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Research Note

Predicting Growth–No Growth of *Listeria monocytogenes* on Vacuum-Packaged Ready-to-Eat Meats

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ABSTRACT

Compliance with U.S. Department of Agriculture (USDA) composition-based labeling standards often has been regarded as evidence of the shelf stability of ready-to-eat (RTE) meats. However, the USDA now requires further proof of shelf stability. Our previous work included development of equations for predicting the probability of *Staphylococcus aureus* growth based on the pH and a_w of an RTE product. In the present study, we evaluated the growth–no-growth during 21°C storage of *Listeria monocytogenes* on 39 vacuum-packaged commercial RTE meat products with a wide range of pH (4.6 to 6.5), a_w (0.47 to 0.98), and percent water-phase salt (%WPS; 2.9 to 34.0). Pieces of each product were inoculated with a five-strain cocktail of *L. monocytogenes* and vacuum packaged, and *L. monocytogenes* levels were determined immediately after inoculation and after storage at 21°C for up to 5 weeks. *L. monocytogenes* grew on 13 of 14 products labeled ''keep refrigerated'' but not on any of the 25 products sold as shelf stable. Using bias reduction logistic regression data analysis, the probability of *L. monocytogenes* growth (Pr) could be predicted as a function of pH and a_w: $Pr = \exp[-59.58 + (4.67 \times pH) + (35.05 \times a_w)]/\{1 + \exp[-59.58 + (4.67 \times pH) + (35.05 \times a_w)]\}$. Pr also could be predicted as a function of pH and %WPS: $Pr = \exp[-20.52 + (4.10 \times pH) - (0.51 \times \%WPS)]/\{1 + \exp[-20.52 + (4.10 \times pH) - (0.51 \times \%WPS)]\}$. The equations accurately predicted *L. monocytogenes* growth (Pr values of 0.68 to 0.99) or no growth (Pr values of <0.01 to 0.26) and with our equations for predicting *S. aureus* growth will be useful for evaluating RTE meat shelf stability.

The U.S. Department of Agriculture (USDA) has used compositional standards to define various ready-to-eat (RTE) meat products. For example, hard salami must have a moisture:protein ratio not greater than 1.9:1 and jerky must have a moisture:protein ratio not greater 0.75:1 *(25)*. Although these USDA standards were not intended to define microbiological shelf stability, compliance with the labeling standard has often been equated with shelf stability for RTE products. However, the USDA now expects processors of shelf-stable RTE meat products to obtain scientific information that validates product shelf stability. Such information may be obtained by performing inoculation studies, but this approach is very expensive and is limited in its usefulness because RTE meat products may vary slightly in composition between batches. For example, when a compositional characteristic of an RTE meat product, such as pH, water activity (a_w), or % water-phase salt (%WPS) is less restrictive of microbial growth in a commercial batch than in a test batch of the same product used in an inoculation study, then the results of the inoculation study cannot be used to prove that the commercial batch is shelf stable. The inflexibility of the inoculation study approach has led to interest in developing scientifically valid computer-based tools that can reliably predict shelf stability of RTE meat products based on key compositional characteristics.

A shelf-stable RTE product can be defined as one having characteristics that prevent the growth of pathogenic microorganisms under nonrefrigerated storage conditions. RTE meat products that have historically been considered shelf stable have reduced pH and a_w and/or elevated %WPS compared with RTE products generally considered not to be shelf stable. Thus, the target pathogenic microorganism(s) used to define shelf stability must be tolerant of increased acid or salt levels and/or decreased a_w. The bacterial pathogen commonly regarded as having the highest tolerance to reduced a_w or increased %WPS is *Staphylococcus aureus (13)*. Mycotoxigenic or antibiotic-producing mold species *(1, 3, 16, 17, 21)* and nonpathogenic spoilage molds *(13)* can grow on RTE products with a_w values that do not permit *S. aureus* growth. However, vacuum packaging or use of in-package oxygen scavengers, which are commonly used by meat processors to extend shelf life, will prevent mold growth. In an earlier study, we developed equations to determine the probability of *S. aureus* growth on vacuum-packaged RTE meat products at 21°C, based on the product pH and either a_w or %WPS *(5)*. However, an additional concern for shelf-stable products would be *Listeria monocytogenes*. This pathogen has a higher minimum a_w for growth

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than does *S. aureus*, 0.92 *(24)* versus 0.86 (under aerobic conditions *(13),* and reportedly can grow at pH as low as 4.4 *(24),* a pH slightly lower than the 4.5 and 5.0 minimum pH values for *S. aureus* toxigenesis under aerobic and anaerobic conditions, respectively *(12)*. Thus, our objectives in this study were to experimentally determine the growth of *L. monocytogenes* at 21°C on a variety of vacuum-packaged RTE meat products with known pH, a_w, and %WPS and to develop mathematical equations for predicting the likelihood of *L. monocytogenes* growth as a function of product composition. In performing this work, we used experimental results from previously published studies *(10, 11)* and work conducted in 2008 and 2009.

