CPS CENTER for PRODUCE SAFETY

CPS 2014 RFP
FINAL PROJECT REPORT

Project Title
Improving pasteurization validation methods for pistachio processing

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Objectives
1. *To develop and test process modifications suitable for existing processing equipment (e.g., flat bed or rotary roasters) that could improve achievement of preventive controls (i.e., log reductions of Salmonella), particularly aimed at achieving lowest-cost solutions for smaller processors.*

2. *To develop, test, and disseminate a "Guidelines for Validation of Pistachio Pathogen Reduction Processes" document to help processors effectively validate various treatments, including dry roasting and thermal pasteurization, via either time-temperature calculations or the use of a non-pathogenic surrogate (Enterococcus faecium).*

FINAL REPORT

Abstract

The new FDA Preventive Controls for Human Foods Rule requires food processors to validate pathogen-reduction steps. Some thermal processes, such as pistachio roasting, are not yet well-characterized with respect to the impact of product and process variables on *Salmonella* lethality. The overall goal of this project was to improve the methods for validating pathogen-reduction processes for pistachios, with particular attention to improving existing processes and enabling processors to reliably validate those processes. This included quantifying and modeling the effects of product and process factors on *Salmonella* lethality for in-shell pistachios, testing those models and a non-pathogenic surrogate (*Enterococcus faecium*) in pilot-scale thermal processes, and developing guidance documents for process validation. In-shell pistachios were inoculated with *Salmonella* Enteritidis PT30 (~8.5 log CFU/g), and thermally treated (dry or presoaked) in both lab-scale and pilot-scale (flat-bed) ovens at various levels of air temperature, process humidity, and product moisture. *Salmonella* survivors, pistachio moisture content, and pistachio water activity were quantified at multiple time points during each treatment. Increasing process temperature or dew point increased *Salmonella* inactivation rates ($P < 0.05$). For dry and presoaked treatments, analyzed separately, initial product moisture content did not affect inactivation rates ($P > 0.05$), which is a novel and important result, relative to establishing critical control factors in process validations. Additionally, when comparing dry against presoaked treatments, inactivation rates were significantly greater ($P < 0.05$) for the presoaked pistachios, which also is especially important in designing and validating roasting processes. Mathematical models then were developed to predict *Salmonella* inactivation in pistachios as a function of product temperature, moisture, and process humidity. The resulting model root mean squared errors were slightly less for dry pistachios (0.82–0.97 log CFU/g) than for presoaked pistachios (0.89–1.06 log CFU/g); however, overall, these models successfully described the effect of product and process variables on *Salmonella* inactivation and are the first published attempts to model pathogen reduction in low-moisture products as a function of multiple dynamic variables. Pilot-scale challenge studies then were conducted to compare *E. faecium* and *Salmonella* lethality, validate the aforementioned models, and quantify lethality variability. Overall, the predictive model and *E. faecium* both were conservative predictors of actual *Salmonella* lethality across all treatments, with mean under-predictions of lethality of ~2 logs. Additionally, the variability of the *Salmonella* lethality outcomes in the pilot-scale trials (~1.5 logs) was much larger than that computed from representative surrogate-based lethality data from commercial flat-bed pistachio roasters (<0.3 log). Based on all of these findings, the impact of product and process factors on *Salmonella* inactivation in pistachios must be considered when designing and validating industrial thermal processes for pathogen reduction. Lastly, pistachio-specific validation guideline documents have been written, which are based on prior research results, interactions with industry partners, and incorporation of these latest research results. These guidelines will be field-tested and updated based on feedback from processors and process authorities working in this area. The ultimate impact of the project will be improved methods for validating pathogen reduction in pistachio thermal processes.

Background

Microbial safety of low-moisture foods is a particularly difficult challenge, as reflected in recent outbreaks and/or recalls associated with *Salmonella*-contaminated nuts and other low-moisture products. Therefore, processing interventions are an emerging imperative to reduce the risk of *Salmonella* in low-moisture products, including pistachios. The new Food Safety Modernization Act (FSMA) Preventive Controls for Human Food rule mandates that the low-moisture food industry implement and validate interventions against identified hazards, such as *Salmonella*. Although a number of pathogen-reduction technologies are available to the pistachio industry (e.g., dry heat, steam, radio-frequency), there are several significant problems: (1) no single technology will be universally applicable, so that product-specific/scalable solutions are needed; (2) the cost of stand-alone pasteurization technologies is an impediment to small processors; and (3) robust validation protocols have not been widely tested or disseminated. Therefore, the overall goal was to improve the methods for validating pathogen-reduction processes for pistachios, with particular attention to improving existing processes and enabling processors to reliably validate those processes. The specific objectives were:

1. To develop and test process modifications suitable for existing processing equipment (e.g., flat bed or rotary roasters) that could improve achievement of preventive controls (i.e., log reductions of *Salmonella*), particularly aimed at achieving lowest-cost solutions for smaller processors.
    a. To quantify the interaction of salt, product water activity, and temperature on inactivation of *Salmonella* during pistachio dry roasting.
    b. To quantify the impact of dynamic humidity control on *Salmonella* lethality during pistachio dry roasting.
    c. To validate the findings from 1a and 1b via pilot-scale, *Salmonella*-inoculated flat-bed roasting studies in the MSU Biosafety Level-2 Pilot Plant.
2. To develop, test, and disseminate a "Guidelines for Validation of Pistachio Pathogen Reduction Processes" document to help processors effectively validate various treatments, including dry roasting and thermal pasteurization, via either time-temperature calculations or the use of a non-pathogenic surrogate (*Enterococcus faecium*).
    a. To quantify, via pilot-scale and commercial-scale testing, the inherent process variability in pistachio pasteurization and roasting operations.
    b. To verify *E. faecium* as a non-pathogenic surrogate for *Salmonella* via inoculated pilot-scale roasting trials.
    c. To develop a science-based validation guidelines procedure that accounts for both process variability and lethality uncertainty to generate statistically-sound validation outcomes.
    d. To introduce and test the validation guidelines via a full-scale field demonstration test at one or more commercial pistachio facilities (with the industry collaborators).

