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Reduction of *Salmonella* on Inoculated Almonds Exposed to Hot Oil

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ABSTRACT

The heat resistance of *Salmonella* inoculated onto almonds was determined after immersion in hot oil. Whole almonds were inoculated with *Salmonella* Enteritidis PT 30 or *Salmonella* Senftenberg 775W and heated in oil. After heating, almonds were drained, transferred to cold tryptic soy broth, and mixed with a stomacher, and samples were plated onto tryptic soy and bismuth sulfite agars. *Salmonella* survivor inactivation curves were upwardly concave. Rapid reductions of 2.9, 3.0, or 3.6 log CFU/g for *Salmonella* Enteritidis were observed after 30 s of exposure to oil at 116, 121, or 127°C, respectively. Thereafter, reduction occurred at a much slower rate. Similar reductions were observed at 127°C for *Salmonella* Senftenberg. The Weibull model was used to predict 4- and 5-log reductions of *Salmonella* Enteritidis after 0.74 and 1.3 min at 127°C, respectively. Neither *Salmonella* serovar could be recovered by enrichment of 1-g samples after almonds inoculated at 5 log CFU/g were exposed to oil at 127°C for 1.5 min. Standard oil roasting times and temperatures that achieve acceptable kernel color and texture should result in much greater than 5-log reductions of *Salmonella* in almonds.

Outbreaks of salmonellosis linked to *Salmonella enterica* serovar Enteritidis PT 30 or PT 9c have been associated with consumption of raw almonds in 2000 to 2001 *(21),* 2003 to 2004 *(10),* and 2005 to 2006 *(29).* Since the 2000 to 2001 outbreak, long-term persistence of *Salmonella* Enteritidis PT 30 has been demonstrated in the production environment *(35), Salmonella* has been found in about 1% of 100-g samples of raw almonds *(13),* and long-term survival with little to no reduction of the organism has been observed in almonds stored under ambient, refrigerated, or frozen conditions *(34).*

The ability of *Salmonella* to survive in the almond production and processing environments and its presence on raw almonds has raised concerns about the potential for additional salmonellosis outbreaks *(12).* One option to prevent further outbreaks is to apply an effective postharvest processing treatment to ensure adequate reduction of *Salmonella* on almonds. Since 1 September 2007, most almonds grown in California and sold in North America (Canada, United States, and Mexico) must be processed with a validated treatment that delivers a minimum 4-log reduction of *Salmonella (17).* However, a 5-log reduction of *Salmonella* is the minimum process for labeling almond bulk packages as ''pasteurized almonds'' *(4).*

Several validated postharvest treatments for almonds have been recognized by the Almond Board of California *(5),* including propylene oxide fumigation *(14),* hot water blanching, and oil roasting. Greater than 4-log reductions of *Salmonella* also have been achieved using steam *(23),* infrared heat *(9),* or high hydrostatic pressure *(38).* Other treatments, such as acidic sprays *(31),* achieved less than 4-log reductions of *Salmonella* and would need to be used in combination with other processes to meet the mandated reduction levels. Although oil roasting is a validated process, no data on the reduction of *Salmonella* on the surface of almonds during oil roasting have been published.

Roasting causes almonds to become more crunchy, and their flavor profile changes. Almonds may be dry roasted, by exposing the kernels to hot air, or oil roasted. A continuous conveyor oil roaster is commonly used for oil roasting. In this process, almonds are loaded onto a stainless steel mesh conveyor, which moves the almonds through a preheated oil pool (kernels are kept submerged in oil). As the roasted kernels are conveyed out of the oil pool to a cooling zone, any residual oil is drained to a catch pan or tray to be recycled. Although industry practices may vary, a treatment of 138 to 177°C for 3 to 15 min is typically used to achieve oil roasted almonds of high quality (low moisture and crunchy kernels) *(4).*

The specific objectives of this study were to (i) evaluate methods for recovery of *Salmonella* from inoculated almonds before and after hot oil treatment and (ii) determine the heat resistance of *Salmonella* in hot oil.

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MATERIALS AND METHODS

Almonds. Raw (untreated) almond kernels were provided by Blue Diamond Growers (Sacramento, CA). Whole Mission almonds of size 25/27 or 27/30 (25 to 27, or 27 to 30 almonds per 28 g) were chosen for the study because they represent the typical variety and sizes of almonds that are commercially oil roasted. Almonds were stored in sealed polyethylene bags (30.5 by 30.5 cm; Bitran, Com-Pac International, Carbondale, IL) inside a tightly sealed plastic tub and held at ambient temperature (24 ± 2°C) for up to 6 months until inoculation.

Inoculum preparation. *Salmonella* Enteritidis PT 30 (ATCC BAA-1045) and *Salmonella* Senftenberg 775W (ATCC 43845) were used in this study. Isolates were stored at −80°C in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) supplemented with 15% glycerol (Fisher, Fair Lawn, NJ). Inoculum was prepared according to procedures described by Danyluk et al. *(14),* with the following modification for cell harvesting. Instead of using swabs to collect the bacterial lawn, approximately 8 to 9 ml of 0.1% peptone (Difco, Becton Dickinson) was added to each petri dish after incubation. The bacterial lawn was loosened with a sterile spreader, and a sterile pipette was used to collect the cells into a sterile container. For every set of three petri dishes, 25 ml of cells was collected, which was sufficient to inoculate 400 g of almonds.

