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Research Paper

Comparing Thermal Process Validation Methods for *Salmonella* Inactivation on Almond Kernels

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ABSTRACT

Ongoing regulatory changes are increasing the need for reliable process validation methods for pathogen reduction processes involving low-moisture products; however, the reliability of various validation methods has not been evaluated. Therefore, the objective was to quantify accuracy and repeatability of four validation methods (two biologically based and two based on time-temperature models) for thermal pasteurization of almonds. Almond kernels were inoculated with *Salmonella* Enteritidis phage type 30 or *Enterococcus faecium* (NRRL B-2354) at $\sim 10^8$ CFU/g, equilibrated to 0.24, 0.45, 0.58, or 0.78 water activity (a_w), and then heated in a pilot-scale, moist-air impingement oven (dry bulb 121, 149, or 177°C; dew point <33.0, 69.4, 81.6, or 90.6°C; $v_{air} = 2.7$ m/s) to a target lethality of ~4 log. Almond surface temperatures were measured in two ways, and those temperatures were used to calculate *Salmonella* inactivation using a traditional (*D, z*) model and a modified model accounting for process humidity. Among the process validation methods, both methods based on time-temperature models had better repeatability, with replication errors approximately half those of the surrogate (*E. faecium*). Additionally, the modified model yielded the lowest root mean squared error in predicting *Salmonella* inactivation (1.1 to 1.5 log CFU/g); in contrast, *E. faecium* yielded a root mean squared error of 1.2 to 1.6 log CFU/g, and the traditional model yielded an unacceptably high error (3.4 to 4.4 log CFU/g). Importantly, the surrogate and modified model both yielded lethality predictions that were statistically equivalent ($\alpha = 0.05$) to actual *Salmonella* lethality. The results demonstrate the importance of methodology, a_w, and process humidity when validating thermal pasteurization processes for low-moisture foods, which should help processors select and interpret validation methods to ensure product safety.

Key words: Lethality; Low water activity; Pathogens; Predictive microbiology; Surrogate

In the last decade, *Salmonella* in low-moisture foods and ingredients has become a recurring problem, as reflected in nationwide outbreaks and recalls involving soy products *(30),* dried hydrolyzed vegetable protein *(27),* cake batter mix *(25),* black pepper *(6),* nuts *(8, 17, 20, 24, 26, 28, 29),* pet food *(32),* and other dry ingredients. The Food Safety Modernization Act (FSMA) preventive controls mandate that the food industry must implement and validate interventions to prevent or control identified hazards, such as *Salmonella* in low-moisture products *(31).*

Therefore, industry has the vital task of ensuring reliable process validation and verification steps. The Grocery Manufacturers Association recommended seven elements for the control of *Salmonella* in low-moisture products *(12).* Two of these are to validate control measures to inactivate *Salmonella* and to ensure that the operation can deliver the critical limits and the processing parameters consistently. The Alliance for Innovation and Operational Excellence also published general validation guidelines for *Salmonella* reduction in heat-processed low-moisture foods *(4).* However, the above reports do not describe how to assess the reliability or repeatability of the methods, which is critical information to evaluate validation and verification processes across different methodologies. For example, the Almond Board of California published validation procedures for blanching *(1, 14),* dry roasting *(2),* and oil roasting *(3, 10),* in which details of the processes and measurement methodologies were explained. The oil roasting guidelines describe methods using a nonpathogenic surrogate organism, aluminum almonds, and *Salmonella* for validation. However, there was no direct comparison among validation methodologies, in terms of reliability or repeatability. Regarding repeatability, the Grocery Manufacturers Association process validation handbook for nut processing mentions that the measured temperature profile data need to be reviewed for consistency across runs, and if there are anomalies or inconsistencies, additional runs must be performed *(13).* Although the handbook pointed out the importance of consistent measurements, no prior study is known to have directly quantified the inherent uncertainty of the various validation methods. Lambertini et al. *(18)* noted that an improved understanding of process variability and pathogen reduction uncertainty also would improve the risk assessments that depend on such information.