Research Methods and Results

The work plan encompassed (1) laboratory-scale experiments subjecting *Salmonella*-inoculated pistachios to a range of thermal (oven) treatments; (2) development of mathematical models to describe *Salmonella* inactivation in terms of product temperature, moisture, humidity, and time; (3) pilot-scale roasting experiments with pistachios inoculated with *E. faecium* or *Salmonella*; and (4) the development of process validation guidelines. This section will summarize the overall methods for each of these project components.

Methods - Laboratory-scale experiments

Overview of experiments. Pistachios were equilibrated to different water activities (a_w), and either directly thermally treated, or first subjected to a presoaking process (to mimic commercial brining), and then thermally treated (Figure 1). Various levels of process temperatures and humidities were used. Upon completion of thermal treatment, *Salmonella* survivors, product a_w, and moisture content were all quantified, and a statistical model was applied to determine the impact of the different product and process factors evaluated.

Pistachios. Raw, in-shell pistachios (21/25 US#1) were obtained from a commercial processor and stored at 4°C for up to 3 mos.

Bacterial strain and inoculation. Pistachios were inoculated with *Salmonella* Enteritidis phage type 30 (ATCC BAA-1045), which was previously shown to be thermally resistant in almond products and to have a similar thermal resistance to strains isolated from pistachios (*S.* Montevideo and *S.* Senftenberg). The culture was stored at −80°C in tryptic soy broth supplemented with 0.6% (wt/vol) yeast extract (TSBYE) and 20% (vol/vol) glycerol until use.
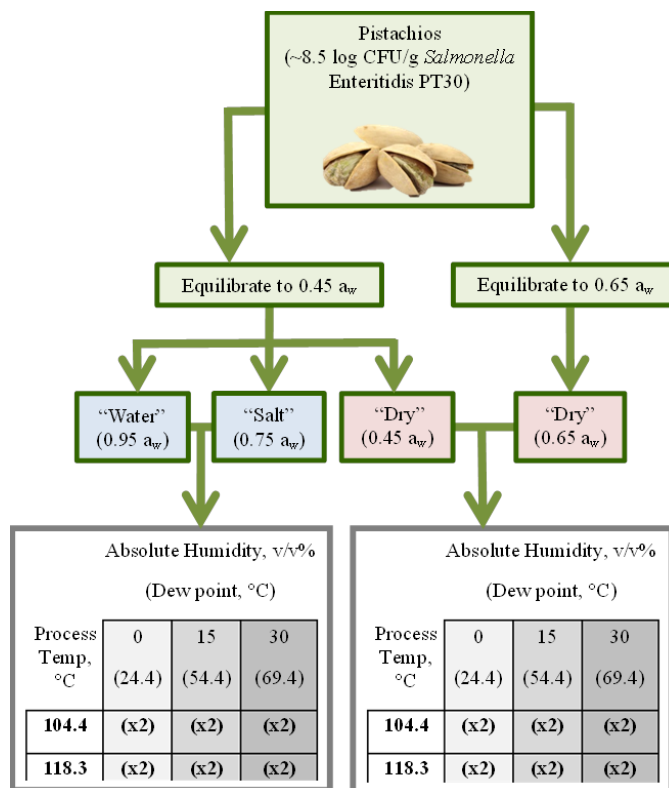
Pistachios
(~8.5 log CFU/g *Salmonella* Enteritidis PT30)

Equilibrate to 0.45 a_w | Equilibrate to 0.65 a_w

"Water" (0.95 a_w) | "Salt" (0.75 a_w) | "Dry" (0.45 a_w) | "Dry" (0.65 a_w)

Process Temp, °C	Absolute Humidity, v/v% (Dew point, °C)		
	0 (24.4)	15 (54.4)	30 (69.4)
104.4	(x2)	(x2)	(x2)
118.3	(x2)	(x2)	(x2)

Process Temp, °C	Absolute Humidity, v/v% (Dew point, °C)		
	0 (24.4)	15 (54.4)	30 (69.4)
104.4	(x2)	(x2)	(x2)
118.3	(x2)	(x2)	(x2)

Figure 1. Overall design of lab-scale experiments, showing sample flow through inoculation, equilibration, pretreatment (i.e., "brining"), and oven conditions.

Pistachios were inoculated according to previously published methods, with minor modifications. For each inoculation conducted, a new culture was started from frozen stock. In preparation for inoculation, two consecutive transfers (24 h each at 37°C) were conducted in TSBYE. The second transfer was spread on three tryptic soy agar plates (150 × 15 mm) supplemented with 0.6% (wt/vol) yeast extract (TSAYE) and incubated for 24 h at 37°C. The three resulting lawn cultures were each harvested with 10 ml sterile 0.1% peptone water (Difco, BD, Franklin Lakes, NJ), and transferred to a sterile container, for a final volume of 25 ml inoculum. The inoculum was added to 400-g batches of in-shell pistachios in a large plastic bag that was sealed, and the resulting mixture was mixed thoroughly by hand. The inoculated pistachios were spread on a tray covered with filter paper and dried overnight in a biosafety cabinet (~20°C). Inoculum homogeneity was tested by plating six subsamples (15.1±1.1 g each) from a 400-g batch of inoculated pistachios.