An appropriate number of 25-ml inoculum preparations (depending upon the total amount of almonds inoculated) were pooled and thoroughly mixed for at least 1 min with a magnetic stir bar and stir plate. The inoculum was kept on the stir plate until all almond samples were inoculated (maximum of 15 min). Inoculum levels were determined by serial dilution in Butterfield's phosphate buffer (BPB) and plating in duplicate onto tryptic soy agar (TSA; Difco, Becton Dickinson) and bismuth sulfite agar (BSA; Difco, Becton Dickinson).

Inoculation procedure. Almonds were inoculated as described previously *(14)*. Each almond sample (400 ± 1 g) was weighed into a polyethylene bag (30.5 by 30.5 cm), and 25 ml of the pooled inoculum was added. The bag was closed and then mixed by inverting the bag manually for 1 min. Almonds were poured out of the bag and spread onto filter paper placed on a metal drying rack inside a large plastic tub. Inoculated almonds were held in the tub (with the tub lid ajar) for 24 ± 2 h at 24 ± 2°C to allow the inoculum to dry.

Inoculated, dried almonds were pooled into a polyethylene bag (40.6 by 40.6 cm; Bitran) and thoroughly mixed by inverting the bag manually for 1 min. Duplicate almond samples (50 ± 1 g) were collected to confirm the inoculation level (5 or 8 log CFU/g). To check the background population on the uninoculated almonds, duplicate samples from untreated controls were plated onto TSA and BSA each time a batch of almonds was inoculated. Inoculated almonds were held at 4°C for a maximum of 3 months. Thermal resistance was stable under these conditions *(2)*. Before treatment, almond samples used for the experiment were removed from 4°C storage and allowed to warm to room temperature for 3 to 4 h.

Measuring almond surface temperature. Experiments were performed to evaluate methods for determining the surface temperatures of almonds during oil roasting. Thermocouples (type K, Omega Engineering, Stamford, CT) were attached to individual almonds, i.e., a separate piece of wire was wrapped around each almond to hold the covered part of the thermocouple wire in place. The exposed tip of the thermocouple was located as follows: (i) on the surface of the almond but not embedded in the pellicle (skin), (ii) embedded in the almond skin, or (iii) inserted (by the manufacturer) into the center of model almonds made of aluminum (FMC Technologies, Madera, CA). Thermocouples also were attached to a wire mesh basket and immersed directly in the oil to monitor the oil temperature. All thermocouples were connected to a data logger (Campbell Scientific, Logan, UT) equipped with an SM192 storage module. The almonds with thermocouples attached were placed inside the mesh basket with 50 g of uninoculated almonds, and the basket was immersed in the hot oil. Measurements were made at three oil temperatures (116, 121, and 127°C), and thermocouple temperatures were monitored every second. Each experiment was replicated three times.

Hot oil treatments. Whole inoculated almonds (50 ± 1 g) were placed in an enclosed wire mesh basket that allowed free movement of the almonds but also ensured that they were completely immersed in oil for the entire treatment. The basket was submerged in a HiTemp Bath (model 160A, Fisher) containing 2.8 liters of safflower oil maintained at a target temperature of 93.3°C (200°F), 104°C (220°F), 116°C (240°F), 121°C (250°F), or 127°C (260°F). Because data were intended for use by the U.S. almond industry, the oil temperature was monitored in degrees Fahrenheit with an HH509 digital thermometer (Omega Engineering) connected with two type K thermocouples. One thermocouple was attached to the basket containing the almonds, and the other was attached at a remote position at the side of the oil bath. The oil bath temperature on the digital display of the heating bath also was monitored before and during heating. Temperature was maintained within 1.1°C (2°F) of the target temperature (as measured on the basket and by oil bath thermocouples) by moving the basket slowly up and down in the oil to promote even temperature distribution. Almonds were heated in hot oil for predetermined times from 30 s to 4 min. The roasting was timed from the moment that the mesh basket was immersed in the hot oil. If the oil temperature moved outside the ±1.1°C target at any time after the first 30 s, the almonds were discarded. The oil was replaced after heating approximately 50 samples.

Recovery of inoculated cells. Almonds were removed from the oil, drained for 10 s, and cooled either by direct addition to cold diluent or by placing them in a plastic bag and immediately placing the bag into a bed of ice. Four methods, i.e., stomaching, blending, mechanical shaking, and hand shaking, were initially used to compare the recovery and level of *Salmonella* Enteritidis from inoculated and heat-treated almond samples. Thereafter, the stomaching method was used. Background microbial levels also were determined on uninoculated almond control samples by the stomaching preparation method. For each method, 50-g samples of almonds were processed:

(i) stomaching: almonds were added to 100 ml of cold TSB in a two-chamber filtering bag (1,600 ml; Nasco, Modesto, CA) and mixed for 2 min at high speed with a Stomacher 400 laboratory blender (Seward, Worthington, UK);

