Food Research International 45 (2012) 1093–1098

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Survival of *Salmonella* Enteritidis PT 30 on inoculated almond kernels in hot water treatments

Linda J. Harris *, Aaron R. Uesugi [1], Shirin J. Abd [2], Kathryn L. McCarthy

Department of Food Science and Technology, University of California, One Shields Avenue, Davis, CA 95616-8598, USA

ARTICLE INFO

Article history:
Received 26 January 2011
Received in revised form 18 March 2011
Accepted 22 March 2011

Keywords:
Almond kernels
Blanch
Salmonella
Thermal process

ABSTRACT

Almonds are blanched by exposure to hot water or steam-injected water to remove the pellicle (skin) from the kernel. This study evaluated the survival of *Salmonella* Enteritidis PT 30, *Salmonella* Senftenberg 775W and *Enterococcus faecalis* on whole raw almond kernels exposed to hot water. Whole, inoculated (7 to 9 log CFU/g) Nonpareil almonds (40 g) were submerged in 25 L of water maintained at 60, 70, 80 and 88 °C. Almonds were heated for up to 12 min, drained for 2 s, and transferred to 80 mL of cold (4 °C) tryptic soy broth. Almonds in broth were stomached at high speed for 2 min, serially diluted, plated onto tryptic soy and bismuth sulfite agars (*Salmonella*) or bile esculin agar (*Enterococcus*) and incubated at 37 °C for 24 and 48 h, respectively. D values of 2.6, 1.2, 0.75 and 0.39 min were calculated for exposure of *S.* Enteritidis PT 30 to water at 60, 70, 80 and 88 °C, respectively; the calculated z value was 35 C°. D values determined for *Salmonella* Senftenberg 775W and *E. faecalis* at 88 °C were 0.37 and 0.36 min, respectively. Neither *Salmonella* serovar could be recovered by enrichment of 1-g samples after almonds inoculated at 5 log CFU/g were heated at 88 °C for 2 min. These data will be useful to validate almond industry blanching processes.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

California accounts for the majority (about 80%) of the world's and all of the U.S.'s commercial almond production (ABC, 2010b). Consumption of raw almonds from California was associated with outbreaks of salmonellosis from 2000 to 2001 (Isaacs et al. 2005), 2003 to 2004 (CDC, 2004) and 2005 to 2006 (Ledet Müller et al. 2007). *Salmonella enterica* serovar Enteritidis phage type 30 (PT 30) and PT 9c were identified as the outbreak strains. Since September 2007, in response to these outbreaks, almonds grown in California and sold in North America (U.S., Canada and Mexico) must be processed to achieve a minimum 4-log reduction of *Salmonella* using a validated process (Federal Register 2007). These processes may either induce desirable sensory characteristics (e.g., roasting or blanching) (Du, Abd, McCarthy, & Harris 2010) or may be designed to retain attributes of the raw almond (e.g., propylene oxide, high pressure, infrared heating, moist air impingement, steam or combination treatments) (ABC, 2007a; Bari et al. 2009; Bari et al. 2010; Brandl, Pan, Huynh, Zhu, & McHugh 2008; Chang, Han, Reyes-De-Corcuera, Powers, & Kang 2010; Jeong, Marks, & Orta-Ramirez 2009; Lee et al. 2006; Willford, Mendonca, & Goodridge 2008; Yang et al. 2010).

Commercial blanching is a process carried out to remove the pellicle or seed coat (skin) of almonds. In general, the skin is easiest to remove from the Nonpareil varieties (Nonpareil, Sonora and Price), which are used as the standard when comparing the blanching potential of other varieties. Blanched almonds are marketed whole, sliced, slivered, diced or as flour.

The water temperature and time of exposure used in commercial blanching processes can vary based on the almond variety, initial almond moisture level and type of blanched product (e.g., whole kernels or pieces) (ABC, 2007b). The blanching process generally consists of multiple steps, including pre-wetting, scalding, peeling and pellicle separation, rinsing, drying and cooling, before products are sorted (graded) and packed. The scalding and drying steps are the only points where heat is applied. In a typical blancher, the almonds enter through a water bath to be wetted for 5 to 20 s, and then the kernels pass through a continuous scalder, where they are submerged in hot water or steam-injected water; the hot water temperatures usually range from 85 to 100 °C with exposure times of 2 to 5 min (ABC, 2010a). After the skins have been removed, almonds are dried with hot air at 104 to 116 °C for 20 to 40 min; exposure time is largely dependent on the size of the dryer (Gray 2010).

Since no published data exist on the behavior of *Salmonella* on the surface of almond kernels during hot water blanching, the specific objectives of this study were to (1) evaluate methods for recovery of *Salmonella* from inoculated almonds before and after exposure to hot water, and (2) determine the heat resistance of *Salmonella* and *Enterococcus faecalis* on almond kernels exposed to hot water.