Equilibration. The inoculated pistachios were held in computer-controlled humidity chambers (3+ days) to equilibrate the samples to target a_w levels of 0.45±0.02 or 0.65±0.02, corresponding to 6.0%±0.2% and 8.9%±0.2% moisture content (dry basis), respectively. Equilibration was confirmed by duplicate measurements immediately before thermal treatment, using an a_w meter (AquaLab 3TE, Decagon Devices, Pullman, WA). Samples were used within 14 days of inoculation. *Salmonella* decline was insignificant during this time. Initial *Salmonella* populations, immediately prior to thermal treatments, were 8.5±0.5 log CFU/g across all inoculated batches.

Presoak treatment. For samples that received a presoak treatment, inoculated pistachios were immersed for 30 s in either deionized water (resulting in an initial a_w of 0.94±0.02, and 21.3%±1.8% moisture content, dry basis) or a 27% NaCl solution (resulting in an initial a_w of 0.77±0.02, and 17.4%±3.1% moisture content, dry basis), drained of excess liquid, then immediately heated. This process, for the NaCl solution, closely matched a typical commercial brining process.

Thermal treatment. Thermal treatments were conducted in a custom, laboratory-scale, moist-air convection oven system. The sample chamber (10 × 10 × 10 cm; Figure 2) contained a two-tiered rack that allowed for two 20-g samples to be treated simultaneously. Treatments were divided into two categories, dry and pre-soaked, with a total of six oven conditions per category, run in duplicate (Figure 1). Samples were treated at two process temperatures (nominally 104.4 and 118.3°C, with an acceptable operational tolerance of ±2°C), three process humidities (~3%, 15%, and 30% moisture by volume, corresponding to dew points of 24.4, 54.4, and 69.4°C, respectively, with an acceptable operational tolerance of ±4°C) and one commercially-relevant air velocity (1.3±0.2 m/s) for a

Figure 2. Inoculated pistachios in lab-scale convection oven.

total of six treatments in duplicate for each of the two treatment categories. The absolute humidity was reported in terms of dew point and was measured with a dew point sensor (DMP246, Vaisala, Woburn, MA). Air velocity, flowing across the single layer of pistachios on the wire racks, was measured using a hot-wire anemometer (Model 407123, Extech Instruments, Nashua, NH).

For treatments using dry pistachios (i.e., no presoak), samples at both initial a_w levels (0.45 and 0.65) were treated simultaneously, and each a_w was randomly assigned to one of the two racks. For the presoaked treatments, preliminary trials showed that initial a_w equilibration before presoaking had no effect on *Salmonella* reduction, so all samples were processed using pistachios equilibrated to a_w of 0.45±0.05. Each of the presoak treatments was randomly assigned to one of the two sample racks. Treatments were randomized within each replication.

Product surface temperature, product a_w, product moisture content, process temperature, and process dew point were monitored throughout the process. Pistachio surface temperature was measured at a frequency of 0.5 Hz by inserting a thin-wire (0.13-mm diameter) thermocouple (5TC-TT-K-36-36, OMEGA Engineering Inc., Stanford, CT) between the shell and the nut at the base of the crack. The thermocouple position was ensured by visual measurement of the length needed to reach the specified location. Additionally, the thermocouple was fixed with a thin zip tie to ensure it was fixed during processing. For each treatment condition, samples were heated for six equally spaced durations, for a total treatment time nominally aimed to achieve a 3- to 5-log reduction of *Salmonella*. The six samples for each condition were run independently, in randomized order. Samples (19.3±0.6 g) were removed from the oven, with a 15.6±0.6 g subsample used for microbial analysis, and the remaining 3.7±0.6 g subsample used for a_w and moisture content analyses (102±2°C for 18±2 h in a laboratory convection oven).

Enumeration of survivors. Treated pistachios were immersed in chilled sterile 0.1% peptone water immediately after thermal treatment, resulting in a nominal 1:1 dilution. Samples were stored in a cooler on ice (0°C) until enumeration (0 to 4 h). Each sample was shaken by hand for 30 s, massaged by hand for 30 s, and shaken again by hand for 30 s. For *Salmonella* enumeration, appropriate ten-fold serial dilutions were plated in duplicate on tryptic soy agar (Difco, BD) supplemented with 0.6% (wt/vol) yeast extract, 0.05% ammonium ferric citrate, and 0.03% sodium thiosulfate (Sigma-Aldrich, St. Louis, MO) (mTSA). After 48±4 h of incubation at 37±2°C, all black colonies were counted as *Salmonella*, and populations were converted to log CFU/g. Log reductions were calculated by subtracting survivor counts from the initial population prior to heating ($t = 0$ sample processed on that day).

Statistical analyses. Salmonella survivor counts (log CFU/g) were first analyzed by analyses of variance (*anovan* in MATLAB; 2014, Natick, MA). The independent variables were time, process temperature, process humidity (measured as the dew point temperature), and initial product a_w (measured at room temperature). Only main effects and two-way interactions of the four variables were considered in the analysis. All independent variables were considered to be continuous.

Two additional two-way ANOVA tests were conducted to determine the impact of initial a_w within dry and presoaked groups. One ANOVA considered *Salmonella* population data only from the dry (not presoaked) samples at 0.45 and 0.65 initial a_w, and a second ANOVA considered data only from presoaked samples (pure water and 27% NaCl solution).

Methods - Mathematical model development

In addition to the statistical analyses described above, the data from the laboratory-scale trials also were used to develop/test multiple microbial inactivation models. Each model was either used directly or adapted from previously published attempts to model *Salmonella* D-values as a function of temperature, combined with either process humidity, product moisture, or both. Product moisture was quantified as either a_w or percent moisture content (dry basis). Each secondary model for the D-value was incorporated into the integral of a log-linear primary model, such as:

$$\log N/N_0 = -t/D[T(t), mc(t), T_{dewpoint}] \qquad (1)$$

Attempts were made to fit models to the entire aggregated data set from all treatments; however, the model parameters did not converge to reasonable values; therefore, based on the results of the prior ANOVAs, the models were fit to two subsets of data, according to whether the pistachios were dry (0.45 or 0.65 initial a_w) or pre-soaked (0.77 or 0.94 initial a_w).