(ii) blending: almonds were added to 450 ml of lactose broth (Difco, Becton Dickinson) or TSB in a 1-liter stainless steel commercial blender (Waring Products, Torrington, CT) and blended for 2 min at low speed;

(iii) mechanical shaking: slightly modified mechanical shaking method *(22)* in which almonds were added to 118-ml sterile polypropylene specimen containers (Fisher), an equal volume (50 ml) of TSB was added, and samples were shaken for 15 min at 150 rpm with a rotary shaker;

(iv) hand shaking: procedure described by Uesugi et al. *(34)* (a modification of a Food and Drug Administration *Bacteriological Analytical Manual* [FDA-BAM] method *(6)*) in which almonds were added to 50 ml of TSB in a 710-ml Whirl-Pak bag (Nasco), shaken vigorously 50 times in a 30-cm arc, allowed to stand for 5 min, and then shaken an additional 5 times.

Untreated samples were plated immediately after preparation, and the treated prepared samples were kept at 4°C for a maximum of 30 min before serial dilution in BPB and plating in duplicate onto TSA and BSA. In addition to plating 0.1 ml of the lowest dilution (10^0 for shaking or stomaching and 10^1 for blending), four spread plates of 0.25 ml each were prepared to improve the detection limit to 1, 2, or 10 CFU/g (0.1, 0.3, or 1.0 log CFU/g) for hand and mechanical shaking, stomaching, and blending, respectively. 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.

Blended samples were also enumerated by a three-tube most-probable-number (MPN) procedure using a modified FDA-BAM method as described below for the end-point procedure.

Confirmation of presumptive *Salmonella* colonies. For some time and temperature treatments, the colony counts on TSA for inoculated almonds dropped to levels equal to background levels of corresponding untreated uninoculated almonds (0.3 to 2.5 log CFU/g, depending on the batch of almonds). In these cases, colonies from the inoculated and treated almonds were confirmed as *Salmonella* by the following procedure. All colonies on TSA were streaked onto Hektoen enteric agar (HE; Difco, Becton Dickinson) plates with sterile toothpicks. After incubation of the HE plates at 35 ± 2°C for 24 ± 2 h, presumptive-positive colonies were restreaked onto HE plates to obtain isolated colonies. One isolated colony from each HE plate was stabbed and streaked into lysine iron agar (LIA; Difco, Becton Dickinson) and triple sugar iron (TSI; Difco, Becton Dickinson) slants and incubated at 35 ± 2°C for 24 ± 2 h. Positive reactions on these slants that were typical of *Salmonella* were confirmed by the *Salmonella* latex test (Oxoid, Ogdensburg, NY). The *Salmonella* count was adjusted as appropriate by subtracting those colonies that were not confirmed as *Salmonella*.

Modified end-point procedure. A modified end-point procedure was used to confirm the reductions of *Salmonella* observed with plate counts *(26)*. The inoculum prepared as described above was diluted in 0.1% peptone to give a target *Salmonella* Enteritidis or *Salmonella* Senftenberg level of 5 log CFU/g after the almonds were dried. Almond samples (50 g) were exposed to 121 or 127°C hot oil as described above. Instead of plating, these samples were enriched for *Salmonella* by a modification of the FDA-BAM method *(7)*. Almonds (50 g) and 450 ml of lactose broth were added to a sterile stainless steel blender jar (Waring) and blended at low speed for 2 min. Three 10-ml portions of the homogenate (each equivalent to 1 g of almonds) were placed into individual sterile test tubes (16 by 150 mm) and incubated at 35 ± 2°C for 24 ± 2 h, and 0.1 ml portions of this culture were removed into tubes containing 10 ml of Rappaport-Vassiliadis (RV) broth (Difco, Becton Dickinson). RV broth tubes were incubated in a circulating, thermostatically controlled water bath at 42 ± 0.2°C for 24 ± 2 h and then streaked onto HE plates. After incubation at 35 ± 2°C for 24 ± 2 h, suspect colonies were picked and inoculated onto both TSI and LIA slants. The slants were incubated at 35 ± 2°C for 24 ± 2 h, and cultures from slants showing a reaction typical of *Salmonella* were confirmed with a *Salmonella* latex test (Oxoid).

Curve fitting with the Weibull model. Survivor curves were fitted with the Weibull model:

$$\log(S) = \log\frac{N}{N_0} = -bt^n$$

where S is the survival ratio at time t, and b and n are the shape and scale parameters, respectively *(32)*. MatLab software (R2008a, The MathWorks, Inc., Natick, MA) was used for nonlinear curve fitting of the survival data, where N_0 is the average number of survivors (CFU per gram) at treatment time (t) zero, determined experimentally. The built-in subroutine *fittype* using model ''*power1*'' yielded the parameters b and n and the adjusted coefficient of determination, R^2. The subroutine *confint* returned the 99% confidence intervals (CIs) for the parameter estimates.