MATERIALS AND METHODS

Inoculum preparation. Four of the *L. monocytogenes* strains used in this study were obtained from the laboratory of Dr. Eric Johnson (Food Research Institute, University of Wisconsin–Madison). Strains Scott A, LM 101, LM 310, and V7 were obtained from a clinical sample, hard salami, goat cheese, and raw milk, respectively. Strain ATCC 51414 was obtained from the American Type Culture Collection (Manassas, VA) and was originally isolated from raw milk associated with a listeriosis outbreak. 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, IL). Working cultures were prepared by culturing each strain twice successively at 35°C for 18 to 24 h in BHIB, streaking each culture on a BHI agar (BHIA; Difco, Becton Dickinson) plate, incubating plates at 35°C for 18 to 24 h, examining colonies for homogeneous morphology, and then storing cultures at 5°C. To prepare inoculum, an individual working culture colony of each strain was transferred to a separate tube of BHIB that was incubated at 35°C for 24 h. The broth cultures were then combined in a 50-ml sterile plastic centrifuge tube and centrifuged for 10 min at 5,000 × *g* at room temperature. After centrifugation, the supernatant was decanted and the pellet was resuspended to 50 ml in Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI). To verify the inoculum cell concentration, serial decimal dilutions were made in BPD, spread plated on BHIA, and incubated for 24 h at 35°C. The inoculum contained about 8 log CFU/ml and was not diluted before product inoculation.

Preparation of meat products. All products were either mailed to the laboratory by processors *(10, 11)* or purchased from local retail markets (work done in 2008 and 2009). To determine pH, a_w, percentage of water, and percentage of sodium chloride, a representative sample of each product was analyzed at a commercial testing laboratory (products 21 through 24 at Silliker Laboratories of Wisconsin, Madison; all other products at Marshfield Clinic Food Safety Services, Marshfield, WI) using standard analytical methods (Table 1). Each product was assigned an identification number and group based on product type. Products were prepared for inoculation by first placing them on a sanitized work surface and then cutting them. In general, products were cut so that interior and exterior surfaces could be inoculated as appropriate. Products 1 (ham stick), 2 (landjaeger), 6 (Cheddar wurst), 7 (smoked bratwurst with cheese), and 16 (Italian sausage) were cut into 6- to 10-cm pieces, and each piece was split longitudinally; the resulting halves were placed such that one had the outer surface up and the other had the inner surface up. Products 3 (summer sausage with cranberries) and 5 (beef salami) were cut into cross-sectional slices 3.0 cm wide. Each sample consisted of a pair of slices. The inedible casing was removed from one slice of each pair, and this slice was then cut in half along the product diameter (6 cm). The resulting half-pieces were placed with the diameter down and the outer surface up. The other full-size piece was laid flat, exposing the internal surface. Products 10 through 15 (various types of sausage) were cut into 3.0-cm-wide slices. Each slice was then split longitudinally, and the resulting half-slices were placed such that one had the outer surface up and the other had the inner surface up. Summer sausage, buffalo sausage, and elk sausage *(10)* (products 21 through 24) were first cut into slices 3.7 cm wide, and the inedible casing was removed. Then each slice was cut just below the outer surface of the slice (the surface just underneath the casing) to yield a thick strip (3.7 by 3.7 by 0.6 cm) that could be laid flat with the outer surface up. Two slices of each commercially presliced product were selected: Cotto salami (products 8 and 9), ham (product 17), Genoa salami (product 18), cervelat summer sausage (product 19), and hard salami (product 20). Beef jerky and pemmican products were either cut into pieces (3.7 by 3.7 cm) *(10)* (product 30) or left as individual strips *(11)* (products 31 through 45).

