Journal of Food Protection, Vol. 72, No. 3, 2009, Pages 539–548

Predicting Growth–No Growth of *Staphylococcus aureus* on Vacuum-Packaged Ready-to-Eat Meats

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MS 08-355: Received 21 July 2008/Accepted 21 October 2008

ABSTRACT

U.S. Department of Agriculture (USDA) composition-based labeling standards for various ready-to-eat (RTE) meat products typically specify maximum product pH and/or moisture:protein ratio and less often maximum water activity (a_w). Compliance with these standards often has been regarded as proof of shelf stability. However, the USDA now requires additional proof, e.g., challenge study results, of shelf stability. The pathogen most likely to grow on vacuum-packaged, reduced-moisture products is *Staphylococcus aureus.* Therefore, vacuum-packaged RTE products that do not support *S. aureus* growth at room temperature could be considered shelf stable. We developed mathematical equations for predicting whether *S. aureus* would grow under such conditions. Twenty-four commercial RTE meat products and 10 intentionally misprocessed products (insufficient drying, fermentation, and/or salt) were inoculated with a five-strain cocktail of *S. aureus,* vacuum packaged, and stored at 21°C. Initial, 7-day, and 28-day *S. aureus* counts were recorded. Product pH, a_w, moisture:protein ratio, and percentage of water-phase salt (%WPS) also were determined. *S. aureus* grew only in the intentionally misprocessed products and in some commercial products labeled "keep refrigerated." Using bias reduction logistic regression data analysis, the probability of *S. aureus* growth (Pr) could be predicted by either of two equations. The first was based on pH and a_w values: $Pr = \exp[-59.36 + (5.75 \cdot pH) + (28.73 \cdot a_w)]/\{1 + [\exp(-59.36 + (5.75 \cdot pH) + (28.73 \cdot a_w)]\}$. The second was based on pH and %WPS: $Pr = \exp[-26.93 + (5.38 \cdot pH) + (-0.61 \cdot \%WPS)]/\{1 + \exp[-26.93 + (5.38 \cdot pH) + (-0.61 \cdot \%WPS)]\}$. These equations accounted for observed *S. aureus* growth–no growth results and will be a useful tool for evaluating the shelf stability of RTE meats.

The U.S. Department of Agriculture (USDA) has published several compositional standards for defining various ready-to-eat (RTE) meats. Products labeled as jerky, for example, must have a moisture:protein ratio (MPR) of ≤0.75:1, and hard salami must have an MPR of ≤1.9:1 *(15).* For a small-scale processor, determining whether a product complies with the USDA standards can be difficult. With experience, processors can establish the relationship between MPR and easily measured product shrink or yield for a specific product formulation, but typically a commercial laboratory must determine the moisture and protein percentages upon which the MPR is based.

The USDA labeling standards are not intended to define microbiological shelf stability. In practice, however, compliance with the labeling standards has been equated with shelf stability for certain products. The situation is further complicated by recent USDA expectations that processors obtain scientific documentation to support claims that products sold at room temperature are shelf stable, regardless of whether the labeling standards are met. One way to obtain this supporting documentation is to conduct a challenge study to confirm shelf stability. This approach is very time-consuming, expensive, and product specific. If a single compositional characteristic limit specified for the tested product, e.g., a maximum pH value, is not met in a subsequent batch of that product, then that batch must be considered deviant and cannot be sold as shelf stable. The deviation results even though other compositional characteristics of the product may be interacting synergistically to inhibit pathogen growth and thereby achieve shelf stability.

Food microbiologists typically evaluate the potential for pathogenic bacterial growth on meat products based upon the product pH and either the water activity (a_w) or percentage of water-phase salt (%WPS). The pH value is relatively easy and inexpensive to monitor. Small-scale processors may be less likely to measure a_w because of the cost of the meter, but the actual analysis is relatively easy. Determination of %WPS is both difficult and expensive and for a small-scale processor would likely require the assistance of a commercial laboratory. Even if a small-scale processor were to build a database relating product formulation and yield to %WPS, the process would be time-consuming and expensive. These considerations have resulted in processor confusion about the purpose of labeling and shelf stability requirements and the different criteria being used by regulators, processors, and scientists to evaluate RTE meat products. Currently, there are no readily available tools that allow interchanging of MPR, a_w, pH, and %WPS measurements for determining whether an RTE meat product is shelf stable.

A shelf-stable product can be defined as one having characteristics that prevent the growth of pathogenic microorganisms under normal storage conditions. Thus, to ex-

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TABLE 1. Staphylococcus aureus *strains used for inoculation*

Strain	Isolated from:	Source[a]
ATCC 12600	Clinical sample	ATCC
ATCC 25923	Clinical sample	ATCC
FRI-100	Cake implicated in illness outbreak	FRI
FRI-472	Turkey salad implicated in illness outbreak	FRI
FRI-1007	Genoa salami implicated in illness outbreak	FRI

[a] ATCC, American Type Culture Collection (Manassas, VA); FRI, Dr. Amy Wong, Food Research Institute (University of Wisconsin–Madison).

perimentally determine shelf stability of RTE meat products that have reduced moisture or pH, and/or added salt, a target pathogen should be identified. The bacterial pathogen commonly regarded as having the highest tolerance to reduced a_w or increased salt concentration is *Staphylococcus aureus (8).* Mycotoxigenic or antibiotic-producing mold species *(1, 2, 9, 10, 12)* and nonpathogenic spoilage molds *(8)* can grow on meat products at lower a_w values than tolerated by *S. aureus.* Thus, these molds also are possible food safety hazards for RTE meat products. However, vacuum packaging, which is used to eliminate aerobic conditions and extend shelf life, prevents mold growth on RTE meats. Although the environmental pathogen *Listeria monocytogenes* also poses a risk for postprocessing contamination of RTE products, it has a higher minimum a_w for growth (0.92) *(13)* than does *S. aureus* (0.86) *(8)* under aerobic conditions. Anaerobic conditions result in a somewhat higher minimum a_w for *S. aureus* growth (0.88) *(6).* Therefore, processing treatments designed to reduce a_w and prevent *S. aureus* growth also should prevent *L. monocytogenes* growth, and the shelf stability of nonrefrigerated semidry sausages and other RTE meat products could be defined strictly in terms of whether *S. aureus* growth occurs. The objectives of the present study were (i) to determine experimentally the growth potential for *S. aureus* on a variety of RTE meat products with known MPR, a_w, pH, and %WPS values and (ii) to develop mathematical tools that are based on critical compositional values and that could be used to predict the likelihood of *S. aureus* growth.

