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Reseach Note

Survival of *Staphylococcus aureus* and *Listeria monocytogenes* on Vacuum-Packaged Beef Jerky and Related Products Stored at 21°C

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ABSTRACT

In the manufacture of beef jerky, a thermal lethality step is followed by drying to prevent growth of pathogenic bacterial postprocessing contaminants on the finished product. Recent guidelines from the U.S. Department of Agriculture have raised the question of the maximum water activity (a_w) in jerky products that will inhibit growth of pathogenic bacteria. The survival of the potential postprocessing contaminants *Staphylococcus aureus* and *Listeria monocytogenes* was evaluated on 15 vacuum-packaged beef jerky and related products with a_w values ranging from 0.47 to 0.87, just below the 0.88 limit reported for anaerobic growth of *S. aureus.* Small individual product pieces were inoculated on the outer surface with five strains each of *S. aureus* and *L. monocytogenes,* repackaged under vacuum, and stored at room temperature (21°C) for 4 weeks. Pathogen numbers were determined before storage and after 1 and 4 weeks. None of the 15 jerky products supported growth of either pathogen. Counts of *S. aureus* fell by 0.2 to 1.8 log CFU after 1 week of storage and by 0.6 to 5.3 log CFU after 4 weeks of storage. Numbers of *L. monocytogenes* fell by 0.6 to 4.7 log CFU and by 2.3 to 5.6 log CFU after 1 and 4 weeks of storage, respectively. Although factors other than a_w may have some effect on pathogen survival, the results of the present study clearly support drying beef jerky to an a_w of ≤0.87 to ensure that bacterial pathogens cannot grow on vacuum-packaged product stored at room temperature.

Current U.S. Department of Agriculture (USDA) standards require products labeled as jerky to have a moisture: protein ratio (MPR) of 0.75:1 or lower *(8).* Traditionally, compliance with this labeling standard was regarded as also ensuring the microbiological shelf stability of these products. However, more recent guidelines from the USDA *(7)* state that shelf stability of jerky products should be evaluated in terms of water activity (a_w) rather than MPR. Therefore, processors of beef jerky and related products may be asked by regulatory personnel to determine product a_w and validate that their product will not support pathogen growth. The USDA guidelines suggest a maximum a_w of 0.80 for jerky products *(7).* However, in the authors' experience, many commercially available jerky products have considerably higher a_w values than that recommended by the USDA.

The bacterial pathogen most tolerant of reduced a_w, *Staphylococcus aureus,* reportedly will not grow aerobically at a_w of ≤0.85 *(4)* or anaerobically at a_w of ≤0.88 *(3). S. aureus* should be present in jerky products only as a result of postprocessing contamination, because the USDA requires thorough thermal treatment early in the jerky manufacturing process *(7).* Another postprocessing contaminant of concern is *Listeria monocytogenes,* but it has a higher minimum a_w for growth, 0.92 *(6).* Processing treatments reducing a_w and thereby preventing *S. aureus* growth should also prevent growth of *L. monocytogenes* and other bacterial pathogens. Although growth by toxin-producing molds is possible on some meat products, mold growth is commonly prevented on commercial jerky products by vacuum packaging. Scientific studies examining the survival of *S. aureus* and *L. monocytogenes* on beef jerky and related products will be of use to processors in validating the safety and shelf stability of their products.

In a recent study of *S. aureus* survival on vacuum-packaged ready-to-eat meat products stored at room temperature (21°C), we found that on four jerky products with a_w ranging from 0.68 to 0.82 *S. aureus* decreased by 1.0 to 2.6 log CFU after 1 week and by 3.2 to 4.5 log CFU after 4 weeks *(2).* In an earlier study *(1),* we found that counts of inoculated *L. monocytogenes* on vacuum-packaged beef jerky (a_w = 0.75) decreased 2.4 log CFU in the first week of room temperature (21°C) storage, and no surviving cells were detected 4 weeks later. These studies provided some useful information about the range of a_w in beef jerky that will not support pathogenic bacterial growth. However, information is still sparse about the maximum a_w that will prevent pathogen growth in jerky products. Therefore, the objective of the present study was to evaluate the survival of *L. monocytogenes* and *S. aureus* on vacuum-packaged

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beef jerky and related products covering a range of a_w values.