Measuring almond moisture content and water activity. The roasted almonds were drained for 10 s and placed in a bag (1,600 ml; Nasco) containing a clean paper towel, and the sealed bag was covered in ice to quickly cool the almonds. Following almond industry practice, an American Association of Cereal Chemists (AACC) approved method (44-15A) *(1)* with some modifications was used to determine moisture. Raw and oil-roasted (127°C for 0.5 and 4.0 min) almonds (50 g) were ground for 20 s in a commercial blender (Waring) and manually shaken through a standard number 12 testing sieve (1.7-mm pore size; VWR Scientific, West Chester, PA). The moisture content of the sieved almond powder was determined with a moisture analyzer (Infrared LJ16, Mettler-Toledo, Columbus, OH), and water activity was measured with a water activity meter (Decagon Devices, Pullman, WA). Moisture and water activity were determined for three separate samples (duplicate measurements were determined for each water activity sample), and the results were averaged.

Statistical analysis. Data were analyzed with Statistical Analysis System (version 8.2) software (SAS Institute, Cary, NC). Analysis of variance with the general linear model procedure and Duncan's multiple range tests was used to find the significant differences among samples at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Salmonella Enteritidis PT 30 was linked to the 2000 to 2001 raw almond outbreak and survived in the almond production and processing environments *(21, 34)*. The Almond Board of California recommended the use of this specific strain of *Salmonella* for validation of almond thermal processes *(3)*; thus, we chose this strain for the current study.

Salmonella Senftenberg 775W is considered unusually heat resistant *(30)*; however, this characterization has been based primarily on experiments in aqueous solutions *(19)*. In chocolate, this strain was less heat resistant than a strain of *Salmonella* Typhimurium *(18)*. Because of the reported discrepancy in heat resistance of *Salmonella* Senftenberg 775W, this strain also was included in the present study.

Almond surface temperature during exposure to hot oil. To find a reliable method to monitor treatment temperatures, we compared measurements obtained from thermocouples attached to the almond surface, embedded (tip only) in the almond skin, and attached to the mesh basket used to immerse the almonds in the oil and measurements obtained from model aluminum almonds

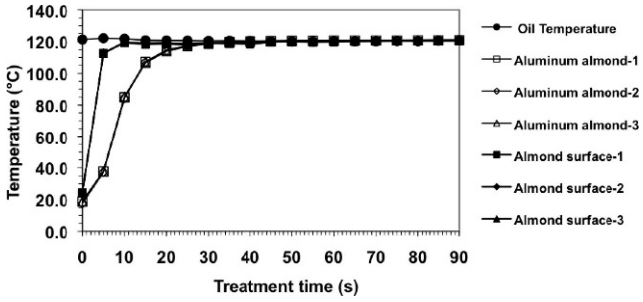

FIGURE 1. *Representative thermal profiles for thermocouples attached to the surface of almonds and in aluminum almonds exposed to hot oil (121°C).*

prefitted with centered thermocouples. Although it was difficult to attach thermocouples to individual almonds, it was more difficult to embed the thermocouple to a consistent depth in the almond skin. Significantly longer times to reach the target temperatures ($P < 0.05$) were observed when the thermocouple was embedded in the almond skin (data not shown).

Thermocouple readings were within 2°C of oil bath temperatures within approximately 10 s for thermocouples attached to the almond surface and within 30 s for aluminum almonds (Fig. 1). The thermocouples on the almond surface gave readings during heating that were the same as those from the thermocouples attached to the mesh basket at all three oil temperatures studied. For all further experiments, oil temperatures during heating were monitored by attaching thermocouples to the mesh basket and to the side of the oil bath.

Almonds most likely become contaminated with *Salmonella* at the kernel surface during or after harvest *(11, 16, 36)*. Thus, the kernel surface temperature rather than the internal temperature is relevant to thermal processes for this product. Heat transfer coefficients for canola, soybean, and palm oils were 250 to 260 $W/m^2 \cdot °C$ at 170°C *(28)*. At heat transfer coefficients of this magnitude, the surface of the almond was estimated to be at the temperature of the oil throughout the entire treatment, which is how the data were analyzed.

Moisture and water activity. The moisture content and water activity of ground almonds were measured after the whole kernels were heated for 0.5 to 4.0 min at 127°C. Almonds dipped in 38°C (warm) oil were used as a control. For all almonds, the carryover of oil was 0.23 to 0.92 g per 50-g sample and was unrelated to time or temperature of oil exposure. The percent moisture decreased linearly for almonds exposed to 127°C oil, whereas water activity fluctuated from 0.46 to 0.50 for control and treated almonds (Table 1). It is possible that moisture and water activity were markedly different at the almond surface, but it was not practical to take such measurements. The moisture content of the raw almonds fell to 3.8% after heating in oil at 127°C for 2 min (Table 1). Almonds with this moisture level would not be considered ''roasted'' based on the industry standard of <3% moisture for lightly roasted product *(20)*.

Recovery methods. The bacterial counts for stomached and plated samples posttreatment were significantly higher than those for samples that were blended and plated or blended and enriched for an MPN analysis (Table 2). For untreated samples (with high levels of *Salmonella*), hand shaking *(6)* and mechanical shaking methods resulted in significantly lower counts than those obtained after either stomaching or blending (Table 3). No significant differences ($P > 0.05$) were observed among the methods after samples were heated for 1 min in oil at 121°C. However, colony counts from blending were significantly lower than those from stomaching or mechanical shaking for samples heated for 3 min at 121°C, a difference which may have been due in part to the higher limit of detection for this method.