MATERIALS AND METHODS

Inoculum preparation. A bacterial cocktail was prepared utilizing five broadly representative strains of *S. aureus* (Table 1). 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 maintained at 4°C on brain heart infusion agar (BHIA; Difco, Becton Dickinson) and were prepared monthly from frozen stock cultures. To obtain working cultures, each strain was cultured twice at 35°C for 18 to 24 h in BHIB, streaked to BHIA, incubated for 18 to 24 h at 35°C, examined for homogeneous colony morphology, and stored at 4°C. Inoculation cultures were prepared for each strain by transferring a single viable colony from the working culture to nonselective nutrient agar (NA; Difco, Becton Dickinson), which was streaked onto a plate to produce lawn-like growth, and the culture was incubated for 18 to 24 h at 35°C. To prepare the five-strain cocktail, as much growth as possible from each plate was aseptically transferred via a disposable inoculation loop (Fisher) to a 50-ml conical centrifuge tube (Falcon, Fisher), and 20 ml of Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI) was added. The tube was then sealed and mechanically agitated until a homogenous suspension was obtained (approximately 30 s). A 1.0-ml volume of suspension was transferred into 9.0 ml of BPD and mechanically agitated (approximately 5 s) to yield the inoculum. A separate inoculum was prepared for each meat sample from different streak plates (i.e., all runs were independent). After preparation, serial BPD dilutions of the inoculum were made, and 0.1 ml was transferred from the 10^{-5} to 10^{-7} dilutions to Baird-Parker agar with egg yolk–tellurite enrichment (BP; Difco, Becton Dickinson), spread, and incubated at 35°C for 48 h. The BP plates were then examined for the typical black, glossy colonies of *S. aureus,* which were counted on plates that had 25 to 250 colonies per plate. For each inoculum sample, one randomly selected presumptive *S. aureus* colony was transferred to NA and incubated at 35°C for 24 h. The resulting colonies were tested for the Gram stain reaction, examined for cellular morphology, and subjected to a latex agglutination test (Oxoid, Inc., Ogdensburg, NY) for presumptive *S. aureus* colonies. Throughout the study, all presumptive isolates were confirmed as *S. aureus.*

Preparation of meat products. During the study, 24 RTE meat products were obtained from local markets during a 3-month period. An additional 10 intentionally misprocessed samples (insufficient drying, fermentation time, and/or salt addition) were prepared upon request by a meat laboratory at the University of Wisconsin–Madison. All products were transported to the laboratory and remained in the manufacturer's packaging at room temperature (approximately 21°C) until they were to be inoculated. The exceptions were summer sausage and salami, which required refrigeration as per the manufacturer's directions; these products (seven samples) were held refrigerated (5°C) until used. All commercial products were prepared and inoculated well before the manufacturer's recommended use-by or expiration date. Intentionally misprocessed products that were not immediately inoculated were held refrigerated (5°C) for a maximum of 12 h before use. All samples were sliced with either a sanitized (70% ethanol, vol/vol) commercial meat slicer (Hobart Corporation, Richmond Hill, GA) or a sanitized knife. All product measurements were obtained with a sanitized clear plastic ruler. For all products and cuts, duplicates of each sample were prepared for each sampling time (0, 14, and 28 days). When a single package of product was of insufficient size to produce all necessary samples, additional samples were obtained from product with the same date, batch, and time stamp (within 3 min) to obtain homogenous product samples. For the intentionally misprocessed samples, a sufficient amount of product was made to ensure that enough samples were available. Three types of summer sausage samples were prepared by taking three cross-sectional cuts. The first cut was a thin slice (approximately 2 mm thick) perpendicular to the long axis of the product, which was designated the slice (S) sample. The second and third cross-sectional cuts were in the same orientation and were 2.54 cm thick. These slices were then filleted parallel to the circumference of the product to a thickness of approximately 1 to 1.25 cm, and the resulting strips were laid flat. The casing was retained on the meat product for the casing (C) samples and removed for the outer (O) samples. The S sample diameter was recorded and C and O sample length and width were recorded to determine surface area. In all cases, only the center portion of the chub was utilized to obtain a consistent diameter throughout all samples and duplicates. Salami samples were prepared using the same procedure as for summer sausage, except when no casing was present (two samples). In these cases, only S and O samples and duplicates were prepared. Jerky samples were prepared by slicing a randomly selected piece of jerky into 2.54-cm square samples; therefore, jerky had only one type of sample cut. Landjaeger sausages were trimmed to a consistent width and length and then cut in half lengthwise to yield an inner (I) and an O sample. Meat sticks were trimmed to uniform length and cut in half perpendicular to the long axis, and the cross-sectional width was measured.

One vacuum-packaged sample from each product (made from three composite random "grabs") was frozen and sent to a commercial laboratory for determination of a_w, pH, and percentages of moisture, fat, protein, and salt. After preparation, pieces for inoculation were placed in a biosafety hood on aluminum foil that had previously been sanitized with 70% (vol/vol) ethanol and a ≥30-min UV light treatment.

Inoculation and plating procedures. To inoculate each RTE meat sample, 0.025 ml of undiluted inoculum (containing 8.3 to 9.3 log CFU/ml) was transferred to the sample's surface and distributed as evenly as possible with a sterile bent plastic spreader (Fisher). The product samples were then allowed to dry for 30 min to allow *S. aureus* attachment to the product surface. Although the small inoculum volume was intended to minimize any changes in pH or a_w (not actually measured), the addition of a small amount of diluent to the product surface would have been more likely to encourage *S. aureus* survival than to reduce it. All 7- and 28-day product samples and duplicates were then vacuum packed (0.8 atm; Food Saver bags and packaging machine, Tilia, Inc., San Francisco, CA) and stored at 21°C. The 0-day product samples and duplicates were immediately analyzed for the number of *S. aureus* cells per sample. Each sample was aseptically transferred to a filter bag (5.25 by 23 cm [6 by 9 in.]; Whirl-Pak, Nasco, Ft. Atkinson, WI) and diluted with 99 ml of BPD. The sample was then stomached at medium (normal) speed for 120 s with a lab blender (Stomacher 400 Circulator lab blender, Fisher) to obtain an initial dilution. Serial dilutions were then prepared in BPD, plated on BP, and incubated at 35°C for 48 h. The BP plates were then examined, and colonies were counted and confirmed as described.

