

Impact of Storage Time and Temperature on Thermal Inactivation of *Salmonella* Enteritidis PT 30 on Oil-Roasted Almonds

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Abstract: Whole Nonpareil variety almonds were inoculated with *Salmonella* Enteritidis PT 30 and stored at 4 or 23 °C for up to 48 wk. At 1, 12, 24, 37, and 48 wk of storage, almonds were heated by immersion in 121 °C oil. After heating for 0.5 to 2.5 min, almonds were drained, transferred to tryptic soy broth, and mixed with a stomacher prior to plating onto tryptic soy and bismuth sulfite agars. Over the 48 wk of storage, *Salmonella* declined by 0.5 and 2.1 log CFU/g at 4 and 23 °C, respectively. The survivor inactivation curves were upwardly concave with rapid initial reductions in the levels of *Salmonella*. For up to 24 wk of storage, the mean counts of the survivors after treatment were not significantly different. The Weibull model predicted 4- and 5-log reductions of *Salmonella* in 0.85 ± 0.16 and 1.8 ± 0.43 min, respectively, for almonds stored at 4 °C, and in 1.6 ± 0.53 and 3.2 ± 1.0 min, respectively, for almonds stored at 23 °C. Refrigerated storage had little impact on heat resistance of *Salmonella* that were inoculated on almonds.

Keywords: almonds, heat resistance, oil roasting, *Salmonella*, storage

Practical Application: This research provides information of value in performing or evaluating validation studies for thermally processed almonds. The sensitivity of *Salmonella* to oil roasting is demonstrated during typical commercial almond storage times and temperatures.

Introduction

Outbreaks of salmonellosis have been associated with consumption of raw almonds in 2000 to 2001 (Isaacs and others 2005), 2003 to 2004 (CDC 2004), and 2005 to 2006 (Ledet Müller and others 2007). In the 2000 to 2001 and 2005 to 2006 outbreaks, *Salmonella enterica* serovar Enteritidis PT 30 was identified as the outbreak strain (CDC 2004; Isaacs and others 2005; Ledet Müller and others 2007). As of September 2007, almond handlers (processors) are required to process California-grown almonds that are sold in North America (Canada, Mexico, and United States) with a validated treatment that delivers a minimum 4-log reduction of *Salmonella* (Federal Register 2007).

The thermal survival curve of *Salmonella* Enteritidis PT 30 and *Salmonella* Senftenberg on almonds exposed to hot oil is an upward concave curve with an asymptotic tail (Du and others 2010). The Weibull model was used to analyze the data and 4- and 5-log reductions were calculated to be achieved in 1.4 and 2.4 min at 121 °C (250 °F), respectively, and 0.74 and 1.3 min at 127 °C (260 °F), respectively.

The validation and efficacy of the oil roasting process and other postharvest treatments, such as propylene oxide (PPO) fumigation and blanching, have been determined on freshly inoculated

and short-term refrigerated almonds (Danyluk and others 2005; Du and others 2010; Harris and others 2011). Based on the standard protocol required for validation studies, almond kernels are inoculated with *Salmonella* Enteritidis PT 30 (or the surrogate *Enterococcus faecium* NRRL B-2354) and dried at ambient temperature (23 °C) for 24 h; inoculated almonds may be stored at refrigeration temperatures before treatment for up to 14 d (ABC 2007a).

In commercial practice, raw almonds may be stored at a broad range of ambient temperatures for 1 y or more before they are processed (Danyluk and others 2006). Uesugi and others (2006) demonstrated the long-term survival of *Salmonella* on inoculated almonds at -20, 4, 23, and 35 °C. However, the impact of storage time and temperature on the subsequent efficacy of thermal inactivation of *Salmonella* Enteritidis PT 30 on oil-roasted almonds has not been previously studied. The objective of this study was to determine the heat resistance of *Salmonella* Enteritidis PT 30 on almonds that are exposed to hot oil after storage at refrigeration or ambient conditions for up to 48 wk.