Stomaching in a 1:2 ratio of almonds to cold diluent provided consistently higher counts at both high and low inoculum levels and before and after heat treatment. An almond/diluent ratio of less than the standard 1:9 was chosen to improve the limit of detection after heating. A 1:1 ratio also was assessed, but this volume of liquid was insufficient to allow for adequate break up of the almonds during stomaching. In addition, stomaching in 100 ml versus blending in 450 ml generates less hazardous waste and requires less labor for preparing diluent and sterilizing, cleaning, and resterilizing blender jars. For these reasons,

TABLE 1. *Moisture content and water activity of almonds before and after oil treatment*

Treatment	Temp (°C)	Time (min)	Moisture content (%)[a]	Water activity[a]
Untreated	25	0.0	4.43 ± 0.09 A	0.48 ± 0.01 C
Oil bath, warm	38	0.5	4.42 ± 0.05 A	0.49 ± 0.01 B
		3.0	4.41 ± 0.08 A	0.47 ± 0.01 D
		4.0	4.51 ± 0.07 A	0.50 ± 0.00 A
Oil bath, hot	127	0.5	4.22 ± 0.14 B	0.49 ± 0.00 B
		1.0	4.05 ± 0.08 C	0.49 ± 0.01 B
		1.5	3.92 ± 0.03 CD	0.50 ± 0.00 A
		2.0	3.80 ± 0.06 DE	0.49 ± 0.01 B
		2.5	3.75 ± 0.15 E	0.49 ± 0.01 B
		3.0	3.67 ± 0.04 E	0.49 ± 0.01 B
		4.0	3.45 ± 0.12 F	0.46 ± 0.01 E

[a] Values are mean ± standard deviation, $n = 3$. Within each column, means with different letters are significantly different ($P < 0.05$).

TABLE 2. *Recovery of* Salmonella *Enteritidis PT 30 from inoculated almonds before and after oil treatment at 121°C for 1 min comparing stomaching, blending, and MPN methods*

Almonds	*n*	Stomaching (100 ml tryptic soy broth)		Blending (450 ml lactose broth)		
		TSA (log CFU/g)	BSA (log CFU/g)	TSA (log CFU/g)	BSA (log CFU/g)	MPN (log MPN/g)
Control	1	8.5	8.4	8.5	8.2	8.4
Treated[a]	6	4.7 ± 0.3 A	4.2 ± 0.4 a	4.1 ± 0.2 B	3.4 ± 0.1 b	3.8 ± 0.3 B b

[a] For treated almonds, values are the mean ± standard deviation. Mean plate counts on TSA or MPN values with different uppercase letters are significantly different ($P < 0.05$). Mean plate counts on BSA or MPN values with different lowercase letters are significantly different ($P < 0.05$).

stomaching was the method chosen to recover *Salmonella* in subsequent experiments.

Almonds were cooled after heating in oil either by direct addition to cold diluent or by placing them in a plastic bag and immediately placing the bag into a bed of ice. No significant differences ($P > 0.05$) were observed in counts obtained by either method (data not shown). For all further experiments, almonds were cooled by direct addition to cold diluent.

Microbial populations on inoculated almonds. *Salmonella* levels determined for undiluted inoculum on TSA were 11 log CFU/ml. After inoculation, *Salmonella* Enteritidis and *Salmonella* Senftenberg levels on the wet almonds (before drying) were 9.3 ± 0.1 and 9.6 log CFU/g, respectively; levels after drying were 8.8 ± 0.2 and 8.9 log CFU/g, respectively. Reductions after drying (0.5 and 0.7 log CFU/g for *Salmonella* Enteritidis and *Salmonella* Senftenberg, respectively) were similar to previously reported results *(14, 34)*.

When the inoculum was diluted before inoculation of the almonds for the end-point determination protocol, greater reductions were observed after inoculum drying for both *Salmonella* serovars. In addition, *Salmonella* Senftenberg was significantly more sensitive to drying ($P < 0.05$) than was *Salmonella* Enteritidis. Therefore, higher inoculum levels were necessary for *Salmonella* Senftenberg to achieve a target level of 5 log CFU/g on dried almonds. Inoculum levels of 7.9 and 8.7 log CFU/ml for *Salmonella* Enteritidis and *Salmonella* Senftenberg, respectively, yielded 6.5 ± 0.3 and 7.4 ± 0.1 log CFU/g, respectively, on wet almonds and 5.1 ± 0.2 and 5.2 ± 0.2 log CFU/g, respectively, on dried almonds.

TSA and BSA were used to enumerate bacterial populations in the inoculum preparation and on uninoculated and inoculated almonds before and after exposure to hot oil. Differences in colony appearance were observed on TSA plates: colonies from uninoculated, untreated control samples were variable in size, color, and shape and included some spreading colonies; colonies from inoculated samples were consistent in size, color, and shape. No colonies were detected on BSA for the uninoculated control samples. Counts on TSA for uninoculated control samples differed among batches, from 0.3 to 2.5 log CFU/g. Colony counts on TSA and BSA differed by 0 to 0.1 log CFU/ml for the inoculum preparation. For inoculated almonds after drying for 24 h, colony counts on TSA and BSA differed by an average of 0.2 log CFU/g, with a maximum difference of 0.3 log CFU/g.