Two pieces of each product that had been vacuum packaged and stored at 21°C were analyzed after 7 and 28 days (two pieces at each sampling time). The log CFU per square centimeter was calculated for each piece analyzed, and the mean value was calculated for each product. When no colonies were present for the least dilute plating, a value of log[(0.9 CFU × 1/dilution factor)/sample area] was assigned based on the conservative assumption of 0.9 CFU *(7).*

Statistical analysis. Four compositional characteristics, a_w, %WPS, pH, and MPR, were studied to determine their effect on the shelf stability of RTE meats. Values for the four compositional characteristics were normalized to a 100-point scale to give equal weight to each characteristic by taking all values in the present study and from an earlier study of RTE meat products *(5)* for each characteristic and determining the range. For a_w, the minimum value was 0.68, and the maximum was 0.98, with a range of 0.30. For %WPS, the minimum value was 1.47, and the maximum was 19.00, with a range of 17.53. For pH, the minimum value was 4.4, and the maximum was 6.6, with a range of 2.2. For MPR, the minimum value was 0.40, and maximum value was 5.40, with a range of 5.0 (Tables 2 through 4). A normalized score for each compositional characteristic was determined for each product by taking the laboratory-determined value for that characteristic and subtracting the minimum value, dividing the difference by the range for that characteristic, and multiplying by 100 (equation 1):

$$\text{characteristic score} = [(\text{actual value} - \text{minimum value})/\text{range}] \times 100 \qquad (1)$$

Normalized pH values and normalized a_w, %WPS, or MPR values were then plotted to explain *S. aureus* growth (Fig. 1). Perfect separation of samples supporting growth of *S. aureus* and samples not supporting *S. aureus* growth was observed.

This lack of overlap indicated a nearly infinite number of possible transitional lines between growth and no growth (with the inflection point being where the probability of growth or no growth = 0.5). Therefore, a bias reduction logistic regression was performed *(3, 11).* This method was originally developed to reduce the bias of maximum likelihood estimates arising from logistic regression analysis and is superior to logistic regression, especially when there are high prediction covariates or small sample sizes *(4).* The bias reduction logistic regression analysis also is applicable to situations in which the two possible outcomes, such as growth and no growth, are perfectly separated by a combination of covariates (Fig. 1). Logistic regression analysis returns unreliable, infinite estimated effects (with infinite confidence intervals) in this situation. The bias reduction logistic regression analysis designated a probability inflection point approximately equal in distance between growth and no-growth characteristics and then weighted points as more likely to display growth or no growth depending upon their proximity to the corresponding area of the plot. Using this technique, an equation for predicting the probability of *S. aureus* growth was developed using nonnormalized pH with a_w, %WPS, or MPR (all nonnormalized) as independent variables.

RESULTS AND DISCUSSION

There was a clear separation between products supporting *S. aureus* growth and those that did not (Fig. 1). Thus, the bias reduction logistic analysis was appropriate for describing the probability of *S. aureus* growth. When the bias reduction logistic analysis was performed to describe *S. aureus* growth as a function of nonnormalized (actual) pH plus a_w, %WPS, or MPR, respectively, the following line equation parameters were determined: for the intercept, −59.36, −26.93, and −30.00; for the slope (pH), 5.75, 5.38, and 4.63; and for the slope (a_w, %WPS, MPR), 28.73, −0.61, and 1.37. These values were then entered into three separate equations that would predict the probability of growth of *S. aureus* based on pH plus a_w, %WPS, or MPR (equations 2, 3, and 4, respectively):

$$\text{estimated probability of growth} = \frac{\exp[-59.36 + (5.75 \cdot \text{pH}) + (28.73 \cdot a_w)]}{1 + \exp[-59.36 + (5.75 \cdot \text{pH}) + (28.73 \cdot a_w)]} \qquad (2)$$

$$\text{estimated probability of growth} = \frac{\exp[-26.93 + (5.38 \cdot \text{pH}) + (-0.61 \cdot \%\text{WPS})]}{1 + \exp[-26.93 + (5.38 \cdot \text{pH}) + (-0.61 \cdot \%\text{WPS})]} \qquad (3)$$

$$\text{estimated probability of growth} = \frac{\exp[-30.00 + (4.63 \cdot \text{pH}) + (1.37 \cdot \text{MPR})]}{1 + \exp[-30.00 + (4.63 \cdot \text{pH}) + (1.37 \cdot \text{MPR})]} \qquad (4)$$

The maximum and minimum values for pH that can be

TABLE 2. *Composition and* S. aureus *growth data for summer sausage stored under vacuum at 21°C*