Materials and Methods

Almonds

Nonpareil almond kernels of size 23/25 or 25/27 (23 to 25, or 25 to 27 almonds per 28 g) were used for this study. Nonpareil almonds are the most common almond variety grown in the United States and are recommended by the Almond Board of California for use in validation studies (ABC 2007a). Almond kernels were provided by Blue Diamond Growers (Sacramento, Calif., U.S.A.). Almonds were stored inside a plastic bag, placed in

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a tightly sealed plastic tub (Rubbermaid, Wooster, Ohio, U.S.A.), and held at ambient temperature (23 ± 2 °C) for less than 2 wk prior to inoculation.

Inoculum preparation

Salmonella Enteritidis PT 30 ATCC BAA-1045, isolated from almonds recalled in association with the *Salmonella* outbreak in 2000 to 2001 (Isaacs and others 2005), was used in this study. Inoculum was prepared according to procedures originally described by Danyluk and others (2005) and with the modification for cell harvesting as described by Du and others (2010). All media were Difco brand obtained from BD (Franklin Lakes, N.J., U.S.A.) unless otherwise specified. The inoculum volume (25 mL) collected from a set of 3 petri plates was used to inoculate 400 g of almond kernels. The required number of 25-mL inoculum preparations was pooled and the collected inoculum was stirred with a sterile magnetic bar until completion of the almond inoculation procedure (less than 30 min). Inoculum levels were determined by serial dilution in Butterfield's phosphate buffer (BPB) and plating onto tryptic soy agar (TSA; tryptic soy broth and 1.5% granulated agar) and bismuth sulfite agar (BSA).

Inoculation procedure

Almonds were inoculated as described by Danyluk and others (2005). Almonds (400 ± 1 g) were weighed into a polyethylene bag (30.5 cm \times 30.5 cm, Bitran, Com-Pac Intl., Carbondale, Ill., U.S.A.) and 25 mL of the prepared inoculum was added. The bag was closed and almonds were rubbed by hand from the outside of the bag for 60 sec to distribute the inoculum. The inoculated almonds were poured out of the bag and spread onto filter paper placed on a metal rack inside a large plastic tub. Almonds were held at 23 ± 2 °C for 24 ± 2 h in the tub (with the lid ajar) to allow the inoculum to dry. Two replicate batches of almonds were inoculated over 2 d.

Storage conditions

Each batch of inoculated, dried almonds was divided in half and placed into 2 separate sterile bags and held at room temperature for 7 d. One bag from each batch was refrigerated (4 °C) and the other was placed inside a tightly sealed plastic tub that was held at ambient conditions (23 ± 2 °C). Sample bags were stored for up to 48 wk.

Hot oil treatments

An oil treatment of 121 °C was chosen to allow for collection of a sufficient number of time points that had counts above the limit of detection (0.3 log CFU/g). Stored inoculated almonds were sampled at 1, 12, 24, 37, and 48 wk to evaluate the effect of storage time and temperature on the heat resistance of *Salmonella*. Samples removed from 4 °C storage were held at room temperature for 3 h prior to treatment to ensure ambient initial product temperature. Almond samples (50 ± 1 g) were placed in an enclosed wire mesh basket to ensure complete immersion in oil. For the roasting treatment, the basket was submerged in a HiTemp Bath (model 160A; Fisher, Fair Lawn, N.J., U.S.A.) containing 2.8 L of safflower oil maintained at a target temperature of 121 °C (250 °F). The basket was moved slowly up and down in the oil to promote even temperature distribution during the treatment.

A type-K thermocouple (Omega Engineering, Stamford, Conn., U.S.A.) was attached to the wire mesh basket holding the almonds. Another thermocouple was placed at the bottom

inside corner of the oil bath to monitor the oil temperature during the experiments.

Almond samples at weeks 1 and 12 of storage were heated in hot oil for 0.5, 1.0, 2.0, and 2.5 min; samples at weeks 24, 37, and 48 of storage were heated for 0.5, 1.0, 1.5, 2.0, and 2.5 min. The roasting was timed from the moment that the mesh basket was immersed in hot oil. If, after the first 30 sec, the oil temperature (as measured by the basket thermocouple) was more than ± 1.1 °C (2 °F) outside of the target temperature (121 °C), the almond sample was discarded. The oil was changed after heating approximately 30 samples.