Parameters were subsequently estimated globally using *nlinfit*, an ordinary least squares nonlinear regression algorithm in MATLAB. The five secondary models that were evaluated were the modified Michigan State University (MSU) model:

$$D_{T,T_d}(t) = D_{ref} \times 10^{\frac{T_{ref}-T(t)}{z_T}+\frac{(T_{d,ref}-T_d)-(T_{ref}-T(t))}{z_M}} \qquad (2)$$

a traditional log-linear temperature model that incorporates log-linear a_w effects:

$$D_{T,a_w}(t) = D_{ref} \times 10^{\frac{T_{ref}-T(t)}{z_T}+\frac{a_{w,ref}-a_w(t)}{z_{a_w}}} \qquad (3)$$

a similar model, replacing a_w with moisture content (MC):

$$D_{T,MC}(t) = D_{ref} \times 10^{\frac{T_{ref}-T(t)}{z_T}+\frac{MC_{ref}-MC(t)}{z_{MC}}} \qquad (4)$$

the modified MSU model, incorporating the log-linear form for a_w:

$$D_{T,T_d,a_w}(t) = D_{ref} \times 10^{\frac{T_{ref}-T(t)}{z_T}+\frac{(T_{d,ref}-T_d)-(T_{ref}-T(t))}{z_M}+\frac{a_{w,ref}-a_w(t)}{z_{a_w}}} \qquad (5)$$

a similar model, replacing a_w with MC:

$$D_{T,T_d,MC}(t) = D_{ref} \times 10^{\frac{T_{ref}-T_s(t)}{z_T}+\frac{(T_{d,ref}-T_d)-(T_{ref}-T_s(t))}{z_M}+\frac{MC_{ref}-MC(t)}{z_{MC}}} \qquad (6)$$

For each parameter, the 95% confidence interval and percent relative error were calculated. Also, each overall model fit was evaluated by the root mean squared error (RMSE) and the corrected Akaike Information Criterion (AIC_C). A lower RMSE indicated a smaller error. A lower AIC_C, when comparing two candidate models, indicates the more-likely-correct model.

Methods – Pilot-scale experiments

For the pilot-scale experiments, the same sample preparation, equilibration, inoculation, and enumeration procedures were followed as described above for the laboratory-scale treatments. The pilot-scale, moist-air impingement oven in the MSU Biosafety Level-2 Pilot Plant (Figure 3) enabled simulation of commercial-scale thermal processing conditions with controlled temperature, humidity, air velocity, and conveyor speed through the oven. In-shell pistachios were inoculated *Salmonella* Enteritidis PT30 or *Enterococcus faecium* (NRRL B-2354), using methods previously reported. *E. faecium* strain NRRL B-2354 has a long history of use for validation of thermal processes for almonds and other foods. This *E. faecium* strain was chosen as the non-pathogenic surrogate for *Salmonella* on pistachios because it has similar thermal resistances on in-shell

Figure 3. Pilot-scale roasting/pasteurization trials.

pistachios in hot oil or hot water (previous study). The inoculated pistachios were placed in a single layer on the conveyor. Four treatments (representing the extreme corners of the overall experimental design) were replicated six times, nominally targeting a 4-log reduction in *Salmonella*. The four treatments (all at 118°C air temperature) were: (1) dry/unbrined nuts in dry air (~16°C dew point); (2) dry/unbrined nuts in humid air (69°C dew point); (3) salt brined nuts in dry air (~16°C dew point); and (4) salt brined nuts in humid air (69°C dew point). The bacterial survivor data, dynamic product temperature, dynamic product moisture content, and process humidity were used to validate the models previously described, compare the surrogate and pathogen outcomes, and quantify process variability.

Methods – Guidance documents

The project had three key partners: Horizon Nut, Buchanan Hollow Nut Company, and the Administrative Committee on Pistachios. Horizon Nut provided critical information, data, and materials for this project. Specifically, they provided: (1) the pistachios used the project; (2) the range of typical brining and roasting conditions, which were used in designing the lab- and pilot-scale thermal treatments; and (3) commercial-scale validation data and reports, which the team has used to evaluate typical process parameters being measured in validation studies and the degree of variability in the reported outcomes. Horizon Nut hosted the project team for two on-site visits and tours of their processing operations, which was extremely helpful in getting the project team up-to-speed on critical factors in flat-bed roasting and pasteurization operations. Buchanan Hollow Nut also hosted the project team for two on-site visits/tours. They provided information and data on operational conditions for rotary roasters, which was helpful in assessing the potential impact of the project research results on different types of roasting systems and different scales of processing operations. The Administrative Committee on Pistachios also organized and hosted a meeting of industry representatives in July 2016, in which the project team reported preliminary results and collected feedback from industry representatives on the relevance and application of those results.

The validation guideline documents have been prepared following the general outline and structure of multiple validation guidelines published by the Almond Board of California. Those guidelines have been in place and updated over a number of years, and are widely referenced in the low-moisture industry. However, they are designed specifically for almond processes. Therefore, the information collected from the industry partnerships in this project, and the research results that have been generated, were used to draft pistachio-specific guidance documents (described further below) for carrying out validations of pathogen-reduction processes.