	Composition[a]						*S. aureus* (log CFU/cm^2) at[c]:		
Processor	MPR	a_w	%WPS	pH	Growth	Cut type[b]	0 days	7 days	28 days
Q	2.7	0.96	5.94	4.9	No	O	5.7 ± 0.9	2.7 ± 0.5	−0.7[d]
						S	5.1 ± 0.2	0.5 ± 0.0	−0.5[d]
						C	4.6 ± 0.1	4.3 ± 0.0	3.8 ± 0.1
	3.0	0.96	5.61	4.9	No	O	5.2 ± 0.1	2.5 ± 0.2	−0.7 ± 0.0
						S	5.4 ± 0.0	0.5 ± 0.0	−0.5[d]
						C	4.9 ± 0.0	3.7 ± 0.1	−0.7[d]
R	2.7	0.97	5.35	4.8	No	O	4.9 ± 0.1	1.7 ± 0.4	−0.7 ± 0.0
						S	5.0 ± 0.1	−0.4 ± 0.0	LE
						C	4.8 ± 0.2	1.5 ± 0.2	−0.7[d]
S	3.1	0.96	5.84	4.8	No	O	5.6 ± 0.0	3.8 ± 0.2	−0.3 ± 0.0
						S	5.2 ± 0.0	0.7 ± 1.4	−0.3 ± 0.0
						C	5.5 ± 0.0	3.9 ± 0.1	−0.3 ± 0.0
T	2.5	0.95	5.08	4.7	No	O	5.2 ± 0.1	2.8 ± 0.1	−0.6 ± 0.0
						S	5.3 ± 0.1	−0.2 ± 0.0	−0.2 ± 0.0
						C	5.3 ± 0.1	3.7 ± 0.1	−0.6 ± 0.0
U	3.1	0.95	5.49	4.9	No	O	5.4 ± 0.0	3.9 ± 0.1	−0.5 ± 0.0
						S	5.2 ± 0.0	1.9 ± 0.4	−0.4 ± 0.0
						C	5.4 ± 0.0	4.0 ± 0.1	−0.5 ± 0.0
V	2.8	0.97	5.66	4.9	No	O	5.2 ± 0.0	−0.7 ± 0.0	−0.7[d]
						S	4.9 ± 0.2	0.7 ± 1.4	LE
						C	5.0 ± 0.1	2.7 ± 0.0	2.3[d]
	2.8	0.96	4.86	4.5	No	O	5.1 ± 0.0	−0.7 ± 0.0	LE
						S	5.1 ± 0.0	0.7 ± 1.4	LE
						C	5.3 ± 0.0	2.2 ± 0.3	−0.7[d]
W	2.8	0.96	4.22	4.8	No	O	5.6 ± 0.1	4.0 ± 0.1	−0.6[d]
						S	5.4 ± 0.0	0.7 ± 1.4	−0.4 ± 0.0
						C	5.5 ± 0.1	4.4 ± 0.1	LE
	2.5	0.97	3.30	4.6	No	O	5.0 ± 0.1	3.4 ± 0.0	−0.6[d]
						S	5.3 ± 0.1	2.8 ± 0.0	−0.4 ± 0.0
						C	5.0 ± 0.1	2.8 ± 0.1	−0.6 ± 0.0
	2.8	0.97	4.07	4.8	No	O	5.6 ± 0.1	4.9 ± 0.3	−0.6 ± 0.0
						S	5.6 ± 0.1	4.4 ± 0.1	−0.4 ± 0.0
						C	5.5 ± 0.1	5.3 ± 0.1	−0.6 ± 0.0
AE[e]	3.0	0.96	3.73	4.9	No	O	5.0 ± 0.1	3.6 ± 0.1	1.4 ± 0.0
						S	4.7 ± 0.1	0.6 ± 0.0	−0.4 ± 0.0
						C	4.9 ± 0.1	3.8 ± 0.2	0.3 ± 1.4
	2.7	0.98	1.47	4.4	No	O	4.8 ± 0.1	4.4 ± 0.1	1.5 ± 0.2
						S	4.7 ± 0.1	3.2 ± 0.4	−0.4 ± 0.0
						C	5.1 ± 0.0	3.0 ± 0.2	−0.7 ± 0.0
	2.8	0.96	3.90	4.7	No	O	5.3 ± 0.1	3.4 ± 0.0	−0.5 ± 0.0
						S	4.4 ± 0.0	3.6 ± 0.2	−0.1 ± 0.0
						C	5.4 ± 0.0	3.0 ± 0.1	−0.5 ± 0.0
	2.6	0.96	3.98	4.5	No	O	5.2 ± 0.0	2.7 ± 0.6	−0.5 ± 0.0
						S	4.4 ± 0.1	1.3 ± 1.9	−0.1 ± 0.0
						C	5.3 ± 0.0	0.8 ± 1.9	−0.5 ± 0.0
	2.5	0.96	3.95	4.4	No	O	5.4 ± 0.1	2.0 ± 0.3	−0.6 ± 0.0
						S	5.4 ± 0.1	0.8 ± 1.4	−0.2 ± 0.0
						C	5.5 ± 0.0	1.5 ± 0.0	−0.6 ± 0.0

[a] Compositional values are for a single representative grab sample. MPR, moisture:protein ratio; a_w, water activity; %WPS, % water-phase salt (brine concentration).

[b] Cut type refers to the surface area that was inoculated: the entire inner surface area of a slice (S), the entire outer surface area of a slice (O), or the entire casing surface area of a slice (C).

[c] Values are the mean ± standard deviation of two samples unless otherwise indicated. When no colonies were observed, the value was based on assumption of 0.9 CFU on the least dilute plate. LE, laboratory error (no data).

[d] Only one sample was measured; therefore, no standard deviation calculation was possible.

[e] Processor AE produced all misprocessed products.

TABLE 3. *Composition and* S. aureus *growth data for other ready-to-eat meat products stored under vacuum at 21°C*

		Composition[a]						*S. aureus* (log CFU/cm^2) at[c]:		
Product category	Processor	MPR	a_w	%WPS	pH	Growth	Cut type[b]	0 days	7 days	28 days
Salami	X	4.2	0.97	3.95	6.0	Yes	O	6.0 ± 0.1	5.4 ± 0.1	5.8 ± 0.1
							S	5.7 ± 0.1	5.6 ± 0.0	6.2[d]
							C	5.8 ± 0.1	5.5 ± 0.0	5.6 ± 0.1
	Y	3.9	0.98	3.02	5.8	Yes	O	5.8 ± 0.0	5.1 ± 0.1	7.0 ± 0.2
							S	5.6 ± 0.0	5.4 ± 0.0	7.2[d]
							C	5.7 ± 0.0	6.0 ± 0.1	6.1 ± 0.2
	Z	1.6	0.90	8.14	4.7	No	O	5.2 ± 0.0	3.9 ± 0.0	3.5 ± 0.1
							S	5.4 ± 0.2	2.5 ± 0.2	−0.3 ± 0.0
	AA	1.4	0.88	10.03	5.1	No	O	6.0 ± 0.1	5.2 ± 0.2	3.5 ± 0.2
							S	6.2 ± 0.0	5.1 ± 0.0	4.1 ± 0.1
Beef sticks	T	1.5	0.91	8.87	5.3	No	O	6.7 ± 0.2	5.7 ± 0[d]	LE
							I	6.6 ± 0.2	3.0 ± 0[d]	0.1 ± 0.0
	Q	2.3	0.93	5.58	5.0	No	O	5.6 ± 0.1	4.2 ± 0.1	−0.3 ± 0.0
							I	5.7 ± 0.0	4.6 ± 0.4	−0.3 ± 0.0
	Q	1.3	0.90	9.74	5.1	No	O	6.4 ± 0.0	5.4 ± 0.1	0.1 ± 0.0
							I	6.4 ± 0.0	1.3 ± 1.7	0.1 ± 0.0
	Q	1.8	0.92	7.98	5.1	No	O	5.9 ± 0.1	0.7 ± 1.4	−0.3 ± 0.0
							I	6.0 ± 0.0	−0.3 ± 0.0	−0.3 ± 0.0
	T	1.9	0.94	5.74	5.0	No	O	6.6 ± 0.0	5.7 ± 0.0	0.2 ± 0.0
							I	6.6 ± 0.1	1.2 ± 0.0	0.2 ± 0.0
	AE[e]	1.9	0.93	3.21	4.6	No	O	5.8 ± 0.1	3.9 ± 0.1	0.8 ± 1.4
							I	5.9 ± 0.0	5.0 ± 0.1	4.2 ± 0.1
Jerky	AB	0.5	0.71	16.87	5.6	No	NA	6.1 ± 0.1	5.6 ± 0.0	4.3 ± 0.1
	AC	0.7	0.79	15.08	6.0	No	NA	6.4 ± 0.0	5.9 ± 0.1	4.8 ± 0.3
	AD	0.7	0.73	14.29	5.3	No	NA	6.4 ± 0.0	3.9 ± 0.1	2.2 ± 0.0
	AC	0.6	0.75	17.45	5.7	No	NA	6.4 ± 0.0	5.4 ± 0.1	2.9 ± 0.1
	AE	0.4	0.72	17.94	6.0	No	NA	6.3 ± 0.1	5.5 ± 0.1	3.2 ± 0.7
	AE	1.0	0.93	5.06	6.1	Yes	NA	6.2 ± 0.0	8.7 ± 0.2	8.3[d]
	AE	1.4	0.96	3.78	6.0	Yes	NA	6.2 ± 0.0	8.8 ± 0.0	8.9 ± 0.1
	AE	1.2	0.92	7.08	6.0	Yes	NA	6.2 ± 0.0	5.8 ± 0.1	6.8[d]