Recovery and enumeration of inoculated cells

Treated almond samples were removed from the hot oil, drained for 10 sec, and placed into a 2-chamber filtering bag (1600 mL; Nasco, Modesto, Calif., U.S.A.) containing 100 mL of TSB (at room temperature). Sealed bags were immediately placed in an ice bath to cool the treated almonds.

For recovery, almond samples were mixed for 2 min at high speed with a Stomacher 400 laboratory blender (Seward, Worthington, UK). Untreated samples were spread plated in duplicate immediately after mixing. The treated samples were held at 4 °C prior to serial dilution in BPB and spread plating in duplicate onto TSA and BSA. In addition, 4 spread plates of 0.25 mL of the lowest dilution were prepared to improve the detection limit to 0.3 log CFU/g.

Plates were counted by hand at 24 ± 2 h (TSA) or 48 ± 2 h (BSA) after incubation at 35 ± 2 °C. Results were reported as the log of the number of survivors per gram of almonds. Counts on TSA included all colonies; counts on BSA were limited to colonies with characteristics typical of *Salmonella* on this medium, namely those with a metallic sheen and black centers.

Confirmation of presumptive *Salmonella* colonies

Some significant differences ($P < 0.05$) in colony counts were observed for treated almonds on TSA and BSA at 37 and 48 wk of storage at 23 °C. For these cases, TSA was replica plated onto xylose lysine desoxycholate (XLD) agar using a replica-plating apparatus and velvet square (14 cm \times 14 cm) (Qbiogene, Carlsbad, Calif., U.S.A.). Black colonies on the XLD plates, which are typical of *Salmonella* colonies on this media, were counted by hand after 24 ± 2 h incubation at 35 ± 2 °C. Presumptive *Salmonella* colonies appearing on XLD agar (up to 10 colonies per sample) were stabbed and streaked into lysine iron agar (LIA) and triple sugar iron (TSI) slants and incubated for 24 ± 2 h at 35 ± 2 °C. Positive reactions on these slants were confirmed by the latex test (Oxoid, Ogdensburg, N.Y., U.S.A.). Based on these results, the *Salmonella* counts were adjusted by subtracting those colonies that were not confirmed as *Salmonella*.

Curve fitting with the Weibull model

Survivor curves were fitted with the Weibull model:

$$\log_{10}(S) = \log_{10} \frac{N}{N_0} = -bt^n,$$

where S is the survival ratio at time t , and b and n are the shape and scale parameters, respectively (Peleg and Cole 1998). MatLab software (R2008a; The MathWorks, Inc., Natick, Mass., U.S.A.) was used for nonlinear curve fitting of the survival data where N_0 is the average number of survivors (CFU/g) determined experimentally at treatment time, $t = 0$. The built-in subroutine

fittype using model “power1” yielded parameters *b* and *n* as well as the adjusted coefficient of determination, R^2 . The subroutine *confint* returned the 99% confidence intervals for the parameter estimates.

Statistical analysis

Two separate batches of almonds were inoculated on different days and stored at 4 and 23 °C. Triplicate samples were processed in hot oil for each of the exposure times ($n = 6$). Analysis of variance (ANOVA), Tukey’s LSD test, and *t*-tests were performed with the JMP 8 software package (SAS Inst., Cary, N.C., U.S.A.). Differences between the mean values were considered significant at $P < 0.05$.

Results and Discussion

Salmonella Enteritidis PT 30 inoculum

The population of *Salmonella* Enteritidis PT 30 in the prepared inoculum was 10.7 and 10.8 log CFU/mL on TSA and BSA, respectively. Immediately after inoculation, the *Salmonella* population was determined to be 9.3 log CFU/g of almond kernels on both TSA and BSA. After the inoculated almonds were dried for 24 h at 23 °C, levels of 8.6 ± 0.0 and 8.5 ± 0.1 CFU/g ($n = 3$) were observed on TSA and BSA, respectively.