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Outcomes and Accomplishments

Results – Laboratory-scale experiments

The overall experimental design resulted in ~144 microbial inactivation results across all the treatments. Each treatment entailed dynamically increasing pistachio temperature, decreasing moisture pistachio moisture, and declining *Salmonella* populations (Figure 4).
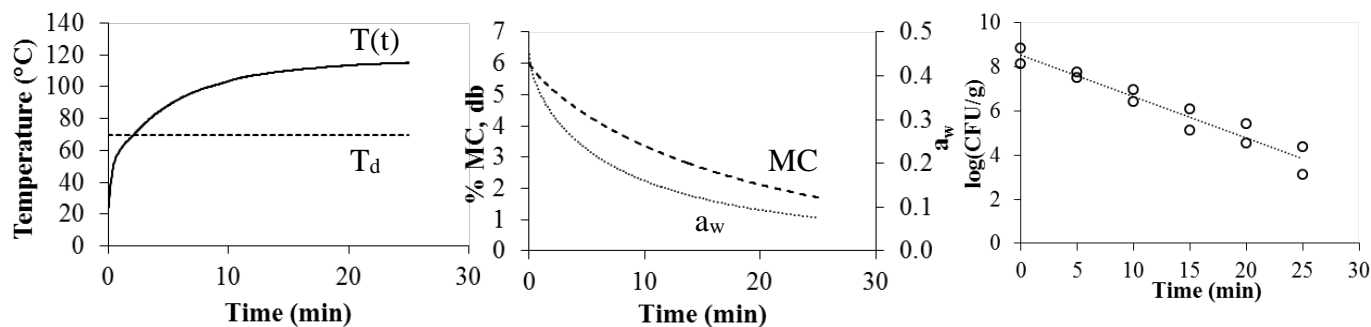

Figure 4. Example temperature (dry bulb and dew point), moisture, and Salmonella *population changes during a lab-scale heating trial (118ºC dry bulb, 69ºC dew point).*

Overall, the results indicated that increasing temperature, humidity, and brining (presoaking) increased *Salmonella* inactivation during hot-air heating (Figure 5). The statistical significance of each of these effects was confirmed through ANOVA tests ($P < 0.05$).

Specifically, increasing process humidity from dry to ~30% moisture by volume (69°C dew point) approximately doubled the *Salmonella* lethality (Figure 5a). Increased inactivation with increasing humidity likely is partially due to vapor condensation on the product when its surface temperature is below the air dew point. With higher humidity, the condensing stage lasts longer. However, even after the surface temperature of the pistachio reached the dew point temperature, inactivation was still enhanced by increased humidity, thus supporting the premise that humidity affects *Salmonella* lethality, independent of condensation and heating effects. In contrast, the initial water activity (or moisture content) of the pistachios did not affect the lethality outcomes within the dry roast or brined treatments (Figure 5b), suggesting that process humidity was more important than product moisture in affecting process lethality.

Additionally, brining the pistachios for 30 s prior to thermal treatment approximately doubled the rate of *Salmonella* reduction during heating (Figure 5c). However, there was no difference between the pure water control and the 27% salt brine (Figure 5d), indicating that the presence of salt in the presoaking treatment did not affect *Salmonella* inactivation during heating.

Lastly, process lethality did increase with dry bulb temperature (104 vs 118°C) (Figure 5e), although that effect (in the range of the study conditions) was smaller than the humidity or brining effects.

Results – Mathematical models

The primary model used in this study was the log-linear model. Both the Weibull model and the log-linear model were evaluated. However, the Weibull model was rejected because parameter relative errors were unacceptably high (much greater than 15% in most cases).

The most appropriate secondary model for each set of data, dry or presoaked, was chosen from a selection of the five models that quantify the D-value as a function of temperature combined with process humidity, product moisture (a_w or percent moisture content, dry basis), or both (eqn. 2–6). A single model could not be fit for both dry and presoaked pistachios as a whole dataset; rather, the
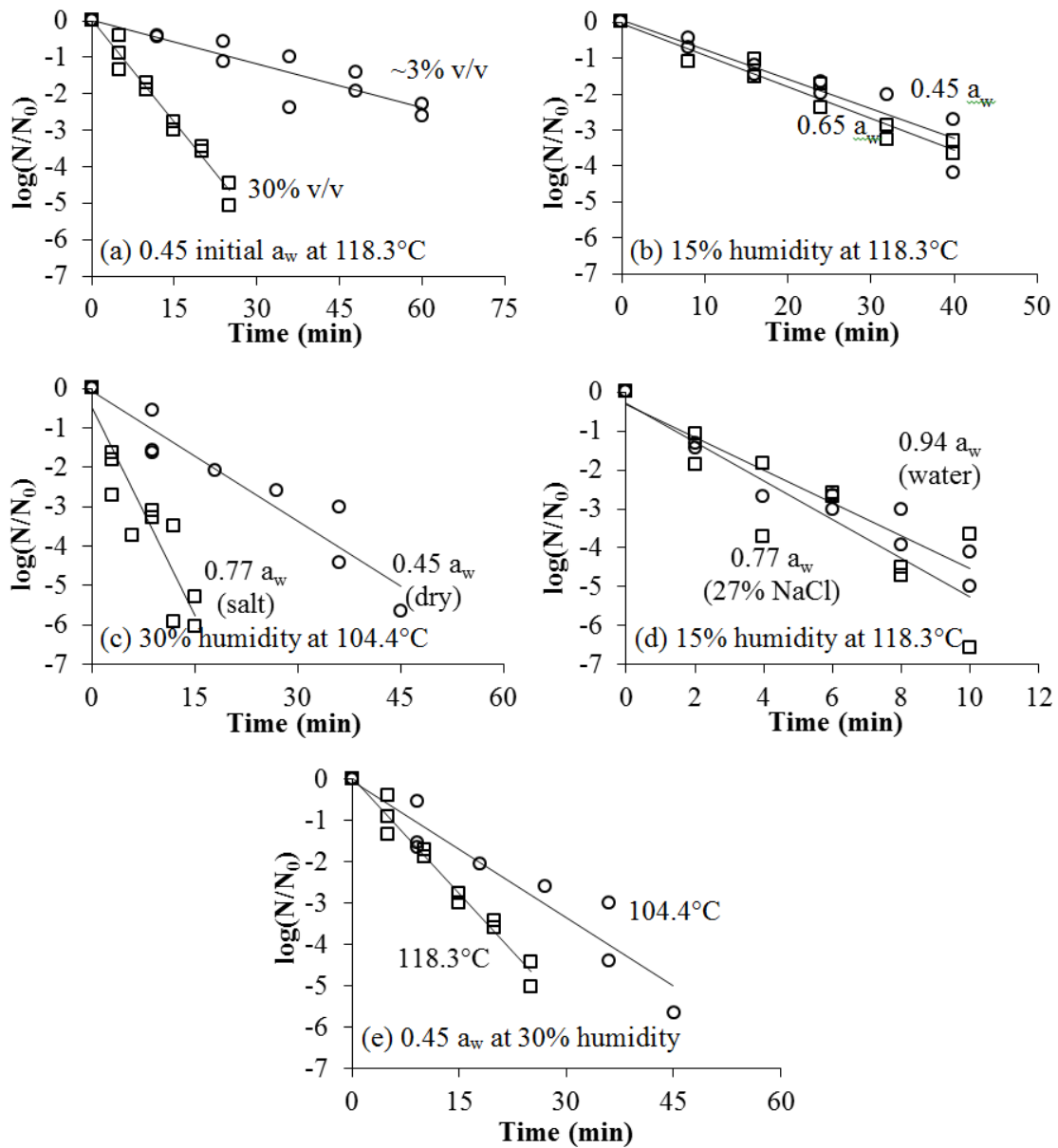
Figure 5. Salmonella *inactivation curves comparing: (a) process humidity, (b) initial a_w for dry pistachios, (c) presoak treatment, (d) addition of NaCl to the presoaking treatment, and (e) temperature.*