[a] Compositional values are for a single representative grab sample. MPR, moisture:protein ratio; a_w, water activity; %WPS, % water-phase salt (brine concentration).
[b] Cut type refers to the surface area that was inoculated: the entire inner surface area of a slice (S), the entire outer surface area of a slice (O), the entire casing surface area of a slice (C), or the inner surface of the slice (I). NA, not applicable (all jerky was cut into square pieces).
[c] Values are the mean ± standard deviation of two samples unless otherwise indicated. When no colonies were observed, the value was based on assumption of 0.9 CFU on the least dilute plate. LE, laboratory error (no data).
[d] Only one sample was measured; therefore, no standard deviation calculation was possible.
[e] Processor AE produced all misprocessed products.

TABLE 4. *Composition and* S. aureus *growth data obtained in a previous study (5) for RTE meat product stored under vacuum at 21°C*

Product category	Processor	Composition[a]				Growth	*S. aureus* (log CFU/cm^2) at[b]:		
		MPR	a_w	%WPS	pH		0 days	7 days	28 days
Jerky	A	0.4	0.68	18.7	5.7	No	5.9 ± 0.2	3.3 ± 0.8	2.3 ± 0.6 (2)
	A	0.7	0.82	10.6	6	No	6.6 ± 0.3	5.1 ± 0.5	2.8 ± 0.5
	B	0.6	0.69	15.4	6.4	No	5.9 ± 0	4.2 ± 0.2	1.4 ± 0.4
	B	0.8	0.76	14.2	6.1	No	5.9 ± 0	4.9 ± 0.1	2.7 ± 0.2
Beef stick	C	2.0	0.88	7.1	4.6	No	5.4 ± 0.2	2.0 ± 0 (3)	0.9 ± 0 (3)
	D	1.7	0.85	9.0	4.9	No	5.9 ± 0.2	2.0 ± 0 (3)	1.2 ± 0.4 (1)
Pepperoni	E	0.9	0.76	19.0	4.9	No	5.9 ± 0.1	2.9 ± 0.1	1.7 ± 0.5
	F	1.7	0.88	11.6	4.9	No	6.3 ± 0.1	2.7 ± 0.1	0.9 ± 0 (3)
	G	1.5	0.86	13.1	4.6	No	6.5[c]	2.0 ± 0.1 (2)	0.9 ± 0 (3)
Salami, dried	J	1.5	0.88	9.3	4.9	No	6.2 ± 0.3	5.8 ± 0	3.2 ± 0.6 (2)
	E	1.1	0.79	16.3	5.1	No	6.4 ± 0.1	3.6 ± 0.1	2.5 ± 0.1
	E	1.0	0.76	17.1	4.8	No	5.7 ± 0.2	LE	0.9 ± 0 (3)
	J	1.7	0.87	9.3	4.9	No	6.2 ± 0.1	LE	2.0 ± 0.1
	E	2.5	0.92	6.5	4.9	No	6.1 ± 0.1	LE	2.2 ± 1.1
	Q	1.6	0.87	8.0	4.8	No	6.1 ± 0.1	LE	3.2 ± 0.3
Summer sausage	D	3.2	0.93	5.8	4.8	No	6.0 ± 0.1	4.9 ± 0.2	0.9 ± 0 (3)
	H	3.3	0.95	4.3	4.5	No	6.3 ± 0.1	3.9 ± 0.4	0.9 ± 0 (3)
	L	3.0	0.93	5.7	4.7	No	6.2 ± 0.2	2.4 ± 0.5	0.9 ± 0 (3)
	C	3.1	0.96	4.7	4.4	No	4.4 ± 0.5	3.0 ± 0 (1)	0.9 ± 0.1 (2)
	P	2.7	0.95	6.5	4.5	No	5.6 ± 0.1	3.0 ± 0 (3)	0.9 ± 0 (3)
	H	2.9	0.96	4.5	4.5	No	5.7 ± 0.1	3.0 ± 0.1 (2)	0.9 ± 0 (3)
	R	3.2	0.94	5.9	4.5	No	5.0 ± 0.3	3.0 ± 0 (3)	0.9 ± 0 (3)
	E	2.4	0.94	6.5	4.9	No	5.8 ± 0.1	5.1 ± 0.3	1.4 ± 0.9 (1)
	D	3.0	0.95	5.2	4.9	No	5.6 ± 0.1	4.7 ± 0.5	3.1 ± 0.8
	D	3.3	0.93	5.9	4.9	No	5.8 ± 0.2	5.3 ± 0.1	4.7 ± 1.0
	K	3.2	0.94	5.1	4.8	No	6.0 ± 0.1	5.3 ± 0.1	4.5 ± 0.1
	K	2.9	0.95	5.0	4.8	No	5.5 ± 0.3	5.1 ± 0.3	4.3 ± 0.5
Bologna	H	5.3	0.97	2.9	6.3	Yes	6.4 ± 0	6.5 ± 0	8.5 ± 0.1
Salami, cooked	I	4.0	0.96	3.7	6.2	Yes	6.1 ± 0.1	6.5 ± 0	7.5 ± 0
	M	4.7	0.96	3.7	6.6	Yes	6.3 ± 0.1	LE	8.9[c]
	N	4.4	0.95	3.9	6.4	Yes	6.2 ± 0	LE	8.3 ± 0.1
	O	5.4	0.97	2.0	6.6	Yes	6.3 ± 0.1	LE	8.5[c]
	O	4.2	0.95	3.8	6.5	Yes	6.3 ± 0.1	LE	8.1[c]
	P	3.8	0.95	5.4	6.2	Yes	6.1 ± 0.1	LE	8.7 ± 0.1