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Several prior studies have investigated the effect of water activity (a_w) on *Salmonella* thermal resistance in low-moisture food products *(5, 11, 19, 21, 22, 33)*. All of these studies evaluated and/or modeled the effect of a_w on thermal resistance based on isothermal and iso-moisture data, or on laboratory-scale data with changing a_w that was not quantified. Application of such models to real-world thermal processes, which are typically nonisothermal and non–iso-moisture, has not been previously reported. Therefore, two critical technical gaps remain in the area of thermal process validations for low-moisture products: (i) how to reliably monitor the critical process parameters, such as the true surface temperature and a_w of the product, and account for them in lethality calculations and (ii) how to quantify uncertainty associated with either surrogate-based test results or model-based lethality calculations and how to account for that uncertainty in validating and ensuring process efficacy. Additionally, although a_w is known to affect *Salmonella* thermal resistance, the relative effect of process humidity and product a_w on lethality has not been quantified.

Therefore, the overall goal of this study was to improve understanding of the uncertainties underlying low-moisture pasteurization validation methods. The specific objective was to quantify the accuracy and repeatability of four validation methods for thermal pasteurization of almonds, including testing an improved thermal inactivation model that accounts for the effect of process humidity on *Salmonella* inactivation. Note that the objective was not to develop or fit an inactivation model, but rather to test the application of multiple process validation methods, in terms of accuracy and repeatability of the evaluated lethality outcomes.

MATERIALS AND METHODS

Almonds. Raw almond kernels (Nonpareils 27/30), previously pasteurized with propylene oxide to reduce background microorganisms, were obtained from a nut processor (Select Harvest USA, Turlock, CA) and were stored in vacuum-sealed plastic bags (4°C) until they were used in this project.

Bacterial strains and inoculation. Two strains were used in this study. *Enterococcus faecium* strain NRRL B-2354 (obtained from Silliker Laboratories, Inc., South Holland, IL) and *Salmonella enterica* serovar Enteritidis phage type 30 (SE PT30) (obtained from Dr. Linda Harris, University of California, Davis) were maintained at −80°C in tryptic soy broth (Difco, BD, Sparks, MD) supplemented with 0.6% (wt/vol) yeast extract (Difco, BD) (TSBYE) and 10% (vol/vol) glycerol.

The inoculation procedure of Danyluk et al. *(9)* was followed, with slight modifications. Before use, *E. faecium* and SE PT30 were subjected to a minimum of two consecutive 24-h, 35°C transfers in TSBYE and then were streaked onto plates (150-mm diameter by 15-mm height) of tryptic soy agar supplemented with 0.6% (wt/vol) yeast extract (Difco, BD) (TSAYE) to obtain uniform bacterial lawn growth. After 24 h at 35 ± 2°C, the bacteria were harvested using 10 ml of sterile 0.1% buffered peptone water (Difco, BD), which was then added to 30 ml of 0.1% buffered peptone water.

Almonds (200 g) were mixed with 16 ml of the *E. faecium* or SE PT30 suspension in a sterile plastic bag (3.8 liter) for 1 min, spread in a single layer in large plastic tubs (50 by 30 by 15 cm), and dried at room temperature (~25°C) in a biosafety hood (v_{air} = ~0.6 m/s) for 2 h.

Immediately thereafter, the inoculated almonds were transferred to a sealed conditioning chamber for equilibration with appropriate salt solutions (with a small fan circulating air within the chamber) until the samples reached the target a_ws of 0.24 ± 0.03, 0.45 ± 0.03, 0.58 ± 0.02, or 0.78 ± 0.02 (mean ± SD of all equilibrated samples, verified at room temperature with an electronic a_w meter [Hygrolab 3, Rotronic Instrument Corp., Hauppauge, NY]), which corresponded to wet basis moisture contents of ~3.3, 3.6, 4.6, and 8.0%, respectively, as determined by an oven drying (gravimetric) method (AOAC 934.01, AOAC, Gaithersburg, MD). Equilibration time to reach the targeted a_w ranged from 8 to 16 days.