Reduction of *Salmonella* after hot oil treatments. Industry oil-roasting practices for almond kernels can vary from 3 to 15 min at 138 to 177°C *(4)*. We used much lower temperatures for this study to facilitate collection of an adequate number of data points; at higher temperatures, the populations of *Salmonella* decreased to below detection in fewer than 2 min. The almond industry also was interested in defining critical temperature limits for

TABLE 3. *Recovery of* Salmonella *Enteritidis PT 30 from inoculated almonds before and after oil treatment at 121°C for 1 and 3 min comparing stomaching, blending, hand shaking, and mechanical shaking methods*

Treatment time (min)	Mean ± SD plate counts (log CFU/g)[a]							
	Stomaching (100 ml TSB)		Blending (450 ml TSB)		Hand shaking (50 ml TSB)		Mechanical shaking (50 ml TSB)	
	TSA	BSA	TSA	BSA	TSA	BSA	TSA	BSA
	Untreated[b]							
0	8.6 ± 0.1 A	8.5 ± 0.2 ab	8.7 ± 0.1 A	8.6 ± 0.1 a	8.1 ± 0.3 B	8.1 ± 0.3 c	8.3 ± 0.3 B	8.2 ± 0.3 c
	Treated[c]							
1	4.6 ± 0.2 A	4.4 ± 0.2 a	4.6 ± 0.3 A	4.0 ± 0.5 a	4.3 ± 0.4 A	3.9 ± 0.4 a	4.5 ± 0.4 A	4.3 ± 0.4 a
3	2.7 ± 0.6 A	2.1 ± 1.1 a	1.9 ± 0.3 B	1.3 ± 0.4 a	2.3 ± 0.4 AB	1.9 ± 0.6 a	2.4 ± 0.3 A	2.1 ± 1.3 a

[a] Within rows, mean plate counts for TSA with different uppercase letters are significantly different ($P < 0.05$) and mean plate counts for BSA with different lowercase letters are significantly different ($P < 0.05$).

[b] $n = 9$ for stomaching, hand shaking, mechanical shaking; $n = 6$ for blending.

[c] After heating in oil, $n = 6$.

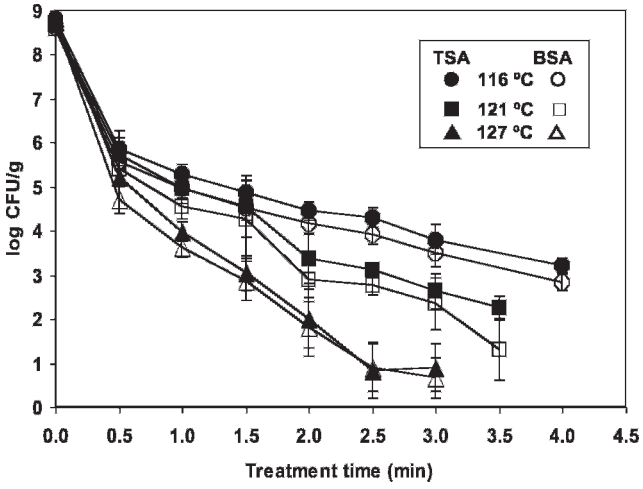

FIGURE 2. *Survival of* Salmonella *Enteritidis PT 30 on inoculated almonds after exposure to hot oil at 116, 121, or 127°C (±1.1°C) (*n = 6*; detection limit = 0.3 log CFU/g).*

oil roasting that were lower than standard operational temperatures.

In most cases, after inoculated almonds were treated in hot oil, colony counts for *Salmonella* Enteritidis on TSA and BSA (Fig. 2) were not significantly different ($P > 0.05$), although counts on BSA were often lower than those on TSA. Significant and rapid reductions of *Salmonella* Enteritidis by 2.9, 3.0, and 3.6 log CFU/g were observed in the first 30 s of heating at 116, 121, and 127°C, respectively (Fig. 2). Thereafter, the reductions occurred at a slower rate. A similar upwardly concave curve was observed for *Salmonella* Senftenberg (data not shown).

Possible explanations for this rapid initial reduction include washing off of loosely attached bacteria as the almonds are placed in the oil, presence of a less protected and more sensitive outer layer of cells, a rapid decrease in water activity at the almond surface, and a release of water vapor at the almond surface during the initial heating period, which would enhance initial microbial reduction.

Almonds were heated for 30 s in 93, 104, 116, 121, and 127°C oil to test the cell wash-off hypothesis. If cells washed off as almonds were placed in the oil, similar 30-s reductions would occur over a broad range of oil temperatures. However, as oil temperature increased, the reduction of *Salmonella* Enteritidis also increased in a linear fashion ($R^2 = 0.99$) (Fig. 3). An extrapolation of this line predicted that a 5-log reduction in *Salmonella* Enteritidis PT 30 would occur at an oil temperature of 145°C (293°F). In a supplementary follow-up experiment, a 5.2-log reduction ($n = 6$) was observed after 30 s when almonds were heated at this temperature (Fig. 3).