[a] Compositional values are for a single representative grab sample. MPR, moisture:protein ratio; a_w, water activity; %WPS, % water-phase salt (brine concentration).

[b] Values are the mean ± standard deviation of three samples unless otherwise indicated. When no colonies were observed, the value was based on assumption of 0.9 CFU on the least dilute plate. The number in parentheses is the number of samples that resulted in no colonies on the least dilute plate. Values of 0.9, 2.0, or 3.0 CFU were assigned when this plate was the 10^{-1}, 10^{-2}, or 10^{-3} dilution, respectively. LE, laboratory error (no data).

[c] Value is the mean of two samples.

reliably entered into these equations are 4.4 to 6.6 (the range of pH values observed in this study). The a_w values must be between 0.68 and 0.98 to be reliable, and the MPR values must be between 0.4 to 5.40 to be reliable. However, these values are found in a vast majority of RTE meat products, and it is highly unlikely that processors would have salable RTE meat products with values below the minimum value in any of these ranges. An RTE meat product with pH and/or a_w, or MPR above the maximum value in any of these ranges would clearly be recognizable as not shelf stable.

These three equations can be expressed in a single form where estimated probability of growth is equal to the odds ratio (o) divided by ($1 + o$). The generalized equation can be expressed as

$$\text{estimated probability of growth} = o/(1 + o) \qquad (5)$$

where o is estimated to be $\exp(\cdots)$ using pH and a_w, %WPS, or MPR values.

When actual product composition values were entered into the bias reduction logistic equations 2 through 4, equations containing pH plus a_w or pH plus %WPS gave reliable growth–no growth predictions (compared with *S. aureus* log counts observed for 28 days; Table 5). A probability value closer to 1 would indicate a higher likelihood of not being shelf stable. The predictive equation utilizing pH and

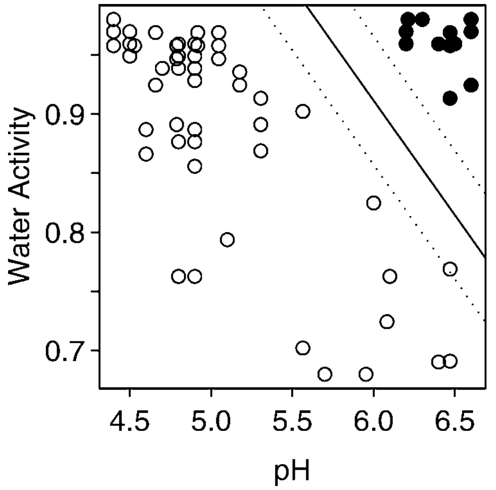

FIGURE 1. *Growth (closed circles) or no growth (open circles) of* S. aureus *on vacuum-packaged RTE meat products at 21°C described as a function of pH and* a_w *for each product. Each symbol represents one product tested either in the present study or in a previous study (5). The 0.50:0.50 growth:no-growth probability estimated boundary line is indicated by the solid line. The dotted lines depict the 0.80:0.20 and 0.20:0.80 growth:no-growth probability estimated boundary lines.*

a_w yielded a growth probability of 0.00 to 0.22 when no growth was observed and a probability of 0.83 to 1.00 when growth was observed. The predictive equation utilizing pH and %WPS yielded a growth probability of 0.00 to 0.25 when no growth was observed and a probability of 0.74 to 1.00 when growth was observed. The predictive equation utilizing pH and MPR yielded probability ranges that overlapped for products supporting and not supporting *S. aureus* growth. When no growth was observed, the growth probability was 0.00 to 0.60, and when growth was observed, the growth probability was 0.35 to 1.00. Therefore, the equation containing pH and MPR was not reliable because of this overlap. However, processors or researchers can reliably estimate the probability of shelf stability when pH and either a_w or %WPS is known. Because it is far simpler for a processor to measure a_w than to measure %WPS, we recommend the use of the equation containing pH and a_w for routine evaluation of product shelf stability.

To determine whether meat type adversely biased predictive results, meat type was plotted versus growth (plot not shown). The results suggested that a bias may exist; no summer sausage, beef stick, or pepperoni samples supported growth of *S. aureus.* However, all summer sausage and pepperoni samples had pH values <5.0 (Tables 2 and 4). This pH level is commonly regarded in the meat industry as indicating shelf stability, regardless of other processing factors. For example, USDA training guidance states that dry shelf-stable meat sticks must have an a_w of ≤0.90 and a pH of ≤5.0, and shelf-stable fermented sausages must have a pH of ≤5.2 and an a_w of <0.95, or only a pH of <5.0, or only an a_w of <0.91 *(14, 16).* For the beef stick samples, although pH levels were >5.0 for three of eight samples, the a_w and %WPS of the product apparently were extreme enough to prevent growth. Bologna is widely recognized as a non–shelf-stable product because of its high MPR, a_w, and pH values compared with those of other RTE meats; therefore, it was not surprising that *S. aureus* growth was both predicted and observed for this type of product. For salami and jerky samples, results for both observed growth and no growth were obtained. For jerky samples, a_w values of the products not supporting growth were all well below the minimum a_w (0.88) needed for anaerobic growth of *S. aureus* (range, 0.68 to 0.82). For the jerky samples where the *S. aureus* growth was predicted and observed, a_w levels ranged from 0.92 to 0.96. Growth was observed in 8 of 16 salami samples. Therefore, we concluded that product type was not a biasing factor for determining shelf stability but rather that shelf stability is determined by product compositional characteristics regardless of product type.