Thermal treatments. Inoculated and equilibrated samples (~25 g each for *Salmonella* and *Enterococcus*) were heated in a pilot-scale, moist-air impingement oven (JBT Food Tech, Sandusky, OH). The treatments consisted of a full-factorial experimental design, composed of (i) four initial a_w levels (described above), (ii) three oven dry bulb temperatures (121, 149, 177°C), and (iii) four oven humidities ("dry" [no added steam], 30, 50, or 70% moisture by volume [%Mv], corresponding to dew points of <33.0, 69.4, 81.6, or 90.6°C). Because the air dry bulb temperature was >100°C, it was important to use an absolute humidity scale (e.g., ppm, dew point, or moisture by volume), rather than relative humidity. In this study, air humidity was measured and reported in terms of moisture by volume (%Mv = p_w/p_T, where p_w is the partial pressure due to water vapor and p_T is the total pressure, which was atmospheric pressure); this is a function of dew point temperature, which was monitored via a dew point temperature sensor (DMP246, Vaisala, Woburn, MA). Note that %Mv is different from % relative humidity (which is defined as $100 \times p_w/p_s$, where p_w is the partial pressure due to water vapor and p_s is the saturation pressure). The impinging air jet velocity was ~2.7 m/s (impinging normal to the belt from both the top and bottom), and nominal target lethality was ~4-log reductions for each treatment. Total heating times ranged from 8.2 to 30.5 min. All treatments were run in triplicate, yielding a total of 144 different runs from 48 distinct treatments.

For each treatment, the *Salmonella*-inoculated sample and the *Enterococcus*-inoculated sample (each in a separate mesh tray, 8 by 8 by 1 cm), three thermocouple-instrumented almonds (described in the next section), and three aluminum almonds (described in the next section) were processed through the oven simultaneously in a shallow bed of carrier almonds (~1 kg) on a mesh tray (36 by 36 by 1 cm), so that all resulting data (i.e., bacterial survivors and time-temperature data) for a given treatment corresponded to identical conditions in the oven (Fig. 1).

Temperature measurements. Assuming that bacterial contamination of nuts is a surface phenomenon, measurement of local conditions right at the product surface is critical for a reliable process validation. For all of the thermal treatments, the surface temperature of the nuts was measured via a thin-wire thermocouple on an almond and an aluminum almond (obtained from JBT Food Tech, Madera, CA) with an embedded thermocouple.

In the first case, a thin-wire thermocouple (K-type, 36-gauge, prefused junction; Omega Engineering Inc., Stamford, CT) was attached just below the surface of an almond. The positioning of the thermocouple junction was ensured by two means. First, the thermocouple wire (insulated portion) was secured rigidly to the almond using a plastic tie. Second, a very small, oblique hole was

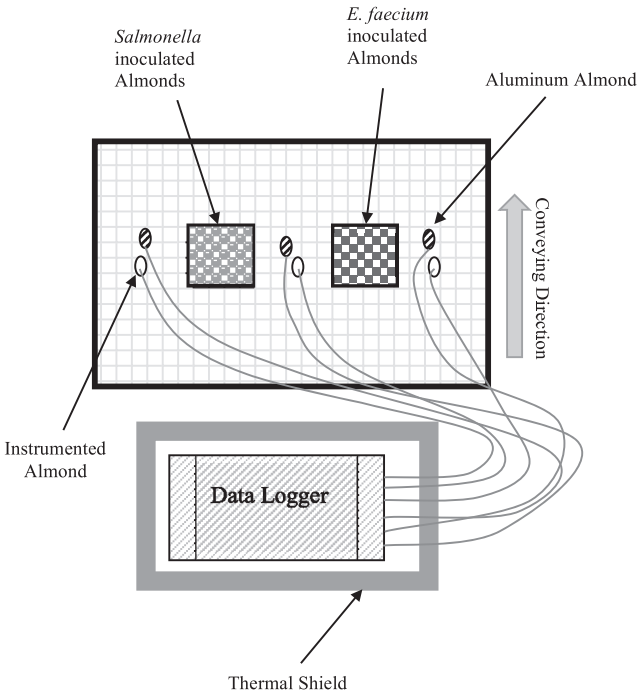


FIGURE 1. *Schematic (top view) showing the arrangement of biological samples (*Salmonella *and* E. faecium*) and the temperature measurement and recording devices on the tray for processing in the pilot-scale oven. The tray contained a uniform bed of almonds (~1 cm thick) around the instrumented and inoculated almonds.*

made into the almond surface with a hypodermic needle (27-gauge, Difco, BD), so that the fused portion (junction and lead wire) of the thermocouple was held just below the almond surface (<0.5 mm), thereby measuring almond temperature at the surface rather than air temperature at the surface. Three instrumented almonds were positioned in the bed of almonds for each run (Fig. 1).