We hypothesized that if an outer layer of sensitive bacterial cells were present, a greater reduction would occur in 30 s for almonds inoculated at 8 log CFU/g than for almonds inoculated at 5 log CFU/g. However, similar reductions of 0.94 and 0.95 log CFU/g were observed for almonds inoculated at 8 and 5 log CFU/g, respectively, in the first 30 s of heating at 93°C. This temperature was

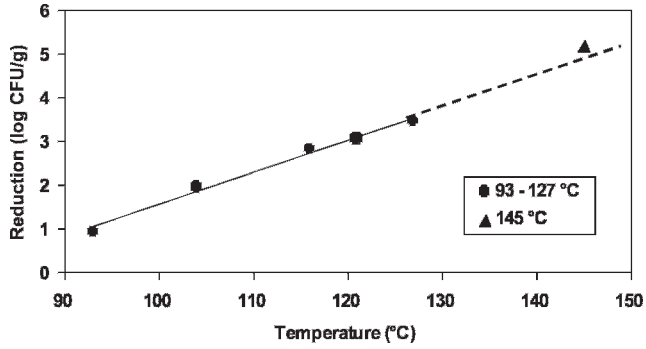

FIGURE 3. *Effect of oil temperature on reduction of* Salmonella *Enteritidis PT 30 on almonds exposed to hot oil for 30 s. Enumeration on TSA (*n = 6*).*

chosen so that counts on the almonds inoculated at the lower level would not approach the limit of detection after heating.

The nonlinear nature of the thermal death curve presents challenges for validation of industry processes. We elected to use two methods to determine process times: the Weibull model and end-point determination.

Weibull model. Survival curves obtained from heat inactivation of *Salmonella* in many foods such as milk chocolate *(18),* peanut butter *(33),* and flour *(8)* are not log linear. To describe nonlinear thermal inactivation of microbial cells, the Weibull model is often used *(24, 33, 37)*; therefore, this model was used to analyze our data.

The suitability of using the Weibull model was evaluated by plotting $\ln(-\ln S)$ versus $\ln t$. Straight lines were observed from the graphs, with R^2 values higher than 0.92 in all cases (data not shown), which indicated that the Weibull model was appropriate *(37)*. The fitted scale (n) and shape (b) parameters were calculated according to the Weibull model for each survival curve (Fig. 4 and Table 4). For all temperatures, n was <1, which is indicative of increasing heat resistance (tailing of survival curve).

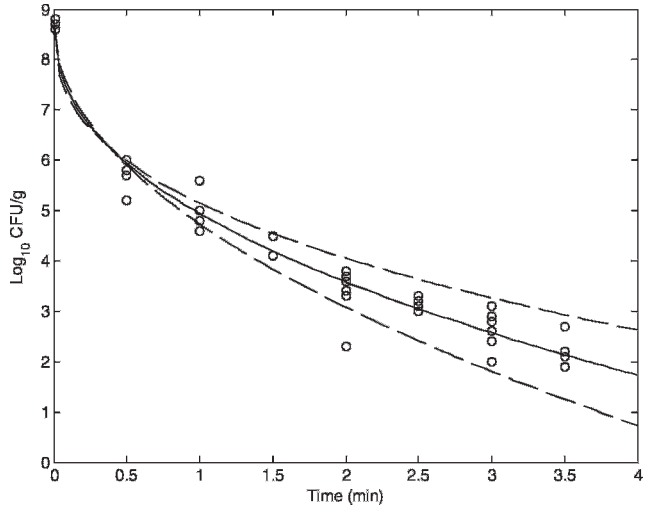

FIGURE 4. *Demonstration of the best fit (—) and 99% upper and lower confidence intervals for the coefficient estimates (---) of the Weibull model to experimental data of* Salmonella *Enteritidis PT 30 on inoculated almonds after exposure to 121°C oil. Enumeration on TSA.*

TABLE 4. *Survival parameters of* Salmonella *Enteritidis PT 30 and* Salmonella *Senftenberg 775W on oil-roasted almonds according to the Weibull model*[a]

Oil temp (°C)	Measure	Power (n)	Coefficient (b) (min^{-n})	R^2
	Salmonella Enteritidis PT 30			
116	Best fit	0.368	3.372	0.952
	99% CI[b]	0.312	3.183	
121	Best fit	0.445	3.759	0.968
	99% CI	0.387	3.552	
127	Best fit	0.494	4.832	0.962
	99% CI	0.418	4.525	
	Salmonella Senftenberg 775W			
127	Best fit	0.457	4.984	0.963
	99% CI	0.387	4.692	

[a] 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 TSA.

[b] Value is the lower bound of the 99% CI (confidence interval).