data were partitioned into dry and presoaked, and models were fit to each of those categories separately.

Based on the model fitting results (Tables 1–2 in Appendix), all the candidate models worked reasonably well, with RMSE values ~1.0 log or less. The MSU model (eqn. 2) is based on only process humidity and dynamic product temperature, but it yielded satisfactory results, with RMSE of 0.86 and 1.02 log CFU/g for the dry and presoaked pistachios, respectively. Based on the AIC_C results, the overall most-likely-correct model was equation 6, which predicts D as a function of product temperature, product moisture content, and process dew point. In dry pistachios, the AIC_c values for equation 5 (with the a_w term) and equation 6 (with a moisture content term) were similar; however, in presoaked pistachios, the AIC was lower for equation 6 than equation 5, suggesting that

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moisture content is a better metric for describing *Salmonella* inactivation in this type of system. For these reasons, equation 6 was determined to overall be the most likely correct model.

These models are the first published attempt to model *Salmonella* inactivation in a low-moisture food as a function of two dynamic variables (temperature and moisture). In addition, due to the nature of the high-moisture presoak condition, a_w was not the best metric for modeling *Salmonella* lethality. Within the range of conditions evaluated, the model parameters generally fell within reasonable expectations based on published data. Unfortunately, due to the highly variable nature of the data gathered for presoaked pistachios, the parameter relative errors were relatively large (just over 15%), with caution necessary if this model is to be used for process validations. In general, the models developed were conservative in their lethality predictions; however, some systematic process effects were observed.

Results – Pilot-scale experiments

The pilot-scale experiments were designed to compare the non-pathogenic surrogate (*E. faecium*) with *Salmonella*, to quantify lethality variability, and to test model performance. Overall, both the model (eqn. 6) and the surrogate were extremely conservative predictors of actual *Salmonella* lethality overall all the treatments (Figure 6).
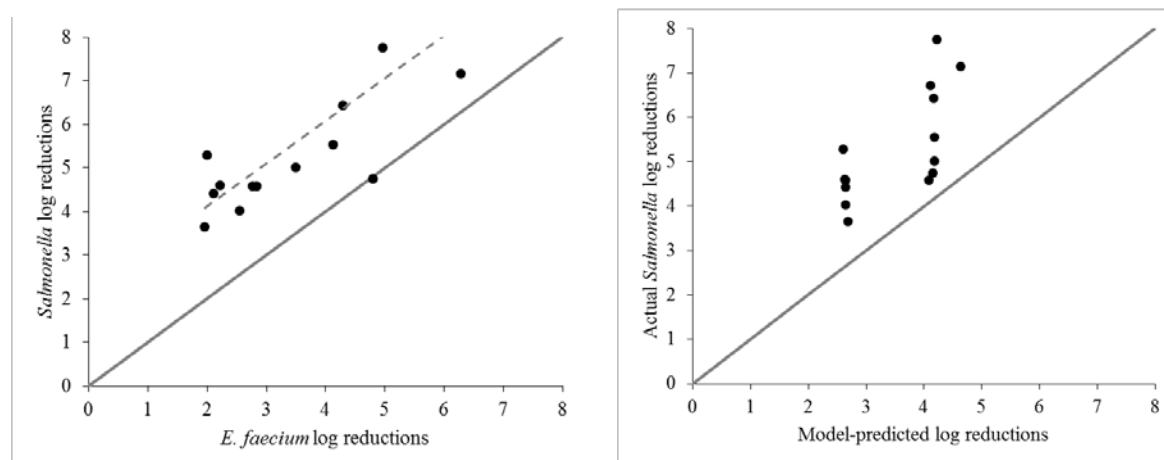

Figure 6.Comparison of actual Salmonella *log reductions in pilot-scale roasting/heating trials to surrogate (*E. faecium*) and model-predicted lethality outcomes.*

Specifically, *E. faecium* under-predicted actual *Salmonella* by a mean value of 2.1 logs. All but one individual test yielded conservative predictions, and that single replication was within 0.06 log. In terms of process variability, the standard deviation of actual *Salmonella* lethality was 1.5 logs, which is a large, but potentially realistic measure of actual process variability that might occur in a commercial system. In terms of the predictive model, there again was a significant conservative bias (~2.2 logs) in predicting the actual *Salmonella* lethality. This was the application of the model (and parameters) estimated from the lab-scale experiments directly to the pilot-scale results. In a recent study by the project PI, the same overall process was followed for almond roasting/pasteurization, and that study yielded similar variability but much closer predictions of pilot-scale outcomes. It is likely that the shell on the pistachio impacts the scalability/applicability of lab-scale inactivation results to pilot- or commercial-scale processes, due to the impact on airflow, water transport, etc.