In the second case, the same type of thermocouples were fixed at the center of aluminum almonds, and three instrumented aluminum almonds were positioned in the bed of almonds for each run. Obviously, this method does not directly measure the true surface temperature of the product but rather measures the core temperature of the aluminum almond. However, given the thermal properties of aluminum (i.e., very high thermal conductivity) and the mass of the aluminum almond, the Biot number (i.e., ratio of internal to external thermal resistance) for this physical model was calculated (analysis not reported here) to be ~0.001. This confirms negligible internal resistance to heat conduction and, therefore, the suitability of a lumped parameter solution for heat transfer in the aluminum almond. This analysis, along with an approximate solution for heat conduction in an actual almond, indicated that the temperature measured in an aluminum almond closely tracks the theoretical true surface for an actual almond over the treatment conditions (time, temperature, and air velocity) used in this project, with the aluminum almond temperature expected to track below the true almond surface temperature by an average value of <10°C. This implies that the aluminum almond is a slightly conservative measure of the true surface temperature of an actual almond. However, the actual measured almond surface temperatures, constrained by the physical limitation of thermocouple placement (i.e., a finite distance below the true surface) and measured as described above, were directly compared with the measured aluminum almond temperatures; those comparisons are reported in the "Results" section.

Temperature data from both measurement systems were recorded in real-time using a radio frequency–transmitting data-logger with a thermal shield (MultiPaq21, DataPaq, Cambridge, UK), which traveled through the oven with the samples. Oven dew point temperature was monitored with an electronic dew point transmitter (DMP246, Vaisala, Woburn, MA).

Recovery and enumeration. After the respective thermal treatments, surviving microorganisms were recovered from the almonds and enumerated *(9)*. Immediately after removal from the oven, the samples were combined with chilled 0.1% buffered peptone water (to halt bacterial inactivation essentially instantaneously), stomached for 180 s, and diluted 1:5 (wt/vol) in 0.1% buffered peptone water. To enumerate *Salmonella* survivors, serial dilutions were plated in duplicate onto TSAYE supplemented with ferric ammonium citrate and sodium thiosulfate. *Salmonella* colonies were differentiated from any surviving background microflora by the characteristic black precipitate in the center of their colonies on this differential medium. *E. faecium* survivors were quantified in the same manner by plating on de Man Rogosa Sharpe medium. The plates were incubated at 35 ± 2°C for 48 h prior to enumeration. Log reductions were calculated by subtracting the survivor counts (log CFU per gram) from the mean initial population level (log CFU per gram) on unheated nuts from the same test day.

Model predictions. The time-temperature data from the instrumented almonds and the aluminum almonds were used to predict process lethality (i.e., log reductions) for *Salmonella* Enteritidis PT30, based on two models. The first, a traditional log-linear Bigelow model, was as follows:

$$D(T) = D_{\text{ref}} \times 10^{\frac{T_{\text{ref}} - T(t)}{z}} \tag{1}$$

$$\log\left(\frac{N}{N_0}\right) = -\int_0^t \left(D\left(T(t)\right)\right)^{-1} dt \tag{2}$$

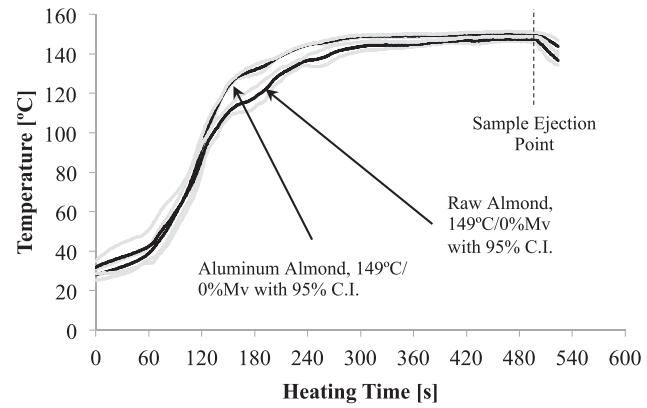
where $D(T)$ is the decimal reduction time (D-value) at temperature T (s), D_{ref} is the reference D-value at T_{ref}, T_{ref} is the reference product surface temperature (°C), $T(t)$ is the product surface temperature at time t (°C), z is the z-value (°C) (i.e., temperature change that results in a 10-fold change in D), N is the number of viable organisms at time t, and N_0 is the initial number of viable organisms at $t = 0$.