Inoculation and enumeration. Pieces of products 1, 2, 3, 5, 6, 7, and 16 were inoculated by distributing 0.025 ml of inoculum with a sterile plastic spreader (Nelson Jameson) over each pair of inner and outer surfaces. The exposed surface of each of two slices of products 8, 9, 17, 18, 19, and 20 was inoculated with 0.025 ml of inoculum distributed with a sterile plastic spreader. After a 30-min period to allow microbial attachment, one slice of each pair was laid atop the corresponding slice with the inoculated surfaces contacting each other. Products 10 through 15 were inoculated by transferring 0.025 ml of inoculum to the exposed internal surface and 0.025 ml of the inoculum to the outer surface and distributing the inoculum with a sterile plastic spreader. The initial inoculation level was 3.7 to 4.6 log CFU/cm^2 for products 1 through 3 and 5 through 20. Products 21 through 24 were inoculated by pipeting 0.025 ml of inoculum onto the outer surface of each piece. The inoculum was distributed over the surface of the piece with a sterile plastic spreader, resulting in an initial inoculation level of 2.0 to 3.2 log CFU/cm^2. A similar procedure was used to inoculate individual jerky pieces: one side of the piece for product 30 (2.1 log CFU/cm^2), and both sides of the piece for products 31 through 45. Inoculation of each side was followed by a 30-min attachment period (3.7 to 5.2 log CFU/cm^2). After inoculation, each piece or pair of pieces was vacuum packaged and stored at 21°C. Enough samples were prepared to allow triplicate analysis at time zero and at 7 and 28 days (all products except products 21 through 24 and 30) or single-sample analysis at time zero and at 7 and 35 days (products 21 through 24). For product 30, triplicate analysis was done at time zero and at 7 and 35 days.

At each sampling time, 99 ml of BPD was added to each opened sample bag, and the sample was pummeled with a stomacher (Stomacher 400 Circulator Lab Blender, Seward, Worthington, UK) for 2 min at medium speed. From the initial sample preparation, 1.0 ml was removed for spread plating among three plates (0.3, 0.3, and 0.4 ml) of *Listeria* selective agar (Oxoid, Ogdensburg, NY) containing *Listeria* selective supplements (Oxford formulation, Oxoid). From the initial sample preparation and each subsequent dilution, 0.1 ml was spread plated on the same selective differential medium, with one plate prepared per dilution. Plates were incubated for 48 h at 35°C, and typical *L. monocytogenes* colonies (small to medium size, gray-brown to black, surrounded by a black precipitate zone) were counted. One typical colony was picked from a plate for each product at each

sampling time for confirmation by Gram reaction, cell morphology, oxidase reaction, and either biochemical characteristics (API *Listeria* kit, bioMérieux, Hazelwood, MO) or hemolysis of blood agar. Throughout the study, all presumptive isolates were confirmed as *L. monocytogenes*.

Statistical analyses. The log CFU per square centimeter was calculated for each piece in the study performed in 2008 and 2009 work, whereas in the 2004 and 2006 studies results were expressed as log CFU per piece. When no colonies were detected at a sampling time on the least dilute plate, a log value of the inverse of the least dilute dilution factor minus 0.1 was assigned, e.g., 0.9 was assigned when no colonies were observed on the plate with a 10^{-1} dilution factor. The mean log CFU per square centimeter or per piece was calculated for each product at each sampling time, up to 35 days. When the 7-day results clearly indicated growth, sampling was discontinued. The mean log value at time zero was subtracted from the value at each sampling time to determine the change in *L. monocytogenes* over time. A positive value indicated that *L. monocytogenes* growth occurred, whereas a zero or negative value indicated that growth did not occur (Table 1). Values for product pH were plotted against product a_w and product %WPS, with one point for each product (Fig. 1). Using different symbols, points representing products that supported *L. monocytogenes* growth were differentiated on the plots from points representing products that did not support *L. monocytogenes* growth. The resulting plots were then evaluated for separation between growth and no-growth points. When adequate separation was observed, the growth and no-growth results and compositional data for each product were analyzed further to estimate the probability of *L. monocytogenes* growth as a function of pH and either a_w or %WPS using a bias reduction logistic regression for generalized linear models. This analysis was performed with the brglm R package *(14, 15, 19)*. The method was originally developed by Firth *(7)* to reduce the bias of maximum likelihood estimates arising from standard logistic regression analysis. This bias is of the order of $1/n$, where n is the sample size. In the case of logistic regression, this approach corresponds to maximizing a penalized likelihood, i.e., the regression coefficients are chosen to maximize the product of the likelihood with the square root of the determinant of the information matrix. The information matrix measures the curvature of the likelihood function and is calculated as the second derivative of the negative log-likelihood function. This method is superior to logistic regression, especially when there are high prediction covariates or small sample sizes *(9)*. Based on the determined probability boundary, equations for predicting the probability of *L. monocytogenes* growth were developed using pH and either a_w or %WPS as independent variables. For each ready-to-eat meat product, predicted probability of *L. monocytogenes* growth was then determined using both equations and compared with the observed growth–no-growth outcome.