* Corresponding author. Tel.: +1 530 7549485; fax: +1 530 7524759.
E-mail address: ljharris@ucdavis.edu (L.J. Harris).
[1] Present address: Kraft Foods, 200 DeForest Ave, East Hanover, NJ 07936, USA.
[2] Present address: The National Food Laboratory, 2441 Constitution Drive, Livermore, CA 94551, USA.

0963-9969/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2011.03.048

2. Materials and methods

2.1. Almonds

Whole raw almond kernels (untreated) were provided by Blue Diamond Growers (Sacramento, CA, USA). Carmel almonds of size 23/25 (23 to 25 almonds per 28 g) were used in initial experiments to evaluate recovery methods. Thereafter, Nonpareil almonds of size 23/25 were evaluated since they represent the typical almond variety and size that is commercially blanched in hot water. In addition, Nonpareil (size 25/27 and 22/24), Carmel (size 20/22), California (size 25/27), Ne Plus (size 25/27) and Butte (size 25/27) almonds were used to determine the blanchability of these different almond varieties. The almonds were stored in plastic bags inside a tightly sealed plastic container for up to 2 months at ambient temperature (between 18 and 24 °C) before use.

2.2. Inoculum preparation

Salmonella Enteritidis PT 30 (ATCC BAA-1045), *Salmonella* Senftenberg 775W (ATCC 43845) and *E. faecalis* (ATCC 29212) were used in this study. All media were obtained from BD (Franklin Lakes, NJ, USA), unless otherwise specified. Isolates were stored at −80 °C in tryptic soy broth (TSB) supplemented with 15% glycerol (Fisher, Fair Lawn, NJ, USA). Inoculum was prepared independently for each strain using the procedure described previously (Danyluk, Uesugi, & Harris 2005), with the following modification for cell harvesting (Du et al. 2010). Following incubation, approximately 9 mL of 0.1% peptone was added to each petri dish (150 by 15 mm); the bacterial lawn was loosened with a sterile spreader, and a sterile pipette was used to collect the cells into a sterile container. A 25-mL suspension of cells was collected from three petri dishes, which was a sufficient volume to inoculate 400 g of almonds. For some experiments the population level in the inoculum was adjusted by diluting in 0.1% peptone prior to inoculating the almonds.

To determine inoculum levels, inocula were serially diluted in Butterfield's phosphate buffer (BPB) and then plated in duplicate onto tryptic soy agar (TSA) and bismuth sulfite agar (BSA) for *Salmonella* or onto TSA and bile esculin agar (BEA) for *Enterococcus*. Plates were incubated for 24 ± 2 h (TSA) or 48 ± 2 h (BSA or BEA) at 35 ± 2 °C.

2.3. Inoculation procedure

Almonds were inoculated as described in more detail previously (Danyluk et al. 2005). Briefly, each almond sample (400 ± 1 g) was weighed into a polyethylene bag (30.5 × 30.5 cm) and 25 mL of the inoculum was added before closing the bag. To mix the contents thoroughly, bags were agitated for 1 min. Inoculated almonds were spread onto filter paper and left to dry for 24 h at 24 ± 2 °C (at approximately 25 to 35% relative humidity). As described previously (Du et al. 2010), the dried almonds were pooled in larger bags (40.6 × 40.6 cm) and thoroughly mixed by inverting and righting bags for 1 min. Inoculum levels were confirmed by plating duplicate samples (40 ± 1 g). To maintain stable populations, inoculated almonds were stored in a sealed plastic bag stored inside a sealed plastic container at 4 °C for a maximum of 3 weeks (Uesugi, Danyluk, & Harris 2006). Prior to blanching treatment, almonds were removed from storage and held at room temperature for 3 to 4 h.

2.4. Measuring almond surface temperature

Different methods for determining the surface temperatures of almonds during hot water blanching were evaluated in experiments similar to those described in detail by Du et al. (2010) for oil roasting. Briefly, the exposed tip of a thermocouple (type K; Omega Engineering, Stamford, CT, USA) was located as follows: (1) on the surface of whole Nonpareil almonds of size 23/25, but not embedded in the skin; (2) embedded (tip only) in the skin of whole Nonpareil almonds of size 23/25; or (3) inserted (by the manufacturer) into the center of model almonds made of aluminum (FMC Technologies, Madera, CA, USA). Thermocouples also were attached to the side of the water bath and to a wire mesh basket that was immersed directly in the hot water. All thermocouples were connected to a data logger (Campbell Scientific, Logan, UT, USA) equipped with an SM192 storage module. The almonds with thermocouples attached were placed inside the wire mesh basket with 40 g of uninoculated almonds, and the basket was immersed in the hot water (88 °C) for 1.5 min. Thermocouple temperatures were monitored every 5 s. The experiment was replicated three times.