The second, modified (MSU) model, based on our previously published work *(15)* was as follows:

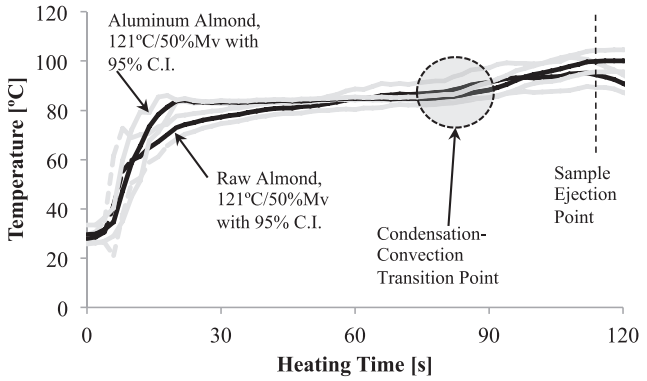
$$D_{Ts,Td}(t) = D_{\text{ref}} \cdot 10^{\left[\left(\frac{T_{\text{ref}} - T_S(t)}{z_T}\right) + \left(\frac{\left(T_{d,\text{ref}} - T_d\right) - \left(T_{\text{ref}} - T_S(t)\right)}{z_M}\right)\right]} \tag{3}$$

$$\log\left(\frac{N}{N_0}\right) = -\int_0^t \left(D_{T_S T_d}(t)\right)^{-1} dt \tag{4}$$

where the variables and parameters different from the preceding model included the following: T_d is the process dew point temperature (°C), $T_{d,\text{ref}}$ is the reference dew point temperature (°C), and z_T and z_M are pseudo z-value–like constants corresponding to temperature and moisture, respectively (C°). For both of the above models (equations 2 and 4), a model fitting was not the objective of this study. Instead, the parameters for each model were previously published *(15, 16)*, so that the pilot-scale data generated in this study were used for independent statistical



(a)



(b)

FIGURE 2. *Example of typical temperature profiles (with 95% confidence interval) measured with thermocouples embedded in or on aluminum almonds and raw almonds. (a) Low humidity condition (~0% Mv, dew point <33°C); (b) high humidity condition (50% Mv, dew point 81.6°C).*

testing and validation of the modeling and process validation methodologies.

Statistical analysis. The resulting model predictions for lethality, along with the experimental results for the surrogate organism (i.e., *E. faecium*), were compared directly to the actual *Salmonella* lethalities for each run. The errors between the predicted and actual outcomes (i.e., residuals) were used to compute the prediction error (i.e., root mean squared error of prediction [RMSE]) and the prediction bias (i.e., mean residual) for each run and method. Additionally, analyses of variance, with Tukey means comparisons, were conducted to test whether initial a_w, process humidity, dry bulb temperature, and validation method significantly ($\alpha = 0.05$) affected the prediction errors. The repeatability of each process validation method was calculated as follows:

$$\text{Repeatability} = \sqrt{\frac{\left[\sum_{j=1}^{m}\sum_{i=1}^{n}\left(\bar{x}_j - x_i\right)^2\right]}{n \times m - m}} \qquad (5)$$

where n is the number of replications, m is the number of conditions, x_i is the log reduction (log CFU/g) of a single replication for a given treatment condition and validation method, and $\bar{x}$ is the mean log reduction (log CFU/g) for a given treatment condition and validation method.

RESULTS

Background microflora, initial inoculum levels, and net lethality. The total aerobic plate count (APC Petrifilm, 3M Corporation, St. Paul, MN) for the preinoculated almonds was 2.5 to 3.1 log CFU/g, but there were no *Salmonella* colonies detected on the modified TSAYE. Bacterial populations in the *Salmonella* and *Enterococcus* inocula were 11.0 ± 0.23 and 10.3 ± 0.08 log CFU/ml, respectively. The postinoculation, preequilibration populations on the almonds were 8.8 ± 0.23 and 8.2 ± 0.14 log CFU/g for *Salmonella* and *Enterococcus,* respectively. The corresponding postinoculation, postequilibration, pretreatment populations were 8.3 ± 0.14 and 7.7 ± 0.35 log CFU/g, respectively, indicating a very small population decline during equilibration. Across all of the thermal treatments in the study, the actual total *Salmonella* lethality was a log reduction of 4.4 ± 1.3 (mean ± SD).