Enumerating *Salmonella* in the presence of a high background population

Background microbial levels in uninoculated almonds were 3.3 ± 0.0 log CFU/g ($n = 3$) on TSA, whereas no colonies were observed on BSA (<0.3 log CFU/g). Because of the high background population, the counts for treated almonds on TSA and BSA were often significantly different ($P < 0.05$), thus TSA counts were inappropriate to enumerate *Salmonella* especially at longer storage and treatment times when counts of the inoculated bacteria were at or below background levels. Therefore, for samples stored at 23 °C for 37 and 48 wk, colony counts of *Salmonella* on TSA were confirmed by the XLD replica-plating technique. Counts on BSA and TSA to XLD were not significantly different ($P > 0.05$); for consistency, BSA counts were used for all calculations and graphs. TSA and BSA have been shown to be appropriate media for recovery of heat-injured *Salmonella* (Gurtler 2009).

Survival of *Salmonella* Enteritidis PT 30 on almonds during storage at 4 and 23 °C

A reduction of 0.4 log CFU/g of *Salmonella* was observed on almonds stored at 4 °C during 48 wk of storage (Table 1). Uesugi and others (2006) observed stable populations of *Salmonella* for almonds stored at 4 °C over 72 wk (18 mo) and others have shown similar behavior for inshell pecans and pecan halves and

pieces (Beuchat and Mann 2010), and in peanut butter (Burnett and others 2000).

By contrast, when almonds were stored at 23 °C, levels of *Salmonella* on unheated almonds declined significantly ($P < 0.05$) by 1 log CFU/g after 12 wk of storage. After 48 wk of storage at 23 °C, the population of *Salmonella* had decreased by 2.1 log CFU/g to 6.3 CFU/g (Table 1). The calculated linear rate of reduction at 23 °C was 0.21 log CFU/month. Similar average declines of 0 or 0.25 log CFU/month at 4 or 23 °C, respectively, were used to develop a risk assessment model to evaluate the annual risk of salmonellosis from consumption of raw almonds (Danyluk and others 2006). The time and temperature of almond storage (in the absence of any other treatment) were shown to be important factors in predicting the number of cases of salmonellosis per year, especially given that almonds may be stored by the handler for a year or more at ambient temperature before processing.

Survival of *Salmonella* Enteritidis PT 30 on almonds stored at 4 or 23 °C and heated in 121 °C oil

Almonds are commercially roasted in oil at typical target temperatures of 138 to 177 °C (280 to 350 °F) for 3 to 15 min (ABC 2007b). We used 121 °C as a test temperature because at higher oil temperatures the population of *Salmonella* dropped to below the limit of detection too quickly to allow collection of an adequate number of data points for comparison purposes. We used a thermocouple attached to the mesh basket to monitor the heating process (Du and others 2010). In most cases, the time for the thermocouple to reach 121 °C was less than 20 sec. If the thermocouple did not reach the target temperature of 121 °C within 30 sec, that sample was discarded.

Almonds are most likely contaminated with microorganisms on the kernel surface during or after harvest (Danyluk and others 2008; Du and others 2007, 2010). Heat transfer coefficients for oils are large (250 to 260 watt/m²·°C at 170 °C; Miller and others 1994) and thus the surface of the almond was estimated to be at the temperature of the oil throughout the entire treatment.

Upwardly concave survivor curves were observed for both storage temperatures and all storage times (Figure 1). As expected, similar curves were observed at week 1 for almonds stored at 4 and 23 °C. Populations of *Salmonella* declined on almonds stored at 23 °C at a rate of 0.21 log CFU/month, which was similar to the rate of 0.2 to 0.3 log CFU/month previously observed (Danyluk and others 2006; Uesugi and others 2006; unpublished data).

As the population of *Salmonella* declined on almonds stored at 23 °C, the impact on the survivor curve was initially (through week 24) limited to the decline observed in the first 30 sec of exposure to the oil. The *t*-test was used to compare the means at each treatment time as a factor of storage temperature. At weeks 12 and 24, the means after time zero at each treatment time were not

Table 1—Population of *Salmonella* Enteritidis PT 30 on inoculated almonds stored for up to 48 wk at 4 or 23 °C.