MATERIALS AND METHODS

Inoculum preparation. Five strains each of *L. monocytogenes* and *S. aureus* were used in this study. Three of the *S. aureus* strains, FRI 100, FRI 472, and FRI 1007, were obtained from the laboratory of Dr. Amy Wong (Food Research Institute, University of Wisconsin, Madison) and were originally isolated from cake, turkey salad, and Genoa salami, respectively, that were implicated in illness outbreaks. The other two *S. aureus* strains, ATCC 12600 and ATCC 25923, were obtained from the American Type Culture Collection (Manassas, Va.) and were originally clinical isolates. The *L. monocytogenes* strains were obtained from the laboratory of Dr. Eric Johnson (Food Research Institute). Strain Scott A is a clinical isolate, strains LM 101 and LM 108 were isolated from hard salami, strain LM 310 was isolated from goat cheese, and strain V7 was isolated from raw milk. Stock cultures were maintained at −20°C in brain heart infusion broth (BHIB; Difco, Becton Dickinson, Sparks, Md.) with 10% (wt/vol) added glycerol (Fisher Scientific, Itasca, Ill.). Working cultures maintained at 4°C on brain heart infusion agar (BHIA; 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 BHIB, streaked onto a BHIA plate, incubated at 35°C for 18 to 24 h, examined for uniform colony morphology, 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 the broth at 35°C for 20 to 24 h. To prepare a five-strain inoculum cocktail of each organism, the BHIB cultures of each organism were combined into one sterile 50-ml plastic centrifuge tube and centrifuged for 12 min at 5,000 × *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, Wis.).

Meat products and inoculation. Fifteen commercial beef jerky and related product samples were received from processors. These products were made by a total of 14 different processors in 11 states in the United States and one country outside the United States. A sample of each product was sent to a commercial testing laboratory for determination of pH (USDA PHM-1), a_w (AOAC 978.18 method, AOAC International, Gaithersburg, Md.), percent moisture (forced air oven method, AOAC 950.46Bb, AOAC International), percent salt (potentiometric method, AOAC 980.25, AOAC International), and percent protein (Kjeldahl method, AOAC 991.20.I, AOAC International). Jerky strips for inoculation ranged in length from 5 to 15 cm. For each product, half of the strips were inoculated with *L. monocytogenes* and half were inoculated with *S. aureus.* For inoculation, a 0.025-ml volume of the undiluted cocktail was pipetted onto the product surface and distributed as evenly as possible with a sterile plastic spreader. The inoculated strips were allowed to dry for 30 min and then turned over, and the other side was inoculated using the same procedures. After drying, one strip inoculated with *L. monocytogenes* and one strip inoculated with *S. aureus* were placed in a Food Saver bag and vacuum-sealed (Food Saver packaging machine; Tilia, Inc., San Francisco, Calif.). Three bags were prepared for each product for each sampling time and were stored at 21°C for 4 weeks.

Enumeration of surviving inoculum cells. At time 0, 1, and 4 weeks, each sample was placed in a Whirl-Pak filter bag (15 by 23 cm; Nasco, Ft. Atkinson, Wis.), 99 ml of BPD was added, and the sample was stomached for 2 min at medium speed (Stomacher 400 Circulator lab blender, Fisher, Pittsburgh, Pa.). This initial dilution was arbitrarily denoted as 10^{-1}. Serial decimal dilutions were made in BPD as needed. For the initial (10^{-1}) dilution, 1.0 ml was distributed for spread plating among three plates (0.3, 0.3, and 0.4 ml) of *Listeria* selective agar base (LSA; Oxoid, Ogdensburg, N.Y.) containing added *Listeria* selective supplements (Oxford formulation; Oxoid). From the original dilution and each subsequent dilution, 0.1 ml was spread onto one LSA plate per dilution and 1.0 ml was spread onto one 3M Petrifilm Staph Express plate (PF-SE; 3M Microbiology, St. Paul, Minn.), and plates were incubated at 35°C for approximately 48 h and 24 h, respectively, and examined for typical *L. monocytogenes* and *S. aureus* colonies. At each sampling time, one presumptive *L. monocytogenes* colony and one presumptive *S. aureus* colony were selected for confirmation testing. Each colony was transferred to BHIA, incubated at 35°C for 20 to 24 h, tested for Gram reaction, cellular morphology, oxidase activity, catalase activity, and either biochemical characteristics (API *Listeria* kit, bioMérieux, Hazelwood, Mo.) or immunological characteristics (DrySlide kit for *S. aureus,* Fisher).