Most of the commercial products studied met applicable USDA compositional standards for product identity. However, whether this result reflects actual marketplace trends is unclear because the actual number of nonstandard products in the marketplace is not known. Of the commercially available jerky products tested (Table 3), all four met the USDA MPR standard of ≤0.75. Each of the jerky products was shelf stable; *S. aureus* decreased by 0.5 to 2.5 log CFU/cm^2 after 1 week and by 1.6 to 4.2 log CFU/cm^2 after 4 weeks. Both dry salami samples tested (processors Z and AA; Table 3) met the USDA standard of an MPR of ≤1.9 and did not support *S. aureus* growth. *S. aureus* on dry salami decreased by 0.8 to 2.9 log CFU/cm^2 after 1 week and by 1.7 to 5.7 log CFU/cm^2 after 4 weeks. The predictive equation utilizing pH and a_w for commercial jerky indicated that *S. aureus* very likely would not grow (probability of growth = 0.00 to 0.10). The predictive equations utilizing pH and a_w for dry salami also indicated that *S. aureus* very likely would not grow (probability of growth = 0.00 to 0.01).

In contrast to the compositional characteristics of beef jerky and dry salami, all commercial beef stick samples (Table 3) were at or above the guideline for an a_w of ≤0.9 and, in some cases, a pH of ≤5.0 *(14, 16),* and no sample satisfied both criteria simultaneously. Nevertheless, *S. aureus* decreased by 0.9 to 6.3 log CFU/cm^2 after 1 week and by 5.9 to 6.5 log CFU/cm^2 after 4 weeks for all samples. At 1 week, O samples had higher levels of *S. aureus* than did I samples for four of five products. However, there was no difference between these two sample types at 4 weeks. The predictive equation utilizing pH and a_w for commercial beef sticks indicated that *S. aureus* very likely would not grow (probability of growth = 0.00 to 0.06).

Commercially available summer sausage samples (Table 2), including samples for which refrigeration was recommended by the manufacturer, all met the USDA guideline *(14, 16)* for nonrefrigerated semidry sausage product with a pH of ≤5.0. *S. aureus* decreased by 0.2 to 5.9 log CFU/cm^2 after 1 week and by 0.8 to 6.4 log CFU/cm^2 after 4 weeks for all samples. Of the four intentionally misprocessed jerky products (Table 3), three had MPR values that exceeded the USDA standard of ≤0.75. For the jerky products that failed to meet the minimum MPR, changes in *S.*

TABLE 5. *Probability of* S. aureus *growth based on compositional characteristics for RTE meats stored under vacuum at 21°C*[a]

	Composition				Growth probability predicted using pH and:			
Processor	pH	a_w	%WPS	MPR	a_w	%WPS	MPR	Growth
Q	4.9	0.96	5.94	2.70	0.03	0.02	0.03	No
Q	4.9	0.96	5.61	2.99	0.03	0.02	0.04	No
R	4.8	0.97	5.35	2.74	0.02	0.01	0.02	No
S	4.8	0.96	5.84	3.12	0.02	0.01	0.03	No
T	4.7	0.95	5.08	2.46	0.01	0.01	0.01	No
U	4.9	0.95	5.49	3.08	0.02	0.02	0.04	No
V	4.9	0.97	5.66	2.79	0.04	0.02	0.03	No
V	4.5	0.96	4.86	2.81	0.00	0.00	0.00	No
W	4.8	0.96	4.22	2.79	0.02	0.03	0.02	No
W	4.6	0.97	3.30	2.49	0.01	0.02	0.00	No
W	4.8	0.97	4.07	2.84	0.02	0.03	0.02	No
AE	4.9	0.96	3.73	2.96	0.03	0.06	0.04	No
AE	4.4	0.98	1.47	2.75	0.00	0.02	0.00	No
AE	4.7	0.96	3.90	2.85	0.01	0.02	0.01	No
AE	4.5	0.96	3.98	2.63	0.00	0.01	0.00	No
AE	4.4	0.96	3.95	2.50	0.00	0.00	0.00	No
X	6	0.97	3.95	4.21	0.95	0.95	0.97	Yes
Y	5.8	0.98	3.02	3.93	0.90	0.92	0.90	Yes
Z	4.7	0.9	8.14	1.62	0.00	0.00	0.00	No
AA	5.1	0.88	10.03	1.41	0.01	0.00	0.01	No
T	5.3	0.91	8.87	1.46	0.06	0.02	0.03	No
Q	5	0.93	5.58	2.31	0.02	0.03	0.02	No
Q	5.1	0.9	9.74	1.32	0.02	0.00	0.01	No
Q	5.1	0.92	7.98	1.85	0.03	0.01	0.02	No
T	5	0.94	5.74	1.86	0.03	0.03	0.01	No
AE	4.6	0.93	3.21	1.86	0.00	0.02	0.00	No
AB	5.6	0.71	16.87	0.46	0.00	0.00	0.03	No
AC	6	0.79	15.08	0.67	0.10	0.02	0.21	No
AD	5.3	0.73	14.29	0.69	0.00	0.00	0.01	No
AC	5.7	0.75	17.45	0.61	0.01	0.00	0.06	No
AE	6	0.72	17.94	0.39	0.02	0.00	0.15	No
AE	6.1	0.93	5.06	1.03	0.92	0.94	0.41	Yes
AE	6	0.96	3.78	1.35	0.94	0.96	0.40	Yes
AE	6	0.92	7.08	1.20	0.83	0.74	0.35	Yes
A	5.7	0.68	18.70	0.40	0.00	0.00	0.04	No
A	6.0	0.82	10.60	0.70	0.22	0.25	0.22	No
B	6.4	0.69	15.40	0.60	0.06	0.14	0.60	No
B	6.1	0.76	14.20	0.80	0.08	0.06	0.33	No
C	4.6	0.88	7.10	2.00	0.00	0.00	0.00	No
D	4.9	0.85	9.00	1.70	0.00	0.00	0.01	No
E	4.9	0.76	19.00	0.90	0.00	0.00	0.00	No
F	4.9	0.88	11.60	1.70	0.00	0.00	0.01	No
G	4.6	0.86	13.10	1.50	0.00	0.00	0.00	No
J	4.9	0.88	9.30	1.50	0.00	0.00	0.01	No
E	5.1	0.79	16.30	1.10	0.00	0.00	0.01	No
E	4.8	0.76	17.10	1.00	0.00	0.00	0.00	No
J	4.9	0.87	9.30	1.70	0.00	0.00	0.01	No
E	4.9	0.92	6.50	2.50	0.01	0.01	0.02	No
Q	4.8	0.87	8.00	1.60	0.00	0.00	0.00	No
D	4.8	0.93	5.80	3.20	0.01	0.01	0.03	No
H	4.5	0.95	4.30	3.30	0.00	0.00	0.01	No
L	4.7	0.93	5.70	3.00	0.00	0.01	0.02	No
C	4.4	0.96	4.70	3.10	0.00	0.00	0.00	No
P	4.5	0.95	6.50	2.70	0.00	0.00	0.00	No
H	4.5	0.96	4.50	2.90	0.00	0.00	0.01	No
R	4.5	0.94	5.90	3.20	0.00	0.00	0.01	No
E	4.9	0.94	6.50	2.40	0.02	0.01	0.02	No
D	4.9	0.95	5.20	3.00	0.02	0.02	0.04	No
D	4.9	0.93	5.90	3.30	0.01	0.02	0.06	No