Results – Guidance documents

Four guidance documents have been drafted and are ready for industry evaluation and testing, as follows:

Guidelines for Validation of Pistachio Pathogen Reduction Processes
1. Biological Hazard Analysis for Pistachios
2. Guidelines for using *E. faecium* in pistachio process validation
3. Guidelines for validation of pistachio dry processes
4. Example validation report

A meeting has been scheduled in the near term with food safety professionals in the pistachio industry, to solicit feedback on the format and content of these guidance documents.

Implementation of these guidelines will continue beyond the end of this project (by the project PI and Co-PI) via support from the industry, and via a major USDA-funded project (led by the PI of this project), focused on enhancing process validation methods across the low-moisture food industry. It is planned to use the implementation of the results from this CPS project as a case study to assess technology decisions, implementation plans, and overall impact on process validation.

Summary of Findings and Recommendations

This project has generated significant new findings, relative to factors affecting pathogen reduction in pistachio thermal processes. The key findings and recommendations are as follows:

1. *Process humidity significantly enhances pathogen lethality.* Pistachio processors should consider monitoring, and perhaps ultimately controlling and enhancing, humidity in the early stages of multi-stage roasting processes, in order to enhance the efficacy of roasting as a pathogen reduction step.

2. *Brining significantly enhances pathogen reduction during roasting.* For roasted, unsalted pistachios, a water pretreatment of some type might be considered, in order to enhance process efficacy. Dry/unbrined nuts in dry roasting conditions yielded, in several conditions, nearly negligible pathogen reduction on the time scales relevant to pistachio roasting.

3. *Initial moisture content may not be a significant factor in process lethality.* Although the data are not yet sufficient to preemptively exclude pistachio moisture content as a critical factor in process validations, they do suggest that, for certain hot-air treatments, it might be possible to do so (with additional supporting evidence).

4. *The non-pathogenic surrogate (E. faecium) appears to be a very conservative tool for validating pistachio hot-air/roasting processes.* Prior work by the project Co-PI has shown a very close correlation between *E. faecium* and *Salmonella* thermal inactivation in water and oil; however, this study suggests that there is a significantly conservative difference in the hot-air treatments.

5. *The predictive microbial inactivation model worked similarly well, with conservative predictions of lethality.* Although not yet sufficient to be the sole tool for a process validation, the predictive model may be a useful tool for evaluating equipment/process modifications (e.g., to improve monitoring and control of existing roasters to support process validations) and for designing validation studies.

6. *The pilot-scale data on lethality uncertainty informs the quantitative microbial risk assessment (QMRA) being published by the project Co-PI.* Using conservative estimates, that study concluded that an overall mean number of cases of pistachio-linked salmonellosis per year in the U.S. would be less than one if a uniform 4 ± 0 log (but not a 3 ± 0 log) reduction were applied to 100% of the domestic product. However, that conclusion increased

to >1 case per year when variability in the pathogen-reduction step increased to 4 ± 1 log reduction. Obviously, there is no such thing as ± 0 uncertainty in real-world processes. Therefore, the lethality uncertainty values reported in the present study suggest that a 1-log uncertainty in lethality outcome is not an unreasonable estimate of the actual results associated with real-world process validations. It is critically important that process controls, monitoring, and variability are properly incorporated into validation strategies for any pathogen reduction process, and that the industry continues to seek ways to maximize the reliability of those processes.

7. ***Next steps.*** Although this project is officially complete, and the team has completed the research tasks proposed, the PI and Co-PI will continue to work on pathogen reduction processes for pistachios, in the following ways:

   a. The Co-PI is meeting in March 2017 with pistachio industry food safety leaders, to solicit feedback on the guidance documents, in terms of content, format, etc.

   b. The Co-PI and PI intend to carry out on-site testing of the guidance documents with (hopefully) two industry partners – one medium/large and one very small processor. The goal will be to "debug" the guidance documents and prepare them for industry-wide/public release.

   c. The PI currently leads a $4.7M USDA-funded project (2015–2020) entitled "Enhancing low-moisture food safety by improving development and implementation of pasteurization technologies." The outreach component of that project includes development of decision-support tools to help the low-moisture food industry evaluate alternatives for pathogen reduction operations, and to reliably validate technologies and processes. The intent is to utilize the results from this CPS project (and the subsequent implementation of the research results and guidance documents) as a unique opportunity to assess impact of new knowledge and tools on industry practices (and ultimately food safety outcomes) within a specific industry sector (i.e., the pistachio industry).

