Journal of Food Protection, Vol. 79, No. 2, 2016, Pages 184–193
doi:10.4315/0362-028X.JFP-15-322

Modeling the Impact of Ingoing Sodium Nitrite, Sodium Ascorbate, and Residual Nitrite Concentrations on Growth Parameters of *Listeria monocytogenes* in Cooked, Cured Pork Sausage

AMANDA M. KING,[1] KATHLEEN A. GLASS,[2] ANDREW L. MILKOWSKI,[1] DENNIS L. SEMAN,[3] AND JEFFREY J. SINDELAR[1]*

[1]*Department of Animal Sciences, University of Wisconsin–Madison, 1805 Linden Drive, Madison, Wisconsin 53706;* [2]*Food Research Institute, University of Wisconsin–Madison, 1550 Linden Drive, Madison, Wisconsin 53706;* [3]*Kraft Heinz Company/Oscar Mayer, 910 Mayer Avenue, Madison, Wisconsin 53704, USA*

MS 15-322: Received 25 July 2015/Accepted 30 October 2015

ABSTRACT

Sodium nitrite has been identified as a key antimicrobial ingredient to control pathogens in ready-to-eat (RTE) meat and poultry products, including *Listeria monocytogenes*. This study was designed to more clearly elucidate the relationship between chemical factors (ingoing nitrite, ascorbate, and residual nitrite) and *L. monocytogenes* growth in RTE meats. Treatments of cooked, cured pork sausage (65% moisture, 1.8% salt, pH 6.6, and water activity 0.98) were based on response surface methodology with ingoing nitrite and ascorbate concentrations as the two main factors. Concentrations of nitrite and ascorbate, including star points, ranged from 0 to 352 and 0 to 643 ppm, respectively. At one of two time points after manufacturing (days 0 and 28), half of each treatment was surface inoculated to target 3 log CFU/g of a five-strain *L. monocytogenes* cocktail, vacuum packaged, and stored at 7°C for up to 4 weeks. Growth of *L. monocytogenes* was measured twice per week, and enumerations were used to estimate lag time and growth rates for each treatment. Residual nitrite concentrations were measured on days 0, 4, 7, 14, 21, and 28, and nitrite depletion rate was estimated by using first-order kinetics. The response surface methodology was used to model *L. monocytogenes* lag time and growth rate based on ingoing nitrite, ascorbate, and the residual nitrite remaining at the point of inoculation. Modeling results showed that lag time was impacted by residual nitrite concentration remaining at inoculation, as well as the squared term of ingoing nitrite, whereas growth rate was affected by ingoing nitrite concentration but not by the remaining residual nitrite at the point of inoculation. Residual nitrite depletion rate was dependent upon ingoing nitrite concentration and was only slightly affected by ascorbate concentration. This study confirmed that ingoing nitrite concentration influences *L. monocytogenes* growth in RTE products, yet residual nitrite concentration contributes to the antimicrobial impact of nitrite as well.

Key words: Ascorbate; *Listeria*; Nitrite; Residual nitrite

Listeria monocytogenes is a significant concern to processors of ready-to-eat (RTE) meat and poultry products. As a ubiquitous organism with the capacity to grow at refrigeration temperatures, *L. monocytogenes* can be present in a post-thermal-processing environment, and if the product becomes contaminated, subsequent growth can occur in RTE meats during refrigerated storage *(20)*. Although listeriosis causes a relatively small proportion of all foodborne illness reported each year, an estimated 1,500 cases, 94% of those cases result in hospitalizations, and 15% result in death *(32)*. A subset of the population is particularly susceptible to infection with this organism, and one of the most at-risk demographics are pregnant women, in whom listeriosis can result in spontaneous abortion *(31)*. RTE meats that support the growth of *L. monocytogenes* are considered high-risk foods for attribution of listeriosis cases *(31, 32)*. Along with environmental and sanitation controls to prevent postprocessing contamination, a number of inhibitors, such as organic acids, have been identified for use in formulations and other postprocessing intervention technologies, such as high pressure processing, have been developed to enhance the safety of these products *(37)*. However, no universal "silver bullet" answer has been discovered for the guaranteed control of *L. monocytogenes* in RTE meats, highlighting the importance of a thorough understanding of the available hurdles to increase overall food safety.

One such formulation-based hurdle is sodium nitrite, an ingredient that has long been used as a preservative and curing agent in formulations that is also responsible for imparting the unique colors and flavors associated with cured meats *(27)*. In addition to significant quality-related contributions, nitrite has also been noted to contribute to the control of pathogens, most notably *Clostridium botulinum (43)*. Owing to the severity of *C. botulinum* toxin production

* Author for correspondence. Tel: 608-262-0555; Fax 608-265-3110; E-mail: jsindelar@wisc.edu.

TABLE 1. *Factors and levels for central composite design*

Factor	Low −1	Middle 0	High 1
Sodium nitrite (ppm)	50	175	300
Sodium ascorbate (ppm)	100	325	550

and the impact of nitrite on mitigating this risk in RTE products, a significant research effort has existed in this subject area for decades. Therefore, the majority of what is currently understood about the antimicrobial impact of nitrite relates specifically to *C. botulinum*. Hustad et al. *(22)* demonstrated the significant effects of ingoing nitrite concentration on botulinal toxin production and reported that frankfurters formulated with 50 ppm of nitrite delayed toxin formation until 56 days of storage at 27°C, as compared with toxin detection in uncured samples at 14 days.

The antimicrobial activity of nitrite is dose dependent and is based on the ingoing nitrite concentrations, but other components of cured meat systems, ascorbate and residual nitrite, in particular, have also been suggested to impact pathogen growth. When added to a meat system, nitrite is extremely reactive and can proceed through one of many chemical pathways with the constituents of meat products *(10)*. As such, the concentration of added nitrite that remains as free nitrite decreases drastically upon addition to a meat mixture and continues to react and deplete throughout processing and storage time *(11, 12)*. Sodium ascorbate, commonly referred to as a cure accelerator, increases the rate of some of these chemical reactions to expedite the development of cured color during processing. As a result of its function to reduce nitrite to nitric oxide, the use of ascorbate also results in a more rapid depletion of residual nitrite during the curing process *(23)*. Although residual nitrite concentrations deplete during processing and storage, regardless of the presence of ascorbate, the loss of added nitrite can be more pronounced when ascorbate is included *(6, 28)*.

The bacteriostatic impact of nitrite on *L. monocytogenes* is less pronounced than its inhibitory properties affecting growth and toxin production by *C. botulinum,* which has been reported to occur at relatively low concentrations (50 to 100 ppm) *(21, 22)*. As a factor of this less prominent effect, some studies have concluded that concentrations of nitrite representative of those used in cured meats were insufficient to effectively control *L. monocytogenes* growth *(17, 29, 33)*. Many studies, however, have evaluated multiple product parameters in combination, including nitrite concentration, salt level, pH, and temperature, and found that nitrite generally enhances bacteriostatic activity in RTE meats *(8, 9, 16, 24)*. Though the relationship between nitrite and *L. monocytogenes* remains ill defined, nitrite does play a contributing role in *L. monocytogenes* control, particularly in combination with additional hurdles. However, with a more detailed understanding of the direct antimicrobial effects of nitrite, its contribution to the safety of RTE meats can be maximized, both alone and in combination with some of the aforementioned hurdles.