Temperature profiles. Generally, aluminum almonds measured the process temperature with a better repeatability when compared to the measurements using the instrumented raw almonds, due to the variability associated with inserting the thermocouple tip into the almond surface (Fig. 2). Additionally, because the thermocouple tip in the actual almond was, by necessity, slightly below the almond surface, the resulting measured temperature tracked below the aluminum almond temperature (and the true almond surface temperature) and, therefore, yielded a more conservative lethality prediction. When comparing the dry condition (Fig. 1a) with the humid conditions (Fig. 1b), a unique condensation-evaporation transition point is distinctively observed in the aluminum almond profile. At this inflection point, the condensed water on the surface of almond starts to be evaporated as the surface temperature increases past the dew point.

Repeatability of validation methods. Overall, the validation methods based on the time-temperature models, when compared with the biological validation methods, yielded a much better (~30% lower) repeatability (equation 5) regardless of the type of model used (Table 1). This indicates less variability resulting from a validation based on good quality temperature measurement and model predictions than from a validation using *Salmonella* or *E. faecium* (biological predictions). Although the use of surrogate organisms tends to be preferred in industry process validations, these results suggest that better validation repeatability can be obtained when a reliable temperature measuring technique is possible and is coupled with robust inactivation models.

Accuracy of validation methods. The biological and mathematical model accuracies (RMSE) were compared with respect to initial product a_ws (Table 2). The surrogate and modified model had the lowest error, and the traditional

TABLE 1. *Repeatability (standard error of replication) of the biological and time-temperature model–based validations*[a]

Validation type	Method	Repeatability (log reduction, log CFU/g)
Biological	*Salmonella*	0.75
	E. faecium	0.46
Temp model	Traditional model	0.24
	Modified (MSU) model	0.26

[a] Biological model validations ($n = 128$); time-temperature model–based validations ($n = 430$). For both model-based methods, the values based on the aluminum almond temperatures were used.

D-z model had an unacceptably high error (i.e., >3.4 log). Although the surrogate (i.e., the biological validation method) had a higher replication error than the model-based predictions, the accuracies of the validations based on the surrogate (average RMSE = 1.3) and the modified model (average RMSE = 1.4 and 1.7 for the aluminum and actual almonds, respectively) were very similar. From a processing perspective, this means that either method can be used for equally accurate process validations, keeping in mind that differences in replication error might necessitate an increased number of replications to achieve equivalent statistical certainty for a given process validation. In terms of the two different temperature measurement methods (i.e., aluminum almond versus instrumented raw almond), both yielded comparable RMSE results, with slightly higher values for the actual almond surface temperature, likely due to the additional experimental variable of thermocouple location.

Effects of product and process conditions on model performance. The accuracies of the models with different temperature measurement techniques also were compared with respect to process temperature or humidity (Table 2). For each process temperature and humidity, lethality calculations based on the aluminum almond temperature showed better accuracy (RMSE) than the calculations based on the instrumented raw almonds, across all of the models. Also, the modified model *(15)* outperformed the traditional model, with 2 to 3 times better overall accuracy. As expected, the modified model showed consistently better accuracies in high humidity ranges (30 to 70% Mv), with the traditional model showing comparable accuracies only for the dry oven condition (<5% Mv). Although the overall prediction accuracy of the surrogate method was the best, the predictive model based method (based on the modified model) was reasonably close to the surrogate validation result in terms of RMSE (Table 2). These results showed the robustness of the modified model and its reliability as a process validation tool for the full humidity range.

