlethality treatment and would allow processors to operate under alternative 1 of the USDA *L. monocytogenes* regulations. Processors should verify similar product composition or conduct challenge studies to validate the postlethality treatment lethality.

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