The time required to achieve 4- or 5-log reductions for *Salmonella* Enteritidis and *Salmonella* Senftenberg on almonds exposed to hot oil was determined using the Weibull model with both the best-fit curves and the lower bound of the 99% CI for the coefficient estimates (Table 5). Differences were observed between the process times determined with TSA and BSA data; TSA data resulted in consistently more conservative times (Table 5). A 4-log reduction of *Salmonella* Enteritidis was achieved after heating for 2.1, 1.4, or 0.74 min at 116, 121, or 127°C, respectively, based on TSA data and the 99% CI. Time to achieve a 4-log reduction of *Salmonella* Senftenberg was calculated as 0.66 min at 127°C, approximately 4 s shorter than the time for *Salmonella* Enteritidis. The time required for a 5-log reduction was 1.7 to 2.0 times that required to achieve a 4-log reduction of *Salmonella* because of the shape of the survivor curve. These data are based on a single study in a single laboratory using one variety of almonds (Mission) and two strains of *Salmonella*. Further studies are underway to evaluate the impact of almond variety, strain, and storage treatment on the heat resistance of *Salmonella* on almonds treated in oil.

Weibull model parameters have been estimated based on inactivation curves for *Salmonella* in heated peanut butter. According to this model, a 5-log reduction of a mixture of three outbreak-associated *Salmonella* Tennessee strains in peanut butter could be achieved in 42 min at 90°C *(24)*; Shachar and Yaron *(33)* obtained a 2.5-log reduction of a cocktail of *Salmonella* serovars Agona, Enteritidis, and Typhimurium in peanut butter heated to the same temperature. In the present study, the lowest temperature used was 93°C. A 1-log reduction was obtained in 30 s at this temperature (Fig. 3). Because this temperature is significantly lower than those typically used to roast almonds, a full thermal inactivation curve was not determined and so a direct comparison of heat resistance of *Salmonella* in the two products was not possible.

TABLE 5. *Time required to achieve 4- or 5-log reductions of* Salmonella *Enteritidis PT 30 and* Salmonella *Senftenberg 775W on almonds exposed to hot oil, according to the Weibull model*

		Time required for reduction (min)			
		4 log		5 log	
Oil temp (°C)	Measure	TSA	BSA	TSA	BSA
	Salmonella Enteritidis PT 30				
116	Best fit	1.6	1.5	2.9	2.7
	99% CI[a]	2.1	1.9	4.2	3.9
121	Best fit	1.2	1.0	1.9	1.7
	99% CI	1.4	1.2	2.4	2.2
127	Best fit	0.68	0.61	1.1	0.99
	99% CI	0.74	0.65	1.3	1.2
	Salmonella Senftenberg 775W				
127	Best fit	0.62	0.60	1.0	0.99
	99% CI	0.66	0.64	1.2	1.2

[a] Value is the lower bound of the 99% CI (confidence interval) for each survival curve (Fig. 4 and Table 4).

End-point determination. An end-point determination is often used as an alternative to plate counts for predicting *D*-values *(26)*. With this method, the presence or absence of the organism is recorded after enrichment of multiple subsamples. We used a variation of this method to validate reductions predicted with *Salmonella* plate count data. Initial levels of *Salmonella* Enteritidis on inoculated almonds were 5.1 and 5.3 log CFU/g before treatment at 121 and 127°C, respectively, as determined by plate counts. After treatment at 121°C for 2.5 min or 127°C for 1.5 min, none of the nine enrichment tubes (each containing 1 g of product) were positive for *Salmonella* Enteritidis (Table 6). Similarly, for almonds inoculated with *Salmonella* Senftenberg to an initial level of 5.2 log CFU/g and treated at 127°C for 1.5 min, none of the nine enrichment tubes (each containing 1 g of product) were positive for *Salmonella* (Table 6). Thus, an approximately 5-log reduction was achieved in less than 1.5 min by heating at 127°C for both *Salmonella* Enteritidis and *Salmonella* Senftenberg, which corresponds to the 1.3 min predicted for the same reduction (Table 5) determined with plate count data and the Weibull equation.

Heat resistance increases as the water activity of the product and the moisture in the heating environment decrease. This effect has been demonstrated in aqueous systems using solutes to control the water activity *(19, 25)* and in low water activity foods such as chocolate *(18),* dried milk *(27),* flour *(8),* and peanut butter *(33)*. Organisms attached to surfaces also exhibit increased heat resistance *(15),* which may be a factor in the high heat resistance for *Salmonella* observed on almonds.

The Technical Expert Review Panel of the Almond Board of California *(4)* used some of the data presented in this article to recommend a minimum process of 2 min of exposure to 127°C oil to achieve a minimum 5-log reduction of *Salmonella* Enteritidis PT 30 in almonds. The term ''pasteurized'' may be used without FDA objection on

TABLE 6. *Effect of treatment time on number of* Salmonella*-positive 1-g almond samples detected after enrichment*

Initial population	Oil temp (°C)	No. of positive samples at[a]: 0.5 min	1.0 min	1.5 min	2.5 min	3.5 min
Salmonella Enteritidis PT30						
5.1 log CFU/g	121			2	0	0
5.3 log CFU/g	127	8	2	0		
Salmonella Senftenberg 775W						
5.2 log CFU/g	127	9	1	0		

[a] Nine 1-g samples were enriched for each inoculation level and temperature.

the labels of almonds that have been treated in this manner *(4)*. Standard industry oil-roasting parameters (138 to 149°C [280 to 300°F] for 3 to 15 min) that achieve acceptable kernel color and texture should result in significantly greater than 5-log reductions of *Salmonella*. Although these data may be indicative of results that could be achieved with other oil-roasted nuts, this supposition should be supported with additional data.

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