For low-moisture products, the performance of validation methods is inevitably affected by changes in product a_w and process humidity condition, which also was assessed in terms of the bias (Fig. 3), with the following observations. (i) The residuals were significantly affected by initial a_w, process humidity, process temperature, and validation method ($P < 0.001$). (ii) There was a significant interaction ($P < 0.001$) between process humidity and validation method. (iii) Validations based on the modified (MSU) model were statistically equivalent to the surrogate-based validations ($\alpha = 0.05$), with biases not significantly different from zero ($\alpha = 0.05$). (iv) Validations based on the

TABLE 2. *Comparison of the prediction accuracy (RMSE) for five different process validation methods for* Salmonella *inactivation on almonds, when grouped by almond initial water activity, oven dry bulb, or oven dew point, and the overall RMSEs*[a]

	RMSE (log CFU/g)[b]				
	Surrogate	Traditional model		Modified (MSU) model	
Conditions	*E. faecium*	AA	RA	AA	RA
Water activity					
0.24	1.2	3.4	3.6	1.1	1.1
0.45	1.1	3.7	3.9	1.4	1.8
0.58	1.4	4.1	4.3	1.4	1.7
0.78	1.6	4.4	4.7	1.5	2.3
Dry bulb temp (°C)					
121	1.2	3.6	3.7	1.2	1.3
149	1.3	4.2	4.4	1.6	2.1
177	1.5	4.0	4.5	1.2	2.0
Dew point temp (°C)					
<33.0 (dry)	1.1	1.3	2.5	1.2	2.5
69.4 (30% Mv)	1.5	4.3	4.7	1.3	1.9
81.6 (50% Mv)	1.3	4.7	4.8	1.5	1.7
90.6 (70% Mv)	1.3	3.8	3.8	1.3	1.3
Overall	1.3	3.9	4.2	1.4	1.8

[a] RMSE, root mean squared error; AA, aluminum almond; RA, instrumented raw almond; %Mv, moisture by volume (%Mv = p_w/p_T, where p_w is the partial pressure of water vapor and p_T is the total pressure, which is atmospheric pressure).
[b] Models were based on previously published data *(15)*.