Storage time (wk)	Mean (\pm SD ^a) plate counts (log CFU/g) ^{b,c}			
	4 °C storage		23 °C storage	
	TSA	BSA	TSA	BSA
1	8.7 \pm 0.1 A a	8.6 \pm 0.1 A a	8.5 \pm 0.1 A ab	8.4 \pm 0.1 A b
12	8.5 \pm 0.1 AB a	8.3 \pm 0.1 B b	7.6 \pm 0.2 B c	7.4 \pm 0.1 B cd
24	8.4 \pm 0.1 AB a	8.3 \pm 0.1 B a	6.9 \pm 0.1 C b	6.7 \pm 0.1 C c
37	8.3 \pm 0.1 AB a	8.1 \pm 0.1 C b	6.5 \pm 0.1 D c	6.4 \pm 0.1 D c
48	8.3 \pm 0.0 B a	8.1 \pm 0.1 C b	6.4 \pm 0.1 E c	6.3 \pm 0.2 E c

^aSD = standard deviation.

^bWithin columns, means with different uppercase letters are significantly different ($P < 0.05$); $n = 6$.

^cWithin rows, means with different lowercase letters are significantly different ($P < 0.05$); $n = 6$.

significantly different ($P > 0.05$) for samples stored at either 4 or 23 °C (Figure 1). This suggests that there may be a subpopulation of *Salmonella* that is more sensitive to ambient storage conditions as well as to heat treatment. Although not measured in this study, it is also possible that differences in moisture content between the stored almonds contributed to this effect. Further study is needed to better define those parameters having the greatest influence on thermal tolerance of *Salmonella* in almonds.

The suitability of the Weibull model to analyze the data for oil-roasted almonds was demonstrated by Du and others (2010) and confirmed here by plotting $\ln(-\ln N/N_0)$ versus $\ln t$. Straight lines were observed on all plots, with R^2 values equal to or higher than 0.92, confirming that the Weibull model could be used to fit the survival curves of *Salmonella* Enteritidis PT 30 on almonds exposed to hot oil. The values for n (power) and b (coefficient) were determined according to the Weibull model for each survival curve (Table 2). For all storage times and temperatures, rapid initial

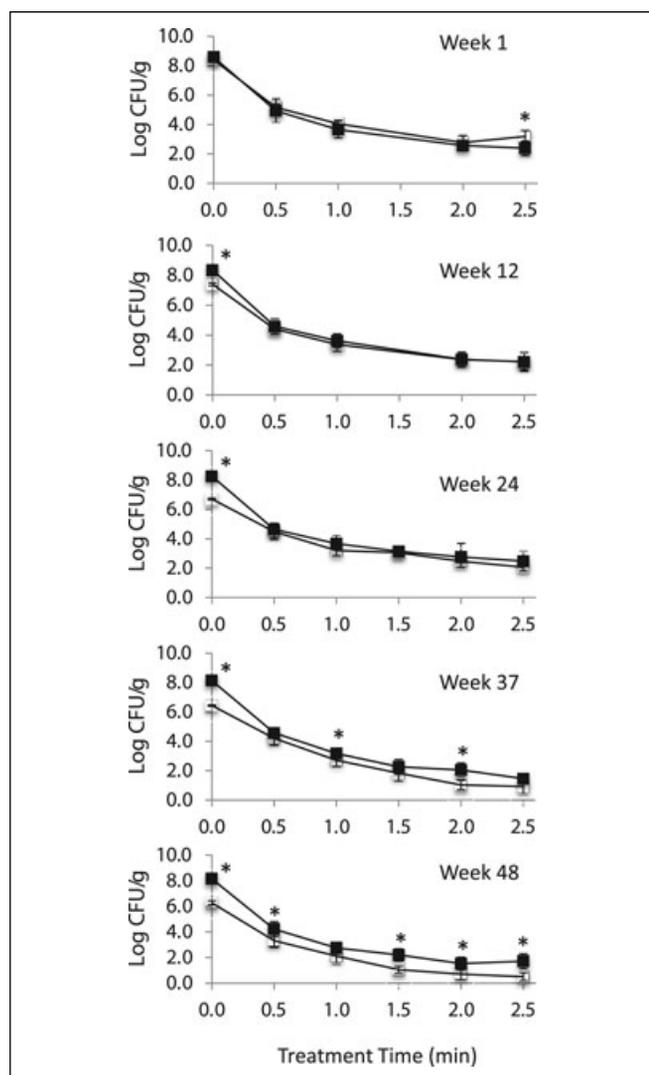


Figure 1—Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds stored at 4 °C (closed square) or 23 °C (open square) for 1 to 48 wk after exposure to hot oil at 121 °C for up to 2.5 min; samples enumerated on BSA ($n = 6$). *Significant difference ($P < 0.05$) between the means at given exposure within storage times.