The high reactivity of nitrite in a meat system makes it a particularly challenging matrix to study in detail. Beyond the documented effect of ingoing nitrite, ascorbate and residual nitrite have both been suggested to have food safety roles. The antimicrobial impact provided by these two factors, either individually, collectively, or synergistically, are less clearly understood than ingoing nitrite concentration and the related pathogen control response. When used with nitrite, ascorbate has been suggested to enhance the control of both *C. botulinum* and *L. monocytogenes (16, 41, 42)*. Tompkin et al. *(41)* demonstrated an additional layer of prevention against botulinal toxin formation when ascorbate was added to cured meats and proposed that the effect was likely due to the ability of ascorbate to chelate some essential cation required for recovery from nitrite-induced injury to *C. botulinum*. Duffy et al. *(16)* demonstrated that 420 ppm of ascorbate used with up to 315 ppm of nitrite significantly improved nitrite's ability to influence *L. monocytogenes* lag time, reporting a difference of 25.4 versus 9.9 days of lag time in cured treatments with and without ascorbate, respectively.

Investigations of the antibotulinal impact of residual nitrite were reported by Tompkin et al. *(40, 44, 45)* and Christiansen et al. *(13),* concluding that in addition to ingoing nitrite concentration, the level of residual nitrite at the point of temperature abuse was important for controlling growth and toxin production and determining the safety of cured products. Because the time of contamination and critical growth period is uniquely different for *L. monocytogenes* than for *C. botulinum,* the question becomes whether the concentration of remaining residual nitrite at the point of contamination impacts the growth of this organism as well. This study was designed to investigate ingoing and residual nitrite concentrations and to model their impact on the growth of *L. monocytogenes* and provide insight to the mechanism by which nitrite contributes to the safety of RTE meats.

MATERIALS AND METHODS

Experimental design. A central composite response surface methodology (RSM) design was used for this two-factor experiment. The two factors, sodium nitrite and sodium ascorbate, and the factor levels used in the study are reported in Table 1. The regulations of the U.S. Department of Agriculture, Food Safety and Inspection Service limit maximum ingoing sodium nitrite concentrations to 156 ppm for comminuted products and 200 ppm for immersion, massaged, or pumped products, on a meat weight basis, and sodium ascorbate can be used in cured products at up to 547 ppm *(46)*. However, to provide insight into mechanistic effects of nitrite and ascorbate on *L. monocytogenes,* ingoing nitrite concentrations were expanded beyond maximum regulatory limits for this study. Although not a specific research objective of this study, the lower levels of the two factors would be considered representative of ingoing concentrations of the natural sources for both ingredients (natural nitrite from celery powder and natural ascorbate from cherry powder) utilized to produce alternatively cured meats in which would be substituted for synthetic sodium nitrite and sodium ascorbate *(34)*. Because ascorbate accelerates nitrite-related reactions and the depletion of residual nitrite, the combination of varying levels of ascorbate with nitrite was

TABLE 2. *Design matrix of the central composite design,* L. monocytogenes *growth parameters, and residual nitrite in cooked, cured pork sausage at 7°C*

						L. monocytogenes growth parameters at initial inoculation[e]			*L. monocytogenes* growth parameters at delayed inoculation[e]		
Trt[a]	Sodium nitrite[b]	Sodium ascorbate[b]	Sodium nitrite (ppm)[c]	Sodium ascorbate (ppm)[c]	Nitrite depletion rate (ppm/day)[d]	Growth rate (log/day)[f]	Lag time (day)[f]	Residual nitrite (ppm)[g]	Growth rate (log/day)[f]	Lag time (day)[f]	Residual nitrite (ppm)[g]
1a	−1	−1	50	100	0.1113	0.574	2.2	34	0.712	3.4	24
2a	−1	1	50	550	0.3891	0.445	3.7	35	0.565	4.7	14
3a	1	−1	300	100	0.0356	0.308	10.1	267	0.368	8.5	137
4a	1	1	300	550	0.0498	0.265	11.5	273	0.461	7.7	109
5a	−1.414	0	0	325	ND	0.762	5.4	0	0.555	3.4	0
6a	1.414	0	352	325	0.0403	0.229	6.6	318	0.399	7.2	157
7a	0	−1.414	175	0	0.0284	0.317	5.5	120	0.817	8.2	112
8a	0	1.414	175	643	0.0572	0.379	9.3	123	0.438	7.0	66
9a	0	0	175	325	0.0449	0.340	6.3	151	0.389	6.7	78
10a	0	0	175	325	0.0438	0.556	8.6	151	0.475	7.1	78
1b	−1	−1	50	100	0.1811	0.668	5.1	25	0.704	3.8	23
2b	−1	1	50	550	0.1891	0.913	6.7	23	0.575	3.6	16
3b	1	−1	300	100	0.0287	0.310	11.4	197	0.239	4.2	160
4b	1	1	300	550	0.0434	0.212	9.2	181	0.234	7.6	123
5b	−1.414	0	0	325	ND	0.580	4.9	0	0.453	3.3	0
6b	1.414	0	352	325	0.0336	0.311	7.6	195	0.194	5.3	187
7b	0	−1.414	175	0	0.0345	0.304	10.5	115	0.362	7.1	92
8b	0	1.414	175	643	0.0427	0.260	6.3	100	0.296	5.1	79
9b	0	0	175	325	0.0419	0.358	7.3	103	0.277	5.8	84
10b	0	0	175	325	0.041	0.359	ND	125	0.265	5.6	87

[a] Trt, treatment; a, replication 1; b, replication 2.
[b] Coded statistical design coefficients for root mean square analysis.
[c] Uncoded factor levels for ingoing sodium nitrite and sodium ascorbate. Ingredients were added as ppm (milligrams per kilogram) on a meat weight basis.
[d] Residual nitrite depletion rate calculated by nonlinear regression and first-order kinetic model. ND, no data.
[e] Initial inoculation, day 0; delayed inoculation, day 28.
[f] Growth rate (μ_{max}) and lag time were calculated by DMFit.
[g] Residual nitrite measured on day 0 and day 28.

expected to yield varied residual nitrite concentrations after processing at the point of inoculation.

The RSM design consisted of four factorial treatments, four star points, and two center points, for a total of 10 treatments (Table 2). The radius for star points was calculated as $2^{n/4}$, where n is the number of factors in the model, resulting in a radius of 1.414. These treatments and the factor levels were determined by using Design of Experiments in JMP (JMP Pro 11, SAS Institute Inc., Cary, NC) and are shown in Table 2. The entire design was repeated a second time, resulting in four total replications of the center point and two replications of each treatment (combination of ingoing nitrite and ascorbate concentration, coded "a" and "b"). Because residual nitrite depletes over storage time, the initial inoculation point was expected to have greater concentrations of residual nitrite than the delayed inoculation point for each of the formulations. Residual nitrite, measured at each inoculation point (day 0 and day 28), was included as a factor in addition to the two-factor RSM design. Therefore, the model was designed to provide knowledge about the mechanistic relationship between nitrite and *L. monocytogenes,* while remaining within an accurate range of ingredient usage in today's processed meat products.