Counts (log CFU) were calculated for each sample, and means and standard deviations were calculated for each sampling time from the three samples of each product. When no surviving cells were detected on the least dilute plate, a value of 0.95 log CFU (9 CFU, or 1 CFU less than the 10 CFU detection limit) was assigned.

RESULTS AND DISCUSSION

The a_w values for the 15 beef jerky and related products ranged from 0.47 to 0.87 (Table 1). Seven of the samples had a_w values above the 0.80 recommended in the USDA guidelines for jerky processors *(7)*; two samples had a_w values greater than 0.85 and, based only on a_w, could potentially support aerobic growth of *S. aureus.* Eight samples had an MPR higher than the USDA labeling limit of 0.75:1. The a_w for these noncompliant products included the extremes of the 15 different products, highlighting the problems associated with using MPR values to predict microbial growth.

The number and type of ingredients used to make the beef jerky and related products ranged widely (Table 2). Of the 13 products for which ingredient statements were available, one contained only two ingredients, beef and salt (product 7), but most products contained water (10 products), one or more sweeteners (8 products), salt (11 products), and various flavoring ingredients, including monosodium glutamate (6 products). Garlic was also a common ingredient (nine products). Nine products were cured with sodium nitrite (often with sodium erythorbate also added). Three products contained soy sauce or teriyaki, five products contained vinegar, and two contained citric acid. Only one product had been dipped in potassium sorbate to prevent mold growth. Ingredients found in only one product included apple juice, papaya juice, Worcestershire sauce, wine, succinic acid, paprika, and tomato powder.

Throughout this study, all presumptive isolates were confirmed as *L. monocytogenes* or *S. aureus.* Therefore, numbers of presumptive organisms are described simply as *S. aureus* or *L. monocytogenes* values. None of the beef

TABLE 1. *Characteristics of beef jerky and related products*

Product code	Processor code	Product description	Whole muscle (yes/no)	a_w	% water-phase salt	pH	Moisture: protein	Meets USDA labeling standard[a]
1	M	Beef jerky	Yes	0.47	16.5	5.6	0.84:1	No
2	M	Jerky teriyaki	Yes	0.63	15.2	5.8	0.92:1	No
3	F	Beef jerky	Yes	0.68	19.5	5.7	0.50:1	Yes
4	B	Peppered beef jerky	Yes	0.73	12.4	6.0	0.57:1	Yes
5	C	Buffalo and beef pemmican	No	0.74	9.6	5.0	0.97:1	No
6	L	Beef jerky	Yes	0.75	23.0	5.3	0.33:1	Yes
7	N	Beef jerky	Yes	0.80	14.7	5.6	0.85:1	No
8	I	Beef jerky	Yes	0.80	34.0	6.3	0.13:1	Yes
9	C	Beef jerky	Yes	0.81	12.7	5.6	0.52:1	Yes
10	H	Beef jerky	Yes	0.81	14.5	5.8	0.59:1	Yes
11	A	Beef jerky	Yes	0.83	13.5	5.4	0.64:1	Yes
12	K	Beef jerky	Yes	0.85	12.6	5.9	0.82:1	No
13	E	Beef jerky	Yes	0.85	11.8	5.8	0.87:1	No
14	D	Beef jerky	Yes	0.86	11.7	5.9	0.89:1	No
15	J	Jerky teriyaki	Yes	0.87	9.8	5.5	0.79:1	No

[a] USDA labeling standard for jerky is MPR $\leq$ 0.75:1.

jerky and related products supported growth of *S. aureus* above inoculation concentrations. Numbers of *S. aureus* fell by 0.2 to 1.8 log CFU during the first week of storage and by 0.6 to 5.3 log CFU after 4 weeks of storage (Table 3). The product with the smallest decrease in *S. aureus* numbers had an a_w of 0.80 (product 7), and the greatest decrease in *S. aureus* counts occurred on products with a_w values of 0.73 and 0.74 (products 4 and 5). These results suggest that factors other than a_w, such as presence or amounts of spices, smoke compounds, or other ingredients, affect *S. aureus* survival.