TABLE 5. *Continued*

Processor	Composition				Growth probability predicted using pH and:			Growth
	pH	a_w	%WPS	MPR	a_w	%WPS	MPR	
K	4.8	0.94	5.10	3.20	0.01	0.01	0.03	No
K	4.8	0.95	5.00	2.90	0.01	0.02	0.02	No
H	6.3	0.97	2.90	5.30	0.99	0.99	1.00	Yes
I	6.2	0.96	3.70	4.00	0.98	0.99	0.98	Yes
M	6.6	0.96	3.70	4.70	1.00	1.00	1.00	Yes
N	6.4	0.95	3.90	4.40	0.99	0.99	1.00	Yes
O	6.6	0.97	2.00	5.40	1.00	1.00	1.00	Yes
O	6.5	0.95	3.80	4.20	1.00	1.00	1.00	Yes
P	6.2	0.95	5.40	3.80	0.97	0.96	0.98	Yes

[a] Probability was calculated based on product pH plus water activity (a_w), % water-phase salt (WPS), or moisture:protein ratio (MPR).

aureus ranged from −0.4 to +2.6 log CFU/cm^2 after 1 week and from +0.6 to +2.7 log CFU/cm^2 after 4 weeks. For these three misprocessed jerky products, the equation utilizing pH and a_w predicted growth of *S. aureus* (probability of growth = 0.83 to 0.94). For the fourth misprocessed jerky sample, this equation predicted no *S. aureus* growth (probability of growth = 0.02). All of the intentionally misprocessed sausage products (Table 2) met the USDA guideline for nonrefrigerated semidry sausage product with a pH of ≤5.0. *S. aureus* decreased by 0.4 to 4.6 log CFU/cm^2 after 1 week and by 3.3 to 6.1 log CFU/cm^2 after 4 weeks (Table 2) for all samples. The predictive equation utilizing pH and a_w for summer sausage indicated that *S. aureus* very likely would not grow on these sausages (probability of growth = 0.00 to 0.04).

In a previous study, Ingham et al. *(5)* examined the shelf stability of several commercially produced RTE meat products. Compositional characteristics for the products in this study are shown in Table 4 with *S. aureus* levels. In that study, none of the products supported a −1.1- to +1.3-log change after 4 weeks. In the present study, four products had a change in *S. aureus* between −1.1 and 1.3 log CFU/cm^2 after 28 days of storage, so the present study helped to fill this data gap. By combining the two data sets, which were produced utilizing identical experimental procedures, a larger body of data was available for analysis, resulting in a more robust predictive tool.

For all products except jerky, multiple product surfaces were sampled to determine whether significant differences in shelf stability were dependent upon the product surface studied. For all summer sausage samples, no significant differences were noted ($P < 0.05$). After 4 weeks, the average decreases in O, S, and C samples were 5.6, 5.4, and 5.2 log CFU/cm^2, respectively. The beef stick samples yielded similar results; for 4-week samples, the average decreases for O and I samples were 5.9 and 5.5 log CFU/cm^2, respectively. For the two commercially produced salami samples in which *S. aureus* grew, the location of sampling did matter. *S. aureus* levels increased on the S sample for both products after 4 weeks. For sample manufacturer X, the S sample was the only cut type that supported growth of *S. aureus.* One could argue that because in two of the three sample types (S, O, and C) net *S. aureus* decreased, the product could be considered shelf stable. This conclusion would be highly erroneous for a number of reasons. First, average net growth of *S. aureus* was observed for all three samples and duplicates. Second, if *S. aureus* was capable of any growth on an RTE meat product at any location on that product, the product could not be considered shelf stable. Because the S sample was least restrictive (i.e., most likely to produce growth of *S. aureus* when the product is not shelf stable), then this type of sample should be utilized. The S cut of summer sausage and salami is common to all RTE meat products, the same cut as the I cut of beef sticks and the sampling method for jerky. Thus for reasons of food safety and consistent sampling, we conclude that the slicing method should be utilized to obtain samples when constructing predictive models for RTE meats.

This research revealed that the compositional characteristics of meat sticks did not meet the limits set forth in the USDA training guidance but did not support *S. aureus* growth. Even though the meat stick samples all failed to meet the minimum guidelines of an a_w of ≤0.9 and a pH of ≤5.0, a decrease in *S. aureus* populations was noted in all the products during the 4-week sample period. This finding suggests that the USDA guidance is too conservative. Ingham et al. *(5)* found similar trends for some products.

Processors are required to validate shelf stability by the USDA, even when labeling identity standards are met, and can utilize the predictive equation based on pH and a_w to assure shelf stability. The shelf stability probability equation accounts for synergism between pH and a_w in predicting shelf stability. This synergism allows processors a much greater degree of flexibility in their processing techniques. The predictive equation could be utilized to replace expensive and time-consuming challenge studies on product shelf stability and allows for processing variations that could be utilized for determination of least-cost formulation calculations. For small and very small processors who already operate on tight budgets, this flexibility represents a potentially huge economic advantage while still ensuring food safety. The final decision concerning whether a product should be utilized, reworked, or destroyed is left to the processor based on these results.

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