Sample preparation. Fresh pork Boston butts were obtained from a commercial supplier and stored vacuum packaged at 2.2 to 4°C until use (within 4 days). The Boston butts were deboned and trimmed to approximately 80% lean (20% fat) and then ground through a 19.05-mm plate and again through a 9.53-mm plate by using a meat grinder (model 4732, Hobart Corp., Troy, OH). The ground pork (13.62 kg of meat block per treatment) was weighed and transferred to a mixer (model 100DA, Leland Detroit Manufacturing Company, Detroit, MI) and mixed with 2% salt for 2 min. A brine solution was made by first dissolving 0.3% sodium tripolyphosphate in 5% water, formulated on a meat weight basis, followed by sodium nitrite (6.25% sodium nitrite, 93.75% sodium chloride; SureCure, Excalibur Seasoning Company, Perkin, IL) and sodium ascorbate according to treatment (defined in Table 2). After salt was incorporated and combined for 2 min, the brine was added to the mixer and blended an additional 3 min.

Each treatment was transferred to a rotary vane vacuum stuffer (VF 608 Plus vacuum filler, Handtmann CNC Technologies Inc., Buffalo Grove, IL) and stuffed into moisture-impermeable plastic casings (diameter: 9.5 cm) to minimize variations in yield and finished product composition. Within 2 h after mixing, treatments were thermally processed by using a step-up steam cooking (100% humidity) schedule in a single truck smokehouse (model 450 MiniSmoker, Alkar Engineering Corp., Lodi, WI) to an internal temperature of 71.1°C. Thermal processing steps included 54.4°C for 30 min, 62.8°C for 30 min, 71.1°C for 30 min, and 79.4°F until the product reached an internal temperature of 71.1°C. After the completion of thermal processing, treatments were transferred to a cooler (2°C) and cooled overnight (16 to 20 h) to an internal temperature <4°C. Thermal processing and cooling

temperature data were recorded by using a temperature-recording logging device (TempTale4, Sensitech, Beverly, MA) to ensure target heating and cooling parameters were met.

Owing to thermal processing space limitations, treatments were randomly assigned to one of two blocks for thermal processing (five treatments per block). Within each block, the treatments were randomized for the order in which they were manufactured. Thermal processing began within 2 h of the beginning of mixing of the first treatment of each block. The stuffed treatments were not held for an appreciable period of time before thermal processing, as that would negate some of the effect of ascorbate on the speed of nitrite reactions occurring in the treatments, which was a primary factor of exploration in the experimental design. After cooling to an internal temperature of <4°C, approximately half of each treatment was aseptically removed from the casing, sliced by using a commercial tabletop slicer (model 919E, Berkel Incorporated, Troy, OH) sanitized with 70% ethanol, to yield approximately 17 g per slice, vacuum packaged in bulk, and stored at 3 to 4°C until transportation to the University of Wisconsin–Madison Food Research Institute for subsequent inoculation (initial inoculation point = time zero). The time zero inoculation time point was 2 days postprocessing. The remainder of each treatment was stored as intact, unsliced chubs in moisture-impermeable plastic casings, under the same conditions (7°C) as inoculated time zero samples. The remaining half of each treatment was sliced after 27 days of storage for inoculation after 28 days of storage at 7°C (delayed inoculation point; 30 days postprocessing). The use of the delayed inoculation allowed for residual nitrite levels to decline over time as a normal occurrence during shelf life of RTE meats, thus providing a different environment with respect to the residual nitrite concentration for the microorganisms between the initial and delayed inoculation points.

Sample proximate analysis. Triplicate uninoculated samples for each treatment were analyzed for moisture (5 h, 100°C vacuum oven method *(3)*), pH (measured in a slurry prepared by homogenizing 10 g with 90 ml of deionized water), NaCl (measured as percentage of Cl^-, $AgNO_3$ potentiometric titration; Mettler DL22 food and beverage analyzer, Columbus, OH), and water activity (a_w; Decagon AquaLab 4TE Water Activity Meter, Pullman, WA) *(3, 38)*.

Objective color analysis. Duplicate uninoculated samples were vacuum packaged and designated for objective color analysis during storage at 7°C, under the same conditions as inoculated samples. At predetermined time intervals (days 0, 14, 28, 42, and 56 for initial inoculation samples and days 0, 14, and 28 for delayed inoculation samples), instrumental color was measured by using a chroma meter (model CR-310, Minolta Camera Co., Ltd., Osaka, Japan; 50-mm aperture, illuminant D_{65}, 0° viewing angle) and a scanning reflectance spectrophotometer (model UV-2401 PC, Shimadzu Corp., Kyoto, Japan), with an attached multipurpose large sample compartment (model MPC-2200, Shimadzu Corp., Columbia, MD). The scanning reflectance spectrophotometer was configured for a sampling interval of 1.0 nm, slit width of 5.0 nm, and fast scan speed at a wavelength range of 500 to 700 nm. Both instruments were standardized by placing the same packaging material as used on the samples over a white standardization tile. Values for the standardization tile were Commission Internationale de l'Eclairage (CIE) L* = 97.06, a* = –0.14, and b* = 1.93. Color was measured by using the CIE L* (lightness), a* (redness), and b* (yellowness) system *(2)*. At each time point and for both color measurement methods, measurements were taken at two locations on each of the two packaged samples for each treatment, and the samples were placed on the white calibration plate during measurement with the chroma meter. Using the spectrophotometer, cured color pigment was estimated using a ratio of the percentage of reflectance at 650 nm divided by the percentage of reflectance at 570 nm *(2)*.

Residual nitrite analysis. Residual nitrite was analyzed on duplicate uninoculated meat samples from each treatment at each designated time point (days 0, 4, 7, 11, 14, 21, and 28). A dedicated high-pressure liquid chromatography (HPLC) instrument was used to quantify residual nitrite, according to methods previously reported by Bryan and Grisham *(7)*. At each predetermined time point, two uninoculated samples per treatment were powdered in liquid nitrogen and then stored at –80°C until analysis. On each day of analysis, 5 g of powdered sample was homogenized with 45 ml of pH 7.4 100 μM phosphate buffer, which was then split into two slurries and centrifuged at 10,000 × *g* at 4°C for 5 min (Avanti J-E with JA-25.50 rotor, Beckman Coulter, Indianapolis, IN). After centrifugation, 400 μl of supernatant from each slurry and 400 μl of methanol were transferred to a 1.5-ml snap-cap centrifuge tube. This mixture was vortexed on high speed for 3 to 5 s and allowed to sit at 4°C for at least 10 min to allow methanol to break down any remaining protein in the sample. The samples were then centrifuged for 8 min at 13,000 × *g* at 4°C (Eppendorf model 5424 centrifuge, Brinkmann Instruments, Inc., Westburg, NY), and supernatant was transferred to a new 1.5-ml snap-cap tube.