In an earlier study *(2),* during 4 weeks of storage we observed similar decreases in *S. aureus* numbers on vacuum-packaged beef jerky products with a_w values ranging from 0.68 to 0.82. These results and those of the present study suggest that an a_w of 0.87 could be considered the limit for preventing *S. aureus* growth.

None of the beef jerky and related products supported growth of *L. monocytogenes* (Table 4). Population decreases were generally greater and more rapid than those for *S. aureus.* Decreases after 1 week of storage ranged from 0.6 to 4.7 log CFU, and decreases after 4 weeks ranged from 2.3 to 5.6 log CFU. The most rapid decreases were on products 11, 4, and 9, with respective a_w values of 0.83, 0.73, and 0.81. For each of these products, no *L. monocytogenes* colonies were recovered on at least one sample at the 1-week sampling time. In contrast, *S. aureus* colonies were detected on all samples after 1 week of storage. After 4 weeks of storage had elapsed, no *L. monocytogenes* colonies were detected on at least one sample of products 4 through 7 and 9 through 14. The a_w values for these products ranged from 0.73 to 0.86. In contrast, the smallest decreases were for products 8, 2, and 1, which had a_w values of 0.80, 0.63, and 0.47, respectively. Clearly, a_w was not the main compositional factor affecting survival of *L. monocytogenes.*

Recent USDA regulations pertaining to the control of *L. monocytogenes* on ready-to-eat meat and poultry products *(5)* require processors to take one or more specific steps to ensure the absence of *L. monocytogenes* from their products. In particular, the rule requires processors to adopt one of three designated alternatives for pathogen control. The alternatives involve different levels of control and are linked to suggested frequencies of mandatory microbiological testing of food contact surfaces. As the level of control increases, the frequency of required testing decreases. As a result, there is an incentive for processors to produce items under alternatives 1 or 2. Under alternative 1, the processor must use a postlethality treatment that reduces or eliminates *L. monocytogenes* and must use an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout product shelf life. Under alternative 2, the processor must use either a postlethality treatment that reduces or eliminates *L. monocytogenes* or must use 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.*

For beef jerky and related products, the reduction of a_w, accomplished through cooking and/or drying, clearly could serve as an antimicrobial process by making the finished product unsuitable for *L. monocytogenes* growth. Compliance guidelines from the USDA state that an effective antimicrobial process will allow no more than a 1.0-log increase in *L. monocytogenes* on an ready-to-eat product throughout its shelf life *(6).* All of the beef jerky and related products met this standard of inhibition and could thus be produced under alternative 2. In addition, for some of the tested products short-term preshipment storage may achieve sufficient lethality to serve as a postlethality treatment (reduction in *L. monocytogenes* numbers by at least

TABLE 2. *Nonmeat ingredients in 13 of the beef jerky and related products*

Ingredient	Product (code)
Water and other liquids	
Water	2, 3, 4, 6, 8, 9, 10, 11, 12, 14
Papaya juice	3
Apple juice	15
Sweeteners	
Sucrose	2, 3, 6, 8, 10, 14, 15
Maple sugar or syrup	10, 14
Fructose	4
Glucose	8
High-fructose corn syrup	6
Acidulants, preservatives, and related ingredients	
Vinegar	2, 3, 6, 9, 11
Citric acid	3, 6
Soy sauce	2, 4
Sodium lactate	4
Teriyaki	15
Worcestershire sauce	6
Wine	2
Succinic acid	2
Potassium sorbate dip	12
Salt	2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 14
Spices and related flavorings	
Black pepper	3, 4, 6, 15
''Flavorings''	2, 6, 14
Garlic	2, 3, 4, 5, 8, 10, 11, 12, 15
Monosodium glutamate	2, 3, 4, 6,[a] 12,[a] 14
Onion	6, 8
Paprika	12
''Spices''	5, 8, 10, 11, 12
Tomato powder	8
Curing agents and related ingredients	
Sodium nitrite	2, 3, 4, 9, 10, 11, 12, 14, 15
Sodium erythorbate	4, 9, 12, 15
Other	
Raisins	5

[a] Listed as hydrolyzed soy and corn protein.