declines in the population were observed as described previously (Du and others 2010) and n (power) was less than 1, which is indicative of an increased heat resistance (tailing of survival curve).

The times required to achieve 4- or 5-log reductions for *Salmonella* Enteritidis PT 30 on almonds exposed to hot oil for each storage time and temperature were determined using both best fit and 99% confidence intervals (CI) (Table 3). Samples tested at week 24 were significantly different from those at other time points, and longer times were predicted to achieve a 4- or 5-log reduction for product stored at 4 or 23 °C (Table 3). The data observed at this time point could not be explained. For all other time points, the data were consistent within the storage temperature, but shorter times to achieve the target reductions of *Salmonella* were predicted with almonds stored at 4 °C. The time required for a 5-log reduction was almost double the time required to achieve a 4-log reduction of the *Salmonella* population due to the asymptotic tailing of the survivor curve, as also observed by Du and others (2010).

For almonds stored at 4 °C, reductions of 4 or 5 logs were achieved after heating for an average time of 0.72 ± 0.09 or 1.3 ± 0.17 min, respectively, based on best fit, and after heating for 0.85 ± 0.16 or 1.8 ± 0.43 min, respectively, based on 99% CI. For almonds stored at 23 °C, reductions of 4 or 5 logs were achieved after heating for an average time of 1.2 ± 0.31 or 2.0 ± 0.50 min, respectively, based on best fit, and after heating for 1.6 ± 0.53 or 3.2 ± 1.0 min, respectively, based on 99% CI. Although the Weibull predicted greater time to achieve 4- or 5-log reductions for almonds stored at 23 °C, the survival curves were virtually identical at week 1 and differed only at time 0 for weeks 12 and 24 (Figure 1).

Previously, survivor curves were determined for inoculated Mission variety almonds that were stored for 12 to 26 d in the refrigerator before treating in oil (Du and others 2010). Times to achieve a 4- or 5-log reduction of *Salmonella* Enteritidis PT 30 in 121 °C oil were predicted to be 1.0 and 1.2 min or 1.7 and 2.2 min using best fit and 99% CI, respectively. The data from that study are the same as or more conservative than data from the current study for Nonpareil almonds stored at 4 °C, with the exception of data for week 24 based on 99% CI (higher by 0.1 and 0.2 min for predicted 4- and 5-log reductions, respectively). For almonds stored at 23 °C, with the exception of week 24 data, times predicted to achieve 4- or 5-log reductions in the current study were within 0.3 min (best fit) of the previous study. Data from Du and others (2010) correlated less well for reduction times predicted when using 99% CI, which may have been due to greater variability in the survival curves observed with the stored almonds.

The Almond Board of California recommends a minimum process of 1.6 or 2.0 min of exposure to 127 °C oil for a 4- or 5-log reduction, respectively, of *Salmonella* Enteritidis PT 30 on almonds (ABC 2007b). No recommendations are given for lower temperatures, in part because the standard roasting temperatures used commercially are much higher. Based on the most conservative results generated by this study, a 4- or 5-log reduction was predicted in 2.5 or 4.7 min, respectively, in 121 °C oil (week 24, 99% CI). These data may be useful for evaluating process deviations where temperatures fall below 127 °C but remain above 121 °C.