This extraction process yielded four subsamples per treatment and time point, which were collected by using an ENO-20 NOx Analyzer (EiCom USA, San Diego, CA) and Data Processor (EICOM EPC-500) and analyzed in PowerChrom (version 2.3, eDaq Ltd., Denistone East, New South Wales, Australia). Analyzer settings were reactor pump 0.22 ml/min, carrier pump 0.66 ml/min, and injection 50 μl. NO_x standards were made from $NaNO_2$ powder diluted with MilliQ water into 0, 20, 40, 60, 80, and 100 μM of $NaNO_2$ solution. Standards and samples were analyzed following the same procedure to determine concentration of $NaNO_2$.

Inoculum preparation. *L. monocytogenes* strains LM 101 (hard salami isolate, serotype 4b), LM 108 (hard salami isolate, serotype 1/2b), LM 310 (goat's milk cheese isolate, serotype 4b), FSL-C1-109 (human isolate, serotype 4b), and V7 (raw milk isolate, serotype 1/2a) were grown individually in 10 ml of Trypticase soy broth (BBL, BD, Sparks, MD) at 37°C for 18 to 20 h. Cells were harvested by centrifugation (2,500 × *g* for 20 min) and suspended in 4.5 ml of 0.1% buffered peptone water (pH 7.2). Equivalent populations of each isolate were combined to provide a five-strain mixture of *L. monocytogenes* to yield a target level of 3 log CFU/g of meat (4 log CFU/50-g package). The purity of each strain was verified by streaking on modified Oxford agar (*Listeria* selective agar base, Difco, BD, Sparks, MD) and Trypticase soy agar (BBL, BD), and populations of each strain and the mixtures were verified by plating on modified Oxford agar.

Inoculation and storage. Each sample consisted of three slices, which were surface inoculated between slices with 250 μl of the *L. monocytogenes* mixture to yield a target level of 3 log CFU/g (equivalent to 3 log CFU/ml of rinse material when using 50-ml rinse for enumeration). Each package was vacuum packaged (Multivac AGW, Sepp Haggemuller KG, Wolfertschwenden, Germany) in gas-impermeable pouches (3-mil high-barrier pouches; oxygen transmission rate, 50 to 70 cm^3/m^2, 24 h at 25°C, and

TABLE 3. *Objective color (CIE L*a*b*) and cured color ratio values for vacuum-packaged, sliced, cooked pork sausage stored at 7°C*

	Objective color[a]			
	L*	a*	b*	Cured color ratio[b]
Cured[c]	58.51 ± 1.35	17.68 ± 0.50	5.41 ± 0.24	2.43 ± 0.20
Uncured	59.82 ± 1.31	10.33 ± 0.62	6.86 ± 0.39	1.58 ± 0.13

[a] CIE L*a*b*: L*, lightness; a*, redness; and b*, yellowness on a 0 to 100 scale.
[b] Cured meat color measurement by reflectance ratio of wavelengths 650 and 570 nm, where no cured color = 1.1; moderate fade = 1.6; less intense but noticeable cured color = 1.7 to 2.0; excellent cured color = 2.2 to 2.6.
[c] All treatments containing ingoing nitrite had similar ($P > 0.05$) color values. Means and standard deviations for all 18 runs with ingoing nitrite are combined as cured and 2 runs without ingoing nitrite were combined as uncured.

60% relative humidity; water transmission rate, 6 to 7.5 g/m^2, 24 h at 25°C, and 90% relative humidity; UltraSource, Kansas City, MO) and stored at 7°C for up to 8 weeks. This study consisted of two inoculation points. As previously stated, approximately half of each treatment was aseptically sliced for inoculation at time zero (initial inoculation), whereas the remaining half was inoculated after 28 days at 7°C (delayed inoculation).

Microbiological enumeration. At time zero and predetermined intervals (days 0, 4, 7, 11, 14, 18, 21, 25, and 28) throughout storage, triplicate inoculated samples were assayed for *L. monocytogenes* populations, and duplicate uninoculated samples were assayed for lactic acid bacteria and pH. Bacterial populations were enumerated in rinse material after adding 50 ml of Butterfield's phosphate buffer to each package and massaging externally by hand for approximately 2 min to release attached cells. The rinse and appropriate 10-fold serial dilutions were surface plated on modified Oxford agar (35°C; 48 h). Populations of lactic acid bacteria were enumerated weekly by plating rinse material on all-purpose Tween agar (Difco, BD) with 0.004% bromocresol purple (25°C; 48 h). Changes in pH of each uninoculated sample were measured weekly by removing a 10-g representative portion of the sample before rinsing in buffer for microbiological analysis. The 10-g portion was homogenized with 90 ml of deionized water with a lab blender (Stomacher 400, A. J. Seward, London, UK), and the pH of the slurry was measured (Accumet Basic pH Meter and Orion 8104 Combination Electrode, Thermo Fisher Scientific, Waltham, MA).

Data analysis. A general linear model function of JMP was used to evaluate difference ($P < 0.05$) in the physicochemical properties (pH, moisture, salt, and a_w) of samples, as well as the objective color measurements. Two microbiological responses were calculated from the collected data: maximum growth rate (μ_{max}; log per day) and lag time of *L. monocytogenes*. Growth responses for *L. monocytogenes* were estimated by using DMFit, an add-on for use with Excel (version 2007, Microsoft Corporation, Seattle, WA), which calculated growth rate (μ_{max}) and lag time for each treatment, and were determined based on the average log CFU per gram from three enumerated samples at each sampling point *(4)*. The statistical analysis of the central composite design was carried out by using the RSM function in JMP. All main effects (ingoing nitrite, ascorbate, and residual nitrite) were left in the models even at $P > 0.05$, because they were the factors of main interest in the initial design of the experiment. Insignificant ($P > 0.05$) two-way interactions and second-order terms were eliminated so that the final models reflected the influence of the main factors and included the significant second-order terms.

The residual nitrite depletion rate was calculated using nonlinear regression in JMP. The first-order decay kinetics model was fit to residual nitrite concentrations measured on days 0, 4, 7, 11, 14, 21, and 28 for each treatment. There were four observations at each time point used to fit the model, with the ingoing concentration set at the initial concentration. Additionally, for more accurate curve fitting, an arbitrary data point was added to each treatment, 10 ppm of residual nitrite at 90 days, to represent the depletion of nitrite that would expectedly occur over time. The value and time point were based on survey data showing that cured products at retail often have $\leq$10 ppm and that 90 days is an acceptable, conservative estimate for shelf life of cured products *(11, 26)*. The depletion rate was analyzed by using the central composite design and response surface modeling in JMP, with ingoing nitrite and ascorbate as main effects and $P < 0.05$. Treatments without ingoing nitrite (treatments 5a and 5b) were omitted from depletion rate analysis.