1.0 log). In these cases, the products could be produced under alternative 1.

To the extent that the 15 beef jerky and related products tested in this study are representative of commercial products, neither *S. aureus* nor *L. monocytogenes* is likely to grow on beef jerky when the a_w is ≤ 0.87 and the product is stored under vacuum at room temperature. In addition, beef jerky and related products probably can be produced under alternative 2 of the USDA *L. monocytogenes* regulations. However, there are clearly compositional factors other than a_w that affect survival of both *S. aureus* and *L. monocytogenes.* For many products tested, added ingredients (e.g., vinegar, citric acid, soy sauce, or salt) may affect potential pathogen survival. If a processor desires to produce beef jerky under alternative 1 of the *L. monocytogenes* regulations, challenge studies with the specific product are probably necessary for process validation.

TABLE 3. Staphylococcus aureus *survival on vacuum-packaged beef jerky and related products stored at 21°C*

Product code	a_w	Mean (SD) *S. aureus* counts (log CFU)[a]		
		0 weeks	1 week	4 weeks
1	0.47	6.2 (0)	5.9 (0.1)	4.0 (0.2)
2	0.63	6.2 (0)	5.4 (0.1)	3.8 (0.5)
3	0.68	6.3 (0)	6.1 (0.1)	3.1 (0.1)
4	0.73	6.3 (0.1)	4.6 (0.2)	1.1 (0.2)
5	0.74	6.3 (0)	5.0 (0)	1.0 (0)[b]
6	0.75	6.2 (0.1)	5.5 (0.2)	3.8 (0.1)
7	0.80	6.1 (0.1)	5.9 (0.1)	5.5 (0.1)
8	0.80	6.3 (0)	5.3 (0.2)	2.7 (0.5)
9	0.81	6.1 (0.1)	4.5 (0.4)	1.6 (0.6)[b]
10	0.81	6.3 (0)	5.9 (0.3)	4.0 (0.4)
11	0.83	6.3 (0)	4.9 (0.1)	1.9 (0.8)[b]
12	0.85	6.2 (0.1)	4.7 (0.3)	2.2 (0.2)
13	0.85	6.3 (0)	5.9 (0.1)	3.7 (0.6)
14	0.86	6.3 (0)	5.5 (0.5)	2.4 (0.5)
15	0.87	6.2 (0)	4.4 (0.3)	1.8 (0.1)

[a] Mean of three samples.
[b] No colonies were detected (assigned value of 0.95 log CFU) for at least one sample.

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TABLE 4. Listeria monocytogenes *survival on vacuum-packaged beef jerky and related products stored at 21°C*

Product code	a_w	Mean (SD) *L. monocytogenes* counts (log CFU)[a]		
		0 weeks	1 week	4 weeks
1	0.47	6.2 (0.2)	5.6 (0.1)	3.7 (0.1)
2	0.63	6.2 (0.3)	4.7 (0.1)	2.8 (0.1)
3	0.68	6.7 (0.1)	5.7 (0.3)	1.2 (0.3)
4	0.73	6.3 (0.1)	2.4 (1.3)[b]	1.0 (0.1)[b]
5	0.74	6.6 (0)	5.3 (0.1)	1.0 (0.1)[b]
6	0.75	5.5 (0.1)	4.2 (0.1)	2.1 (0.2)[b]
7	0.80	6.5 (0.1)	4.7 (0.2)	1.0 (0)[b]
8	0.80	5.3 (0.1)	4.9 (0.1)	3.0 (0.2)
9	0.81	6.1 (0.1)	1.8 (0.5)[b]	1.0 (0)[b]
10	0.81	6.4 (0.2)	4.5 (0.3)	1.1 (0.3)[b]
11	0.83	6.5 (0.2)	1.8 (1.5)[b]	1.0 (0)[b]
12	0.85	6.5 (0)	4.9 (0.5)	1.0 (0.1)[b]
13	0.85	5.6 (0.1)	4.1 (0.3)	2.0 (0)[b]
14	0.86	6.4 (0.1)	3.7 (0.7)	1.0 (0)[b]
15	0.87	5.9 (0.1)	4.1 (0.1)	2.0 (0)

[a] Mean of three samples.
[b] No colonies were detected (assigned value of 0.95 log CFU) for at least one sample.

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