APPENDICES

Publications and Presentations

1. Casulli KE. 2016. Improving pathogen-reduction validation methods for pistachio processing. M.S. thesis. Department of Biosystems and Agricultural Engineering. Michigan State University. East Lansing, MI.
2. Casulli KE, Dolan KD, Marks BP. 2016. Performance of *Enterococcus faecium* as a nonpathogenic surrogate for *Salmonella* during pistachio roasting. Abstract 17. Presented at the Conference on Food Engineering. Columbus, OH. September 12–14, 2016.
3. Casulli K, Garces-Vega F, Dolan K, Harris LJ, Marks B. 2016. Modeling the effect of product temperature, moisture, and process humidity on thermal inactivation of *Salmonella* in pistachios. Abstract P2-19. Presented at the Annual Meeting of the International Association for Food Protection. July 31 – Aug 3, 2016. St. Louis, MO.
4. Casulli, KE, Garces-Vega FJ, Dolan KD, Marks BP. 2016. Impact of temperature, moisture, and humidity on thermal inactivation of *Salmonella* in pistachios heated under dynamic processing conditions. Presented at the XIII Latin American Congress of Microbiology and Food Hygiene. September 27–30, 2016. Medellín, Colombia.
5. Harris LJ and Marks BP. 2016. The impact of water and product composition on pathogen survival and inactivation. Symposium S21. Presented at the IAFP European Symposium on Food Safety. May 11–13, 2016. Athens, Greece.
6. Marks BP. 2016. Validating legacy processing systems: application of thermal resistance data and models to predict log reductions in dynamic processes. Symposium 096. Presented at the Annual Meeting of the Institute of Food Technologists. July 19, 2016. Chicago, IL.

7. Casulli KE, Dolan KD, Marks BP. 2017. Modeling the effect of product temperature, moisture, and process humidity on thermal inactivation of *Salmonella* on pistachios during hot-air heating. *Journal of Food Protection.* To be submitted.
8. Casulli KE, Garces-Vega FJ, Dolan KD, Harris LJ, Ryser ET, Marks BP. 2017. Impact of process temperature, humidity, and initial product moisture on thermal inactivation of *Salmonella* Enteritidis PT30 on pistachios during hot-air heating. *Journal of Food Protection.* To be submitted.

Budget Summary

Overall, the project expenditures have followed the proposed budget quite closely. The only notable difference was that less was spent on travel than expected, with those funds supporting additional salary needed for personnel working on the project, particularly the pilot-scale trials in the MSU Biosafety Level-2 Pilot Plant.

Suggestions to CPS

Overall, the structure of the CPS grants program has ensured accountability that is appropriate for grants of this nature. Additionally, direct engagement with industry partners enhanced project relevance and outcomes. The only suggestion is that it would be helpful to somehow optimize the requirement to write two different reports (CDFA and CPS) for each reporting period. It would be ideal if a single reporting structure could serve the accountability and reporting purposes of both entities.

Tables and Figures

Table 1. Parameter estimates for *Salmonella* inactivation data from dry pistachios using secondary models integrated into the log-linear primary model.

Eqn	Reference Conditions	Parameter	Estimate	Percent Rel. Error	RMSE Log (CFU/g)	Mean of Residuals log(CFU/g)	AIC_c
(2)	$T_{ref}=105°C$ $T_{d,ref}=65°C$	D_{ref} (min)	8.32	4.10	0.86	-0.36	-14.2
		z_T (°C)	19.6	8.73			
		z_M (°C)	38.2	7.99			
(3)	$T_{ref}=105°C$ $a_{w,ref}=0.3$	D_{ref} (min)	4.12	8.64	0.95	-0.17	-8.0
		z_T (°C)	37.1	14.0			
		z_{aw}	0.26	6.59			
(4)	$T_{ref}=105°C$ $MC_{ref}=5\%$ MC	D_{ref} (min)	5.60	6.19	0.97	-0.17	-3.7
		z_T (°C)	31.7	11.9			
		z_{MC} (%MC)	4.64	6.77			
(5)	$T_{ref}=105°C$ $T_{d,ref}=65°C$ $a_{w,ref}=0.3$	D_{ref} (min)	4.35	8.13	0.82	-0.22	-45.9
		z_T (°C)	22.1	8.37			
		z_M (°C)	70.2	13.9			
		z_{aw}	0.41	13.1			
(6)	$T_{ref}=105°C$ $T_{d,ref}=65°C$ $MC_{ref}=5\%$ MC	D_{ref} (min)	5.78	5.94	0.83	-0.23	-41.9
		z_T (°C)	22.0	8.13			
		z_M (°C)	67.6	14.0			
		z_{MC} (%MC)	8.54	16.1			

Table 2. Parameter estimates for *Salmonella* inactivation data from presoaked (brined) pistachios using secondary models integrated into the log-linear primary model.

Eqn	Reference Conditions	Parameter	Estimate	Percent Rel. Error	RMSE Log (CFU/g)	Mean of Residuals	AIC$_c$
(3)	T_{ref}=80°C $T_{d,ref}$=65°C	D_{ref} (min)	3.42	4.31	1.02	-0.22	11.6
		z_T (°C)	92.2	26.3			
		z_M (°C)	55.8	6.22			
(4)	T_{ref}=80°C $a_{w,ref}$=0.7	D_{ref} (min)	4.31	3.99	1.06	-0.13	23.0
		z_T (°C)	181	42.4			
		z_{aw}	0.50	7.22			
(5)	T_{ref}=80°C MC_{ref}=10	D_{ref} (min)					
		z_T (°C)	No results (failure to converge)				
		z_{MC} (%MC)					
(6)	T_{ref}=80°C $T_{d,ref}$=65°C $a_{w,ref}$=0.7	D_{ref} (min)	3.43	3.97	0.93	-0.13	-14.9
		z_T (°C)	98.0	23.1			
		z_M (°C)	99.8	15.4			
		z_{aw}	0.96	18.4			
(7)	T_{ref}=80°C $T_{d,ref}$=65°C MC_{ref}=10	D_{ref} (min)	4.42	5.39	0.89	-0.11	-25.6
		z_T (°C)	77.9	17.9			
		z_M (°C)	74.3	7.02			
		z_{MC} (%MC)	24.0	13.2			