Model performance. To evaluate the strength of the *L. monocytogenes* growth rate and lag time models, bias and accuracy factors developed by Ross were used *(30)*. These two values were calculated by comparing the responses predicted by the models with the observed responses. The first of the two, the bias factor ($10^{(\Sigma\log(\text{predicted/observed})/n)}$), indicates whether the points tend to lie above or below the line of equivalence, on average. This factor also takes into account the distance from that line of equivalence. Thus, if the observed values and predicted values were in perfect agreement, the bias factor would be 1. If the bias factor is greater than 1, for example 1.1, then the predictions exceed the observations by 10%. The second evaluation factor, the accuracy factor ($10^{(\Sigma\log(\text{predicted/observed})/n)}$), indicates the spread of the results about the prediction, and similar to the bias factor, a value of 1 represents a perfect fit of the model with the observations. An accuracy factor of 2, for example, would indicate that the predictions are different from the observations by a factor of 2, either half as large or twice as large *(30)*.

RESULTS AND DISCUSSION

Production of samples. Chemical analysis revealed similar composition across all formulations, with an average 65.0% ± 1.8% moisture, 1.84% ± 0.06% NaCl, pH 6.59 ± 0.05, and a_w 0.979 ± 0.004, respectively. The low variability in moisture, NaCl, pH, and a_w across treatments suggested that their respective influences on either *L. monocytogenes* or the effects of nitrite and ascorbate, or both, could be ignored in this study.

TABLE 4. *Analysis of variance for residual nitrite depletion rate*[a]

Source	Degrees of freedom	Sum of squares	*F* ratio	Probability
Ingoing $NaNO_2$	1	0.061720	23.2097	0.0003
Ascorbate	1	0.008371	3.1479	0.0978
Ingoing $NaNO_2{}^2$[b]	1	0.035430	13.3236	0.0026
Total error	14	0.037229		

[a] Residual nitrite depletion rate (ppm per day). The model was condensed by eliminating all nonsignificant ($P > 0.05$) two-way interactions and second-order terms; $r^2 = 0.74$; root mean square error = 0.05; lack of fit $P = 0.25$.

[b] Ingoing $NaNO_2{}^2$ denotes a second-order term for ingoing sodium nitrite.

Objective color analysis. Objective color was measured in this study to determine if nitrite and ascorbate had an impact on cured color intensity and color stability over storage time. Results reported in Table 3 confirmed that the combinations of nitrite and ascorbate used were sufficient to develop and maintain cured color throughout this study. According to the American Meat Science Association Color Measurement Guidelines, cured color ratios of approximately 1.1 to 1.6 indicate no cured color or moderate fade, and values of approximately 2.2 to 2.6 indicate excellent cured color *(2)*. Therefore, these results indicate that not only did all treatments containing nitrite develop cured color, but that cured color was similar across all factor combinations, at a range of ingoing nitrite from 50 to 352 ppm, confirming that 50 ppm of nitrite is a sufficient level to develop cured color *(19)*. Because these samples were vacuum packaged and stored in the dark, cured color fading was not expected, and data showed that a minimum of 50 ppm of nitrite and 100 ppm of ascorbate were sufficient to maintain cured color over 8 weeks storage time under these conditions *(1, 15)*.

Residual nitrite. Concentrations of residual nitrite for both the initial inoculation point on day 0 and the delayed inoculation point on day 28 are shown in Table 2. The concentration of residual nitrite was different based on inoculation point and was also dependent upon the ingoing nitrite concentration ($P < 0.05$). Analysis of variance indicated that ascorbate did not affect residual nitrite concentration on day 0 (initial inoculation) but was a significant factor for residual nitrite on day 28 (delayed inoculation; $P < 0.05$). At initial inoculation, an average of 69% of ingoing nitrite was remaining, with a range of 46 to 91%, whereas an average 45% with a range of 28 to 64% was recovered at the point of delayed inoculation. Analysis of variance showed that both ingoing nitrite and the squared term of ingoing nitrite were significant for the nitrite depletion rate (Table 4; $P < 0.05$).

Previous studies have reported that up to 50% of ingoing nitrite is lost during processing, including mixing, stuffing, and thermal processing *(10)*. Hustad et al. *(22)* measured residual nitrite concentrations in vacuum-packaged wieners made with 50, 100, 150, 200, and 300 ppm of ingoing nitrite and recovered <50% after thermal processing. Furthermore, the authors observed that concentrations continued to decline during storage at 7°C to ≤12 ppm after 28 days. Depletion rates in this study agree with previous literature that reported that nitrite concentrations deplete rapidly during processing, heating, and early in shelf life, and the rate of depletion slows during extended storage *(5, 18, 25)*. Compared with the literature, the increased concentrations recovered in this study could be attributed to a number of factors, including strict control of processing procedures, such as minimizing holding time before thermal processing and the use of plastics casings that prevented moisture loss during thermal processing. Additionally, samples to be used for nitrite analysis were held at −80°C, a temperature that prevents further nitrite-related reactions to occur. The use of the dedicated ENO-20 HPLC system provided increased sensitivity, and preliminary studies showed that residual nitrite was not lost during the extraction (sample preparation) procedures (data not shown). This also could have contributed to somewhat higher than expected residual nitrite concentrations.

TABLE 5. *Analysis of variance for lag time (day) of* L. monocytogenes[a]

Source	Degrees of freedom	Sum of squares	*F* ratio	Probability
Ingoing $NaNO_2$	1	0.560408	0.2053	0.6534
Ascorbate	1	0.519305	0.1902	0.6655
Ingoing $NaNO_2{}^2$[b]	1	14.820742	5.4296	0.0259
Residual $NaNO_2$	1	14.525276	5.3213	0.0273
Total error	34	92.80731		

[a] The model was condensed by eliminating all nonsignificant ($P > 0.05$) two-way interactions and second-order terms; $r^2 = 0.54$ root mean square error = 1.65; lack of fit $P = 0.17$.

[b] Ingoing $NaNO_2{}^2$ denotes a second-order term for ingoing sodium nitrite.

TABLE 6. *Regression coefficients for predicting the lag time (day) of* L. monocytogenes *in cured meat products*

Factor	Regression coefficient
Constant	4.847229
Ingoing $NaNO_2$	0.002307
Ascorbate	0.000570
Ingoing $NaNO_2{}^2$[a]	−0.000053
Residual $NaNO_2$	0.016884

[a] Ingoing $NaNO_2{}^2$ denotes a second-order term for ingoing sodium nitrite.

Lag time model analysis. Lag times for each run were estimated by using DMFit and are shown in Table 2, along with the design factors and levels for each run. DMFit was able to estimate a lag time for each run, which ranged from 2.2 to 11.5 days for all runs and both inoculation points. The analysis of variance for the lag time model indicated that the squared term of ingoing nitrite and residual nitrite were significant factors in determining lag

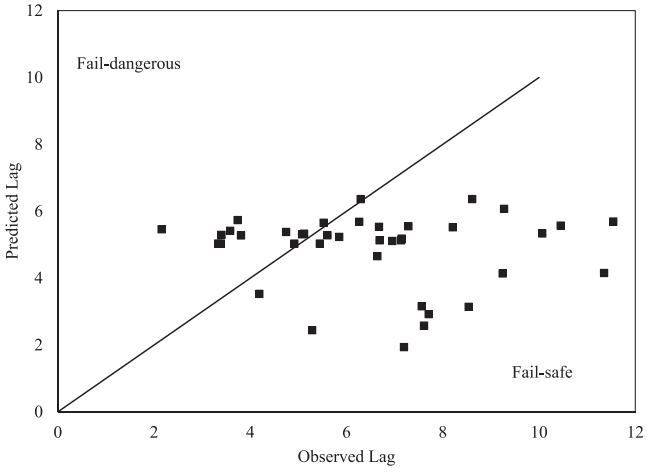

FIGURE 1. *Simple regression of observed versus predicted lag time (day) for* L. monocytogenes. *The bias factor for this model was 0.78, and the accuracy factor was 1.55.*

time ($P < 0.05$; Table 5). The model had an $r^2 = 0.54$, root mean square error was 1.65, and lack of fit was not significant ($P > 0.05$).