RESULTS AND DISCUSSION

Perfect separation of the two types of points (products supporting *L. monocytogenes* growth and products not supporting *L. monocytogenes* growth) was observed in plots of product pH versus a_w or %WPS (Fig. 1A and 1B). A similar plot of product a_w and %WPS had much less distinct separation between products supporting *L. monocytogenes* growth and those that did not (Fig. 1C).

This lack of overlap between the two types of points indicated a nearly infinite number of possible transitional lines between growth and no growth, with the inflection point where the probability of growth or no growth is equal to 0.5. Therefore, a bias reduction logistic regression for generalized linear models was performed using the brglm R package *(14)* to estimate a probability boundary approximately equal in distance between growth and no-growth characteristics *(15, 19)*. This method was originally developed to reduce the bias of maximum likelihood estimates arising from logistic regression analysis and is superior to logistic regression when there are high prediction covariates or small sample sizes *(9)*. The bias reduction logistic regression analysis is applicable in situations when the two possible outcomes, such as growth and no growth, are perfectly separated by a combination of covariates. In contrast, standard logistic regression analysis returns unreliable, infinite estimated effects (with infinite confidence intervals) in this situation. Based on the determined probability boundary, equations for predicting the probability of *L. monocytogenes* growth were developed using pH and either a_w or %WPS as independent variables. For each product, the predicted probability of *L. monocytogenes* growth was then determined using both equations, and the prediction was compared with the observed growth–no-growth outcome.

L. monocytogenes grew only in products that were not intended to be shelf stable, i.e., those that were labeled ''keep refrigerated'' (Table 1). Glass and Doyle *(8)* reported similar findings with inoculated RTE meat products that were vacuum packaged and stored at 4.4°C for up to 12 weeks. Under these conditions, *L. monocytogenes* grew on ham, bologna, sliced poultry products, wieners, bratwurst, and roast beef. However, little or no growth was observed on two different summer sausage products (pH 4.8 and 4.9, and %WPS $\geq$ 5.6). The results of the Glass and Doyle study indicated that addition of sodium nitrite is unlikely to cause noticeable inhibition of *L. monocytogenes*. Likewise, several of the products that supported *L. monocytogenes* growth in our study contained sodium nitrite, suggesting that this common meat ingredient is not notably inhibitory to *L. monocytogenes* growth.

In general, in our study *L. monocytogenes* rapidly decreased on the products that were sold as shelf stable. Product 19, cervelat summer sausage, was labeled ''keep

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[a] a_w, water activity; %WPS, percent water-phase salt.
[b] Predicted growth probabilities were calculated from product pH and either a_w or %WPS.
[c] Estimated value; no colonies observed on the least dilute plate. Growth reported is log CFU of detection limit minus 0.1.
[d] NT, not tested; growth already observed at the preceding sampling time.
[e] TNTC, too numerous to count; uncountable high number of colonies on the most dilute plate. Growth reported assumes 250 colonies on the most dilute plate.

TABLE 1. *Observed growth–no growth of* Listeria monocytogenes *and predicted probability of* L. monocytogenes *growth on vacuum-packaged ready-to-eat meat products stored at 21°C*