Moisture, water activity, and relative humidity have been shown to impact the heat resistance of *Salmonella* on almonds (Kaur and Harris 2010; Jeong and others 2011), with observations of increased sensitivity to heat treatment on almonds at higher moisture levels or at higher relative humidity. It is possible that the refrigerated almonds in the current study had a higher moisture or

Table 2—Survival parameters of *Salmonella* Enteritidis PT 30 on inoculated almonds stored at 4 or 23 °C, according to the Weibull model.^a

Storage time (wk)	Measure	4 °C storage			23 °C storage		
		Power (<i>n</i>)	Coefficient (<i>b</i>) (min ^{-<i>n</i>})	R ²	Power (<i>n</i>)	Coefficient (<i>b</i>) (min ^{-<i>n</i>})	R ²
1	Best fit	0.400	4.532	0.925	0.392	4.031	0.929
	99% CI ^b	0.290	4.137		0.288	3.696	
12	Best fit	0.395	4.491	0.931	0.408	3.743	0.932
	99% CI	0.291	4.122		0.301	3.429	
24	Best fit	0.380	4.294	0.899	0.461	3.113	0.938
	99% CI	0.268	3.938		0.356	2.880	
37	Best fit	0.428	4.677	0.956	0.547	3.544	0.943
	99% CI	0.346	4.401		0.430	3.250	
48	Best fit	0.388	4.934	0.922	0.507	4.090	0.947
	99% CI	0.288	4.573		0.382	3.796	

^aThe nonlinear regression was done with Matlab software. The fitted Weibull model was in the form of $\log S = -bt^n$, where *S* is the survival ratio, *t* is treatment time, and *b* and *n* are constants. Enumeration on BSA.

^bValue is the lower bound of the 99% CI (confidence interval).

Table 3—Time required to achieve 4- or 5-log reductions of *Salmonella* Enteritidis PT 30 on almonds heated in 121 °C oil after storage at 4 or 23 °C, according to the Weibull model.

Storage time (wk)	Measure	Time required for reduction (min)			
		4 °C storage		23 °C storage	
		4 log	5 log	4 log	5 log
1	Best fit	0.73	1.3	0.98	1.7
	99% CI ^a	0.89	1.9	1.3	2.9
12	Best fit	0.75	1.3	1.2	2.0
	99% CI	0.90	1.9	1.7	3.5
24	Best fit	0.83	1.5	1.7	2.8
	99% CI	1.1	2.4	2.5	4.7
37	Best fit	0.69	1.2	1.2	1.9
	99% CI	0.76	1.4	1.6	2.7
48	Best fit	0.58	1.0	0.96	1.5
	99% CI	0.63	1.4	1.2	2.1
		Average time required for reduction (min) ± SD			
	Best fit	0.72 ± 0.09	1.3 ± 0.17	1.2 ± 0.31	2.0 ± 0.50
	99% CI	0.85 ± 0.16	1.8 ± 0.43	1.6 ± 0.53	3.2 ± 1.0

^aValue is the lower bound of the 99% CI (confidence interval) for each survival curve (Table 2).

water activity than the almonds stored under ambient conditions. Although moisture and water activity were not monitored in the current study, other work in this laboratory with walnuts, almonds, and pistachios has shown that nuts may gain moisture when stored under refrigeration conditions (data not shown) either by exposure to the generally higher relative humidity in the refrigerator or from moisture condensation after removal of the product from the refrigerator. The ambient humidity in the laboratory where these studies were performed was typically less than 40%; moisture condensation on these almonds was less likely. However, the possibility for moisture condensation on refrigerated nuts used for validation studies should be considered when higher ambient relative humidity is observed. Inoculated almonds prepared for validation studies are often stored at refrigeration temperatures because populations of *Salmonella* are stable on almonds at 4 °C. The Almond Board of California recommends refrigerated storage of inoculated almonds for no longer than 2 wk for validation studies (ABC 2007a) unless specified requirements for initial populations, moisture levels, and heat resistance are met. In the current study, heat resistance was unchanged over 12 wk of refrigerated storage.

Conclusion

This study evaluated the impact of storage temperature and time on the thermal sensitivity of *Salmonella* inoculated onto almonds

and exposed to hot oil. Storage temperature, but not time, had an impact on predicted 4- and 5-log reductions of *Salmonella* inoculated onto almonds.

Acknowledgments

This research was supported by the Almond Board of California. We are thankful for the technical assistance of Wen-Xian Du and the editorial assistance of Sylvia Yada.

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