The final regression equation (Table 6) to predict *L. monocytogenes* lag time in RTE cooked, cured pork sausage, vacuum packaged and stored at 7°C, is as follows:

$$\begin{aligned}\text{Predicted } L.\ monocytogenes \text{ lag time} &= 4.847229 - 0.002307 \cdot [\text{ingoing } NaNO_2] \\ &+ 0.000570 \cdot [\text{ascorbate}] \\ &- 0.000053 \cdot [\text{ingoing } NaNO_2^2] \\ &+ 0.016884 \cdot [\text{residual } NaNO_2]\end{aligned}$$

The scatterplot of predicted lag versus observed lag time is shown in Figure 1. As evident from this plot, there is more scatter around the line of equivalence for the lag model than the rate model. Because the bias factor for this model was 0.78, less than 1, this index indicates that the model over predicted lag time, and by a magnitude of approximately 22%, thus making it a fail-safe model. The accuracy factor of 1.55 indicates that the predicted values are within 55% of the observed values.

Note that when evaluating this model that microbiological enumerations were done twice per week (days 0, 4, 7, 11, 14, 18, 21, 25, and 28) throughout the storage time and compared with weekly or biweekly sampling, this sampling scheme provides more accurate data for calculating lag time. However, the sampling interval should be kept in mind when considering the precision of this calculation and model for lag time.

TABLE 7. *Analysis of variance for growth rate of* L. monocytogenes[a]

Source	Degrees of freedom	Sum of squares	*F* ratio	Probability
Ingoing $NaNO_2$	1	0.079606	4.6800	0.0372
Ascorbate	1	0.024592	1.4458	0.2371
Residual $NaNO_2$	1	0.008899	0.5232	0.4742
Total error	36	0.612354		

[a] Growth rate (μ_{max}; log per day); the model was condensed by eliminating all nonsignificant ($P > 0.05$) two-way interactions and second-order terms; $r^2 = 0.52$; root mean square error = 0.13; lack of fit $P = 0.50$.

TABLE 8. *Regression coefficients for predicting the growth of* L. monocytogenes *in cured meat products*[a]

Factor	Regression coefficient
Constant	0.665951
Ingoing $NaNO_2$	−0.000866
Ascorbate	−0.000124
Residual $NaNO_2$	−0.000416

[a] Regression coefficients for predicting growth (μ_{max}; log per day).

The μ_{max} model analysis. Estimated growth rates (μ_{max}) for *L. monocytogenes* are also presented in Table 2. Note that all factor combinations resulted in growth of *L. monocytogenes,* so all runs have $\mu_{max} > 0$. The μ_{max} ranged from 0.194 to 0.913 across both initial and delayed inoculation points. Analysis of variance for μ_{max} indicated that of the three main factors (ingoing nitrite, ascorbate, and residual nitrite), only ingoing nitrite significantly impacted the μ_{max} of *L. monocytogenes* ($P < 0.05$; Table 7). The overall model, including coefficients for ingoing nitrite, ascorbate, and residual nitrite, resulted in $r^2 = 0.52$ and root mean square error = 0.13. Lack of fit was not significant for this model ($P > 0.05$). The final regression equation (Table 8) to predict *L. monocytogenes* growth rate in RTE cooked, cured pork sausage, vacuum packaged and stored at 7°C, is as follows:

$$\begin{aligned}\text{Predicted } L.\ monocytogenes \text{ growth rate } (\mu_{max}) &= 0.665951 - 0.000866 \cdot [\text{ingoing } NaNO_2] \\ &- 0.000124 \cdot [\text{ascorbate}] \\ &- 0.000416 \cdot [\text{residual } NaNO_2]\end{aligned}$$

It is not unexpected that increasing concentrations of nitrite would impact growth rates through an inverse relationship. A coefficient of 0.001 would mean that growth rate would be affected by 0.1 log/day for every 100 ppm change in ingoing nitrite, so with the coefficient for ingoing nitrite in this model of 0.000866, the effect is slightly less than that. Therefore, the magnitude of ingoing nitrite's impact on growth rate observed in this study corresponds to previous reports that a relatively small impact on growth is achieved at concentrations allowed for use in RTE meats. Meanwhile, though not significant factors, residual nitrite concentration had approximately half the impact as ingoing nitrite concentration (coefficients of –0.000416 and –0.000866, respectively) and ascorbate had an even more minor impact.

To evaluate the model performance, a scatter plot was created with predicted versus observed growth rates (Fig. 2). This figure shows not only the scatter of the data about a line of equivalence, which indicates a perfect fit of the model predictions with the observed data, but also shows if the points tend to lie in a fail-dangerous or fail-safe zone. For a

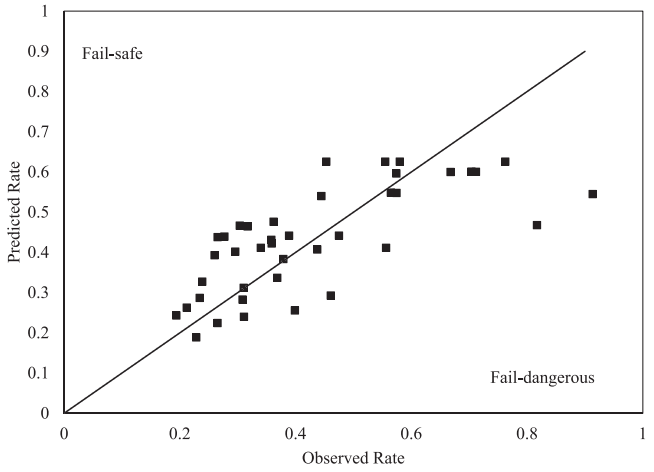

FIGURE 2. *Simple regression of observed versus predicted* L. monocytogenes *growth rates (μ_{max}; log per day). The bias factor for this model was 1.03, and the accuracy factor was 1.25.*

rate model such as this, an observed rate that exceeds that of the prediction is considered fail-dangerous, as the model underestimates risk, whereas an observed rate that is less than the predicted rate is considered fail-safe. Performance indices were calculated based on these data and resulted in a bias factor of 1.03 and accuracy factor of 1.25. A bias factor >1 for a rate model indicates it is a fail-safe model, and in this case, the predicted growth rate exceeds the observed rate by 3% on average. The accuracy factor of 1.25 for this model shows that on average, predicted rates are within 25% of the observed rates.