Product type	Product no.	Composition[a]			*L. monocytogenes* growth			
					Growth change (log CFU/cm^2)		Predicted probability[b]	
		pH	a_w	%WPS	7 days	28 or 35 days	a_w	%WPS
Ham stick	1	4.8	0.91	6.2	−5.5	−4.9[c]	<0.01	0.02
Landjaeger	2	5.0	0.93	6.6	−2.8	−4.5[c]	0.03	0.04
Summer sausage with cranberries	3	5.0	0.95	4.8	−1.7	−1.9	0.05	0.08
Summer sausage	21	4.9	0.95	5.2	−3.2	−3.3[c]	0.03	0.05
	22	4.8	0.96	5.0	−1.9	−2.5[c]	0.03	0.03
Cervelat summer sausage (keep refrigerated)	19	4.7	0.95	8.0	−3.8[c]	−4.8[c]	0.01	<0.01
Elk sausage	24	5.3	0.96	4.5	−1.6	−3.1[c]	0.23	0.26
Buffalo sausage	23	5.2	0.95	6.5	−2.2[c]	−2.2[c]	0.12	0.08
Genoa salami	18	4.6	0.93	7.3	−3.8[c]	−4.8[c]	<0.01	<0.01
Hard salami	20	4.8	0.89	9.1	−4.8[c]	−4.8[c]	<0.01	<0.01
Beef jerky	30	5.6	0.75	14.4	−2.4	−2.7[c]	<0.01	<0.01
	31	5.6	0.47	16.5	−0.6	−2.5	<0.01	<0.01
	32	5.8	0.63	15.2	−1.5	−3.4	<0.01	0.01
	33	5.7	0.68	19.5	−1.0	−4.5	<0.01	<0.01
	34	6.0	0.73	12.4	−3.9	−5.3	<0.01	0.10
	36	5.3	0.75	23.0	−1.3	−3.4	<0.01	<0.01
	37	5.6	0.80	14.7	−1.8	−5.5[c]	<0.01	<0.01
	38	6.3	0.80	34.0	−0.4	−2.3	0.11	<0.01
	39	5.6	0.81	12.7	−4.3	−5.1[c]	<0.01	0.02
	40	5.8	0.81	14.5	−0.9	−5.3	0.02	0.02
	41	5.4	0.83	13.5	−4.7	−5.5[c]	<0.01	<0.01
	42	5.9	0.85	12.6	−1.6	−5.5	0.10	0.06
	43	5.8	0.85	11.8	−1.5	−3.6	0.06	0.06
	44	5.9	0.86	11.7	−2.7	−5.4[c]	0.13	0.10
	45	5.5	0.87	9.8	−1.8	−3.9	0.03	0.05
Buffalo and beef pemmican	35	5.0	0.74	9.6	−1.3	−5.6	<0.01	<0.01
Beef salami (keep refrigerated)	5	5.6	0.98	3.4	−0.2	NT[d]	0.72	0.68
Cheddar wurst (keep refrigerated)	6	6.3	0.96	3.4	>0.5 (TNTC)[e]	NT	0.97	0.98
Bratwurst with cheese (keep refrigerated)	7	6.4	0.96	3.8	>0.5 (TNTC)	NT	0.98	0.98
Cotto salami	8	6.4	0.96	3.3	>0.5 (TNTC)	NT	0.98	0.98
(keep refrigerated)	9	6.4	0.95	3.8	>0.5 (TNTC)	NT	0.97	0.98
Ring bologna (keep refrigerated)	10	6.5	0.96	4.0	>0.5 (TNTC)	NT	0.98	0.98
Smoked sausage	11	6.2	0.96	3.9	>0.6 (TNTC)	NT	0.96	0.96
(keep refrigerated)	14	6.0	0.96	3.0	>1.1	NT	0.88	0.92
Turkey kielbasa (keep refrigerated)	12	6.5	0.97	3.3	>0.7 (TNTC)	NT	0.98	0.97
Beef kielbasa (keep refrigerated)	13	6.3	0.97	3.6	>0.7 (TNTC)	NT	0.98	0.97
Potato sausage (keep refrigerated)	15	6.3	0.97	3.3	>0.7 (TNTC)	NT	0.98	0.97
Italian sausage (keep refrigerated)	16	6.5	0.98	2.9	>0.8 (TNTC)	NT	0.99	0.99
Ham (keep refrigerated)	17	6.3	0.98	3.1	>0.6 (TNTC)	NT	0.98	0.97