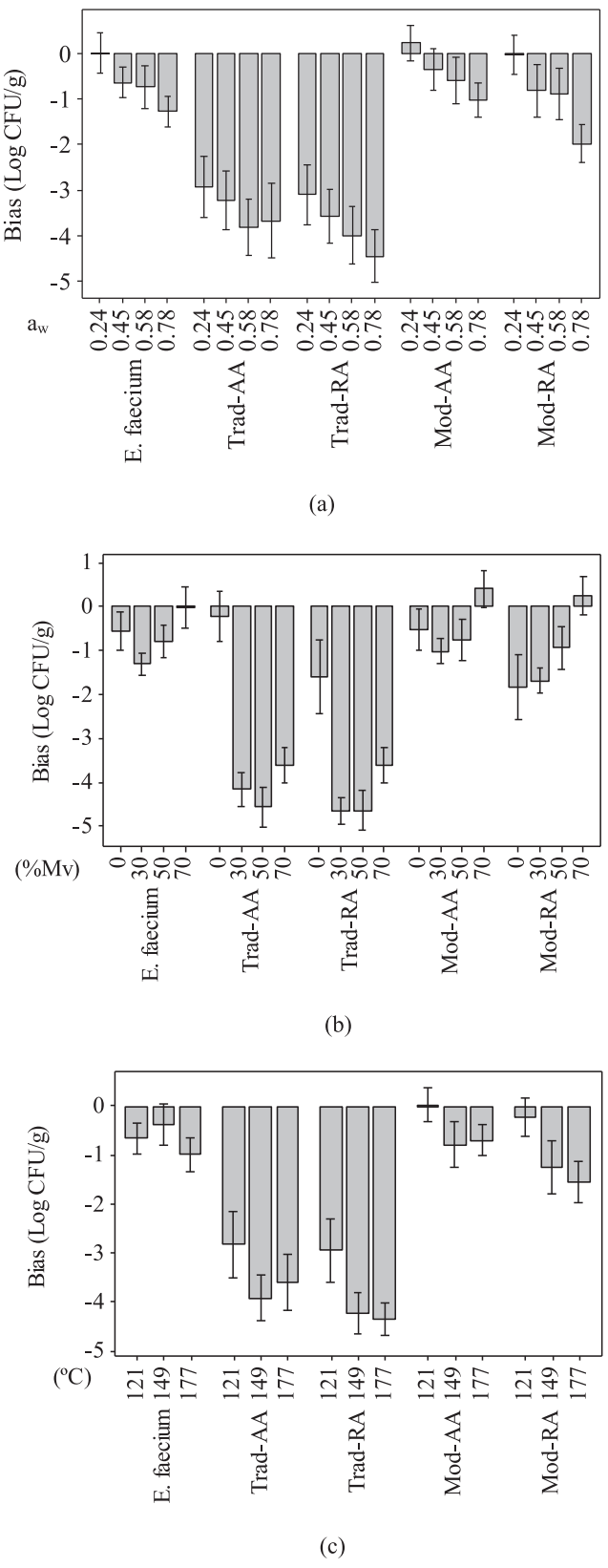


FIGURE 3. *The effects of initial almond water activity (a_w) and oven humidity on the prediction bias (mean residuals ± 95% CI) for process lethality (i.e., log reductions) based on the surrogate, the traditional model, and the modified (MSU) model, all versus actual* Salmonella *lethality (bias <0 implies overestimation; bias >0 implies underestimation by process validation method). (a) The effect of water activity; (b) the effect of oven humidity (%Mv); (c) the effect of oven temperature. Trad, traditional model; Mod, modified (MSU) model; AA, aluminum almond; RA, instrumented raw almond.*

traditional *D*-*z* model yielded nonzero ($P < 0.05$) negative bias values (i.e., underpredictions). (v) Last, although the traditional (*D*-*z*) inactivation model yielded significant, fail-dangerous predictions in this application, the process validations based on the modified model and surrogate were both statistically equivalent to the actual *Salmonella* lethality across the range of thermal treatments applied in this study.

DISCUSSION

Few prior studies have documented the accuracy, uncertainty, or repeatability of pathogen-reduction validations at the pilot or commercial scale for low-moisture products, which was the motivation for the present project. Recently, several studies have documented validations of baking processes; although the formulated products did not have low a_ws, *Salmonella* was introduced into the products by inoculating low-moisture ingredients (e.g., flour or seeds) prior to product formulation. Channaiah et al. *(7)* validated a hamburger baking process (for both *Salmonella* and *E. faecium*), in which they documented process sufficiency and acceptability of the surrogate. They also documented *D*- and *z*-values for both organisms in a formulated dough. Similarly, Shrestha et al. *(23)* compared the efficacy of dry and humid baking processes on *Salmonella* reduction in bread and on sesame seeds. They documented significant humidity-enhanced lethality and also reported surviving *Salmonella* on sesame seeds on bread surfaces after dry baking. However, neither of the above studies applied inactivation models to compute a predicted lethality for the nonisothermal, non–iso-moisture processes. The results of both studies did indicate standard deviations of lethality outcomes that were on the order of ~1 log, which is on the same order of magnitude as the *Salmonella* outcomes in the present study. Such results support the importance of understanding the true variability inherent to any validation method, in order to set appropriate levels of confidence in process outcomes.

Although biological validation (i.e., using a nonpathogenic surrogate organism) has been regarded as a reliable validation method, the validation based on a time-temperature model using the modified lethality model *(15)* in the present study was statistically equivalent to the *E. faecium* biological validation and was more accurate than the traditional model. In the same context, the repeatabilities of the validations based on a time-temperature model (traditional and modified) were equivalent and were much better than the biological methods (*Salmonella* and *E. faecium*). However, given that initial product a_w slightly affected the accuracy of the modified inactivation model, accurate and consistent validation outcomes may require measurement and utilization of process humidity and dynamic product a_w data, in addition to time-temperature data. As such, the ideal secondary inactivation model for

thermal pasteurization of low-moisture foods may need to account for both process humidity and product a_w (and/or moisture content).

Overall, the results of this study should not be used to conclude that any specific validation method for thermal pasteurization of low-moisture foods is universally the best method. The selection of a validation method depends on a variety of factors, including cost, feasibility of temperature measurements, accessibility to microbiological facilities, etc. However, the results of this study should be helpful to processors in selecting a suitable validation method and applying that method to yield reliable results to ensure product safety. Although almonds were the test case in this study, the general observations should be applicable to pathogen reduction processes for other nuts (and other low-a_w products, in general) that have already experienced problems with *Salmonella* or that are at risk for similar problems in the future, such as peanut products, pistachios, walnuts, or cereal grain products *(13),* and that will be subject to FSMA preventive controls rules. Ultimately, the results of this study demonstrate that variability and uncertainty are critical prerequisite information when choosing and applying a particular process validation methodology.

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