When considering the relative impact of these two parameters, lag time and growth rate of *L. monocytogenes,* on the overall product safety, neither can be ignored. However, with the wide variability in the infectious dose, any increase in *L. monocytogenes* populations could be potentially hazardous to a consumer. Therefore, although preventing initial contamination is most critical, extending lag time could be considered more important than limiting the exponential growth rate to ensure the ultimate safety of RTE meats.

This study investigated the effects of ingoing nitrite, ascorbate, and residual nitrite on lag and growth of *L. monocytogenes* and determined that ingoing nitrite was the prominent factor impacting growth rate of *L. monocytogenes,* whereas the squared term of ingoing nitrite and the residual nitrite concentration were significant for lag time ($P < 0.05$). The residual nitrite depletion rate did not affect *L. monocytogenes* growth rate or lag time ($P > 0.05$). Previous reports have shown somewhat conflicting results as to the magnitude of impact ingoing nitrite concentration has on *L. monocytogenes*. An early study on the impact of nitrite on *L. monocytogenes* by Shahamat et al. *(39)* suggested that nitrite was only inhibitory at pH ≤ 5.5 and $>3\%$ salt. However, since then, a number of models have reported that ingoing nitrite concentration is a significant factor in *L. monocytogenes* growth characteristics in RTE meat products *(8, 9, 16, 24)*. Nitrite is known to have less effect on *L. monocytogenes* than on *C. botulinum,* and this less definitive effect may account for the variability in results reported, suggesting that perhaps the levels of nitrite used in RTE meats fall within a precarious area in which the impact on *L. monocytogenes* growth is dependent upon a number of other product parameters *(43)*. A number of studies have proven this hypothesis and have shown that pH, salt, atmosphere, and temperature interact with nitrite in different ways to affect *L. monocytogenes* growth in RTE meats *(9, 24)*.

Although the enhancing effect of ascorbate in conjunction with nitrite has been shown in the inhibition of spore formers, the literature is less conclusive in regard to *L. monocytogenes* control *(14, 41, 42)*. Farber et al. *(17)* tested 0 and 200 ppm of ingoing nitrite, 0 and 550 ppm of sodium erythorbate, 1 and 3% salt, and 4 and 10°C storage temperatures in liver pate inoculated with *L. monocytogenes* and found that of those factors tested, only temperature significantly impacted growth. On the other hand, Duffy et al. *(16)* found that 420 ppm of sodium ascorbate significantly increased the inhibitory effects of nitrite against *L. monocytogenes* in vacuum-packaged RTE meats (beef, pork, and poultry sausages). The authors defined lag in this study as the time to a 3-log increase and reported that vacuum-packaged cooked meats with 70 to 140 ppm of residual nitrite, at the point of inoculation, extended lag time to twice as long as similar products made without nitrite at 5°C, and this effect was potentiated by the addition of ascorbate *(16)*.

In a preliminary study (data not shown), the residual nitrite concentration was a covariate of the ingoing concentration, not isolated as its own factor, whereas the use of ingoing and delayed inoculation points in this study design allowed for each ingoing combination of nitrite and ascorbate to yield two residual nitrite concentrations at the time of inoculation, with other parameters held constant. The growth rate model showed that the concentration of residual nitrite at inoculation did not impact rate of growth, yet the lag model suggested that it could play a role in determining the time until *L. monocytogenes* begins exponential growth. These combined conclusions suggest that nitrite itself may stress *L. monocytogenes* cells, but it is also plausible that other nitrosated species, generated by reactions of nitrite with components of a cured meat system, may play a role by directly interfering with *L. monocytogenes* growth or by eliminating the required cofactors for growth *(35, 36)*. Much of the mechanistic investigation of nitrite as an antimicrobial has been done with its activity relating to *Clostridia,* and results have pointed to nitric oxide, generated by the reduction of nitrite, as a key active molecule that may inhibit iron-sulfur proteins essential to metabolism *(45, 47, 48)*.

Nevertheless, both ingoing nitrite and residual nitrite were shown by this study to contribute to the control of growth of *L. monocytogenes*. Ingoing ascorbate concentration did not directly contribute to *L. monocytogenes* control, though by influencing residual nitrite concentration, provided an inadvertent impact on microbial growth. Overall, the results of this study suggest that growth rate of *L. monocytogenes* is largely influenced by ingoing

concentrations and, therefore, would imply that maximizing ingoing nitrite concentrations yields peak antimicrobial efficacy of this ingredient. Additionally, results indicate that the amount of residual nitrite in the product at the point of inoculation is important to the onset of exponential bacterial growth. Although this study reinforces ingoing nitrite concentration as a determining factor in controlling *L. monocytogenes* growth, results additionally demonstrate that the reaction between nitrite and the pathogen is complex and can also be affected by residual nitrite concentrations in RTE meats.