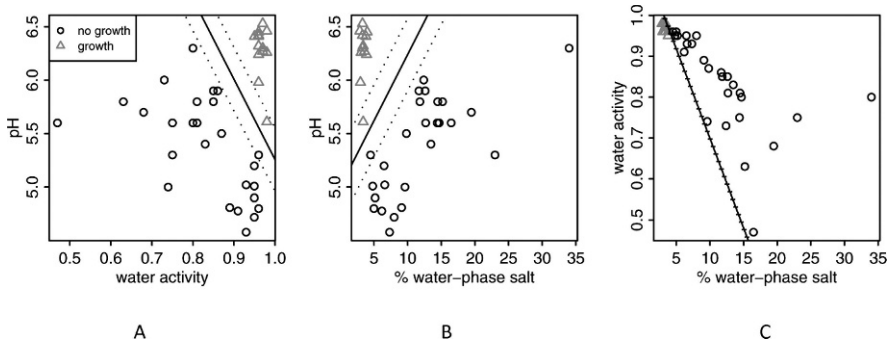

FIGURE 1. *Growth (△) and no growth (○) of* L. monocytogenes *on vacuum-packaged ready-to-eat meat products stored at 21°C. Each point represents one product and is plotted as a function of pH and water activity (A), pH and percent water-phase salt (B), or water activity and percent water-phase salt (C). Solid lines represent the estimated region where growth and no growth have equal probability (0.5). Dashed lines in A and B represent the regions where the probability of growth is estimated to be 0.8 (upper line) and 0.2 (lower line).*

refrigerated'' but clearly did not support *L. monocytogenes* growth. The processor of this product may have chosen to label it as a non–shelf-stable product so that it could be displayed in the same area of the market as the processor's many other non–shelf-stable RTE products or to maximize the time during which the product retained desirable organoleptic qualities. In comparing the findings of the present study to our previous results with *S. aureus (5), L. monocytogenes* levels decreased at similar or slightly greater rates compared with those of *S. aureus*. Therefore, a processor either could use *S. aureus* as the target pathogen for evaluating shelf stability of vacuum-packaged RTE meat products or could take a conservative approach and use both *L. monocytogenes* and *S. aureus* as shelf-stability indices.

The 21°C storage temperature in the present study was chosen because it is broadly representative of the temperatures at which RTE shelf-stable meat products are retailed. Storage temperatures other than 21°C would be expected to result in different reductions in *L. monocytogenes* numbers in fermented shelf-stable products, with the extent of reduction increasing with product acidity and storage temperature *(18)*. Conversely, for non–shelf-stable products *L. monocytogenes* growth would be expected to be proportional to temperature *(4, 6)* within the expected range of refrigeration and room temperature storage.

When the bias reduction for generalized linear models analysis was performed to describe *L. monocytogenes* growth as a function of pH and either a_w or %WPS, line equation parameters (intercept and slope values) were obtained. These values were then used to make two equations that predicted Pr, the probability of *L. monocytogenes* growth:

$$\Pr = \exp[-59.58 + (4.67 \times \text{pH}) + (35.05 \times a_w)]/\{1 + \exp[-59.58 + (4.67 \times \text{pH}) + (35.05 \times a_w)]\} \quad (1)$$

$$\Pr = \exp[-20.52 + (4.10 \times \text{pH}) - (0.51 \times \%\text{WPS})]/\{1 + \exp[-20.52 + (4.10 \times \text{pH}) - (0.51 \times \%\text{WPS})]\} \quad (2)$$

These two predictive equations had residual deviance values of 3.44 and 3.57, respectively, and Akaike information criterion values of 9.44 and 9.57, respectively. The ranges of pH, a_w, and %WPS values that can be reliably entered into these equations are 4.6 to 6.5, 0.47 to 0.98, and 2.9 to 34.0, respectively. Most RTE meat products fall within these ranges. Products with pH < 4.6, $a_w < 0.47$, or %WPS > 34.0 would likely be recognized as shelf stable by trained regulators, whereas products with pH > 6.5 and $a_w > 0.98$ would be clearly recognized as not shelf stable. However, it may not be obvious whether a product with %WPS < 2.9 is shelf stable.