REFERENCES

1. Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2012. Principles of meat science, 5th ed. Kendall Hunt, Dubuque, IA.
2. American Meat Science Association. 2012. AMSA meat color measurement guidelines, 2nd ed. American Meat Science Association, Champaign, IL.
3. Association of Official Analytical Chemists. 1990. Moisture in meat. Official method 950.46, p. 931. *In* Official methods of analysis. Association of Official Analytical Chemists, Arlington, VA.
4. Baranyi, J., and Y. Le Marc. 2006. DMFit 2.1. Available at: http://www.combase.cc/index.php/en/downloads/file/53-dmfit-30. Accessed 18 June 2013.
5. Barbieri, G., M. Bergamaschi, Ge. Barbieri, and M. Franceschini. 2013. Kinetics of nitrite evaluated in a meat product. *Meat Sci*. 93:282–286.
6. Brown, C. L., H. B. Hedrick, and M. E. Bailey. 1974. Characteristics of cured ham as influenced by levels of sodium nitrite and sodium ascorbate. *J. Food Sci*. 39:977–979.
7. Bryan, N. S., and M. B. Grisham. 2007. Methods to detect nitric oxide and its metabolites in biological samples. *Free Radic. Biol. Med*. 43:645–657.
8. Buchanan, R. L., and J. G. Phillips. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. *J. Food Prot*. 53:370–381.
9. Buchanan, R. L., H. G. Stahl, and R. C. Whiting. 1989. Effects and interactions of temperature, pH, sodium chloride, and sodium nitrite on the growth of *Listeria monocytogenes*. *J. Food Prot*. 52:844–851.
10. Cassens, R. G. 1979. Reactions of nitrite in meat. *Food Technol*. 33:46–57.
11. Cassens, R. G. 1997. Composition and safety of cured meats in the USA. *Food Chem*. 59:561–566.
12. Cassens, R. G., G. Woolford, S. H. Lee, and R. Goutefongea. 1976. Fate of nitrite in meat, p. 95–100. *In* B. Krol and B. J. Tinbergen (ed.). Proceedings of the Second International Symposium on Nitrite in Meat Products. Pudoc, Zeist, The Netherlands.
13. Christiansen, L., R. Tompkin, and A. Shaparis. 1978. Fate of *Clostridium botulinum* in perishable canned cured meat at abuse temperature. *J. Food Prot*. 41:354–355.
14. Christiansen, L. N. 1980. Factors influencing botulinal inhibition by nitrite. *Food Technol*. 34:237–239.
15. Draudt, H. N., and F. E. Deatherage. 1956. Studies on the chemistry of cured meat pigment fading. *J. Food Res*. 21:122.
16. Duffy, L. L., P. B. Vanderlinde, and F. H. Grau. 1994. Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, a_w, nitrite and ascorbate. *Int. J. Food Microbiol*. 23:377–390.
17. Farber, J. M., R. C. McKellar, and W. H. Ross. 1995. Modelling the effects of various parameters on the growth of *Listeria monocytogenes* on liver pate. *Food Microbiol*. 12:447–453.
18. Fox, J. B., and R. A. Nicholas. 1974. Nitrite in meat. Effect of various compounds on loss of nitrite. *J. Agric. Food Chem*. 22:302–306.
19. Froehlich, D. A., E. A. Gullett, and W. R. Usborne. 1983. Effect of nitrite and salt on the color, flavor and overall acceptability of ham. *J. Food Sci*. 48:152–154.
20. Glass, K., and M. P. Doyle. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl. Environ. Microbiol*. 55:1565–1569.
21. Hauschild, A. H. W., R. Hilsheimer, G. Jarvis, and D. P. Raymond. 1982. Contribution of nitrite to the control of *Clostridium botulinum* in liver sausage. *J. Food Prot*. 45:500–506.
22. Hustad, G. O., J. G. Cerveny, H. Trenk, R. H. Deibel, D. A. Kautter, T. Fazio, R. W. Johnston, and O. E. Kolari. 1973. Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in wieners. *Appl. Microbiol*. 26:22–26.
23. Izumi, K., R. G. Cassens, and M. L. Greaser. 1989. Reaction of nitrite with ascorbic acid and its significant role in nitrite-cured food. *Meat Sci*. 26:141–153.
24. McClure, P. J., T. M. Kelly, and T. A. Roberts. 1991. The effects of temperature, pH, sodium chloride and sodium nitrite on the growth of *Listeria monocytogenes*. *Int. J. Food Microbiol*. 14:77–92.
25. Nordin, H. R. 1969. The depletion of added sodium nitrite in ham. *Can. Inst. Food Technol. J*. 2:79–85.
26. Nuñez De González, M. T., W. N. Osburn, M. D. Hardin, M. Longnecker, H. K. Garg, N. S. Bryan, and J. T. Keeton. 2012. Survey of residual nitrite and nitrate in conventional and organic/natural/uncured/indirectly cured meats available at retail in the United States. *J. Agric. Food Chem*. 60:3981–3990.
27. Pegg, R. B., and F. Shahidi. 2000. Nitrite curing of meat: the *N*-nitrosamine problem and nitrite alternatives. Food & Nutrition Press, Inc., Trumbull, CT.
28. Pérez-Rodríguez, M. L., N. Bosch-Bosch, and M. Garciá-Mata. 1996. Monitoring nitrite and nitrate residues in frankfurters during processing and storage. *Meat Sci*. 44:65–73.
29. Qvist, S., and N. Bernbom. 2000. Effect of sodium lactate, sodium nitrite, pH and temperature on growth of *Listeria monocytogenes*. Presented at the Second NSF International Conference on Food Safety, Savannah, GA, 11 to 13 October 2000.
30. Ross, T. 1996. Indices for performance evaluation of predictive models in food microbiology. *J. Appl. Bacteriol*. 81:501–508.
31. Ryser, E. T., and R. L. Buchanan. 2013. *Listeria monocytogenes,* p. 503–546. *In* M. P. Doyle and R. L. Buchanan (ed.), Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC.
32. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis*. 17:7–15.
33. Schlyter, J. H., K. A. Glass, J. Loeffelholz, A. J. Degnan, and J. B. Luchansky. 1993. The effects of diacetate with nitrite, lactate, or pediocin on the viability of *Listeria monocytogenes* in turkey slurries. *Int. J. Food Microbiol*. 19:271–281.
34. Sebranek, J. G., and J. N. Bacus. 2007. Cured meat products without direct addition of nitrate or nitrite: what are the issues? *Meat Sci*. 77:136–147.
35. Sebranek, J. G., R. G. Cassens, W. G. Hoekstra, W. C. Winder, E. V. Podebradsky, and E. W. Kielsmeier. 1973. ^{15}N tracer studies of nitrite added to a comminuted meat product. *J. Food Sci*. 38:1220–1223.
36. Sebranek, J. G., and J. B. Fox. 1985. A review of nitrite and chloride chemistry: interactions and implications for cured meats. *J. Sci. Food Agric*. 36:1169–1182.
37. Sebranek, J. G., A. L. Jackson-Davis, K. L. Myers, and N. A. Lavieri. 2012. Beyond celery and starter culture: advances in natural/organic curing processes in the United States. *Meat Sci*. 92:267–273.
38. Sebranek, J. G., S. M. Lonergan, M. King-Brink, and E. Larson. 2001. Meat science and processing. Peerage Press, Zenda, WI.
39. Shahamat, M., A. Seaman, and M. Woodbine. 1980. Influence of sodium chloride, pH and temperature on the inhibitory activity of sodium nitrite on *Listeria monocytogenes,* p. 227. *In* G. W. Gould and J. E. Corry (ed.), Microbial growth and survival in extremes of environment. Academic Press, New York.
40. Tompkin, R. 1978. The role and mechanism of the inhibition of *C. botulinum* by nitrite—is a replacement available?, p. 135–147. *In*

Proceedings of the Reciprocal Meat Conference, American Meat Science Association, Storrs, CT, 18 to 22 June 1978.
41. Tompkin, R., L. Christiansen, and A. Shaparis. 1978. Antibotulinal role of isoascorbate in cured meat. *J. Food Sci.* 43:1368–1370.
42. Tompkin, R., L. Christiansen, and A. Shaparis. 1978. Enhancing nitrite inhibition of *Clostridium botulinum* with isoascorbate in perishable canned cured meat. *Appl. Environ. Microbiol.* 35:59–61.
43. Tompkin, R. B. 2005. Nitrite, p. 169–236. *In* P. M. Davidson, J. N. Sofos, and A. L. Branen (ed.), Antimicrobials in food. CRC Press, Boca Raton, FL.
44. Tompkin, R. B., L. N. Christiansen, and A. B. Shaparis. 1978. Causes of variation in botulinal inhibition in perishable canned cured meat. *Appl. Environ. Microbiol.* 35:886–889.
45. Tompkin, R. B., L. N. Christiansen, and A. B. Shaparis. 1978. The effect of iron on botulinal inhibition in perishable canned cured meat. *J. Food Technol.* 13:521–527.
46. U.S. Department of Agriculture. 1995. Processing inspectors' calculations handbook. U.S. Government Printing Office, Washington, DC.
47. Woods, L. F. J., and D. F. Wood. 1982. A note on the effect of nitrite inhibition on the metabolism of *Clostridium botulinum*. *J. Appl. Bacteriol.* 52:109–110.
48. Woods, L. F. J., J. M. Wood, and P. A. Gibbs. 1981. The involvement of nitric oxide in the inhibition of the phosphoroclastic system in *Clostridium sporogenes* by sodium nitrite. *J. Gen. Microbiol.* 125:299–406.