When actual individual product compositional values were entered into equations 1 and 2, accurate growth predictions were obtained for all products (Table 1). For the 13 products on which *L. monocytogenes* growth was observed, the two equations predicted probability values for *L. monocytogenes* growth ranging from 0.68 to 0.99. For 25 of the 26 products on which *L. monocytogenes* did not grow, the two equations predicted probability values for *L. monocytogenes* growth ranging from <0.01 to 0.13. Product 24 (elk sausage) did not support *L. monocytogenes* growth, but the equations based on a_w and %WPS yielded probability values for *L. monocytogenes* growth of 0.23 and 0.26, respectively. This product had the highest pH, highest a_w, and lowest %WPS of any of the acidified or fermented products tested (products 1 through 3 and 18 through 24). When the pH and %WPS values for the two summer sausage types studied by Glass and Doyle *(8)* were entered in our pH and %WPS equation (equation 2), a <0.01 probability of *L. monocytogenes* growth was predicted. Although a direct comparison is not possible because of the different storage temperatures employed, the lack of *L. monocytogenes* growth on summer sausage observed in our study suggests that the predicted probability for *L. monocytogenes* growth on the summer sausages studied by Glass and Doyle *(8)* is accurate. The predictive equations could clearly be used with conservative cutoffs, such as growth = Pr ≥ 0.70 and no growth = Pr ≤ 0.20. Products with a calculated probability of *L. monocytogenes* growth between 0.20 and 0.70 should then be evaluated using inoculation studies.

One compositional factor not accounted for in our predictive equations is the presence or absence of lactate and/or diacetate salts added for inhibition of *L. monocytogenes* growth. The importance of these compounds for inhibiting *L. monocytogenes* growth was highlighted in a 2002 study by Seman et al. *(20)*. These authors found that the addition of 1.5% (wt/wt) potassium lactate plus 0.15% sodium diacetate or 2.5% potassium lactate plus 0.15% sodium diacetate prevented *L. monocytogenes* growth on refrigerated bologna, Cotto salami, smoked cooked ham, and wieners. When these products were made without lactate and diacetate, *L. monocytogenes* growth occurred within 4 to 8 weeks. In the present study, only products 6 (Cheddar wurst), 8 and 9 (Cotto salami), and 14 (smoked sausage) contained lactate and diacetate salts, but these products still supported *L. monocytogenes* growth at 21°C. These results suggest that the lactate and diacetate salts may inhibit *L. monocytogenes* in these products only in combination with proper refrigeration.

There are several ways meat processors could use the predictive equations developed in this study. In performing the hazard analysis required under the USDA hazard analysis critical control point regulations *(22),* processors could determine the pH and either a_w or %WPS for their product(s), enter the values into the appropriate equation, and then make an informed judgment on whether *L. monocytogenes* growth on the finished products is likely to occur based on the resulting probability value. Under the USDA *Listeria* regulations *(23),* processors of RTE meat products must choose one of three alternatives for preventing product contamination with and/or growth of *L. monocytogenes*. Under alternative 1, the processor must employ a postlethality treatment that reduces or eliminates *L. monocytogenes* and use an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout the product shelf life. Under alternative 2, the processor must use either the postlethality treatment or the antimicrobial agent and/or process but not both, and under alternative 3 the processor relies only upon sanitation measures to control *L. monocytogenes*. The predictive equations developed in this study could be used to support processors who describe their *Listeria* control programs as falling under alternative 2 (the product compositional characteristics serve as antimicrobial agents preventing *L. monocytogenes* growth). A meat processor could also use the predictive equations to determine target compositional characteristics for achieving shelf stability in products. For example, a processor considering the shelf stability of a meat product with pH 5.7 or 5.2 (a common target pH for fermented products *(2)*) could insert the pH value into either equation and select a maximum allowable probability of *L. monocytogenes* growth, e.g., 0.20, and solve for either a_w or %WPS.

The equations developed in this study for predicting *L. monocytogenes* growth could be used with those developed for predicting *S. aureus* growth *(5)* to obtain a comprehensive assessment of product shelf stability. Prediction of shelf stability in this manner would enable processors to minimize or eliminate expensive and time-consuming inoculation studies and to validate product shelf stability even when compositional characteristics vary slightly from batch to batch. For small and very small processors with limited financial resources, the resulting savings could be significant. To use either of the shelf stability predictive equations, processors would have to either purchase and use their own pH meter or hire a commercial laboratory to do the pH analysis. Processors wanting to use an a_w-based equation would have to purchase and use an a_w meter or hire a laboratory to measure a_w. Measurement of percent water and percent salt, needed to calculate %WPS, would typically not be feasible for a small or very small processor, so processors choosing to use an equation based on a_w and %WPS would need to hire a laboratory to obtain supporting compositional information.

In summary, predictive equations are versatile tools that can be used when planning strategies for controlling *L. monocytogenes* in RTE meat products, for formulating such products, and for validating their shelf stability.

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