2.5. Hot water treatments

Whole inoculated or uninoculated almonds (40 g) were placed in a wire mesh basket that allowed free movement of the almonds and ensured complete submersion in water for the entire treatment. The basket was submerged in 25 L of hot water in a water bath maintained at a target temperature of 60, 70, 80 or 88 °C. Temperature was maintained within 0.2 °C of the target temperature. A heating unit (Isotemp 2150; Fisher) circulated the water, which also caused the almonds to be gently agitated during treatment. The digital display of the heating unit was monitored before and during heating of the almonds. A digital thermometer (Omega HH509; Omega Engineering) connected to a type K thermocouple was used to monitor water temperature; the thermocouple was attached to the basket containing the almonds. Almonds were heated for predetermined times ranging from 30 s to 12 min, timed from the moment that the mesh basket was immersed in the hot water. If the water temperature moved outside the ± 0.2 °C target at any time after the first 30 s, the almond sample was discarded. Between treatments, any water that had evaporated was replaced so as to maintain the volume at 25 L.

Although higher blanching temperatures are more commonly used by the industry, they were not evaluated here because of laboratory equipment limitations (higher temperatures were not stable) and because preliminary results (not shown) indicated that the decrease in *Salmonella* was too rapid at temperatures above 88 °C and only one or two measurements could be made.

2.6. Recovery of inoculated cells

Three methods, i.e., stomaching, mechanical shaking and hand shaking, were initially compared for recovery and enumeration of *Salmonella* Enteritidis PT 30 from almonds (10-g and 50-g samples). The stomaching method was used in subsequent experiments and to determine background microbial levels on uninoculated almonds (control samples). The procedures of each method are described below.

Stomaching: almonds were added to a double volume of BPB (w/v) in a two-chamber filtering bag (1600 mL; Nasco, Modesto, CA, USA) and macerated for 2 min at high speed with a Stomacher 400 laboratory blender (Seward, Worthington, UK).

Mechanical shaking: a modification of the method described by King and Jones (2001) was used. Almonds and an equal volume (w/v) of sterile BPB were placed into 118-mL sterile polypropylene specimen containers (Fisher); samples were placed on a rotary shaker and shaken for 15 min at 150 rpm.

Hand shaking: a procedure described by Uesugi et al. (2006) was used, which is a modification of the Food and Drug Administration *Bacteriological analytical manual* (FDA-BAM) method (Andrews & Hammack 2003), in which almonds were added to an equal volume of BPB 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.

For most experiments after hot water treatment, almonds (40 g) were removed from the water, drained in the wire mesh basket for

approximately 2 s, and transferred to 80 mL of cold (4 °C) TSB in a 710-mL Whirl-Pak bag. No attempt was made to separate loose skins from the almonds; microbial counts were determined for almond kernels and skins together. Samples were macerated for 2 min at high speed using a Stomacher 3500 laboratory blender (Seward), then held on ice for 3 to 5 min, vigorously shaken by hand five times, and then serially diluted in BPB.

Sample aliquots (0.1 mL for spread plating) were immediately plated in duplicate onto TSA and BSA for *Salmonella* or onto TSA and BEA for *Enterococcus*. In addition to plating 0.1 mL of the lowest dilution (10^0), four spread plates of 0.25 mL each were prepared to improve the detection limit to 2 CFU/g (0.3 log CFU/g). Colonies were counted at 24 ± 2 h (TSA) or 48 ± 2 h (BSA and BEA) after incubation at 35 ± 2 °C. Results were reported as the log of the number of survivors per gram of almonds. When some counts were below the limit of detection, averages were calculated using the limit of detection for those samples.

2.7. Confirmation of presumptive Salmonella colonies

When almonds were blanched at higher temperatures and longer times, counts on TSA dropped to the levels of the background population (≤2.0 log CFU/g). In these cases, presumptive *Salmonella* colonies were confirmed using the procedure described by Du et al. (2010). All presumptive *Salmonella* colonies on TSA were streaked onto Hektoen enteric agar (HE) plates with sterile toothpicks; plates were incubated at 35 ± 2 °C for 24 ± 2 h. Presumptive positive colonies were re-streaked onto HE plates to obtain isolated colonies. One isolated colony from each HE plate was stabbed and streaked onto lysine iron agar (LIA) and triple sugar iron (TSI) 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, USA). The *Salmonella* count was adjusted, as appropriate, based on these results.

2.8. Modified end-point procedure

Reductions of *Salmonella* on additional almond samples inoculated with *Salmonella* Enteritidis PT 30 and *Salmonella* Senftenberg 775W (to a level of approximately 5 log CFU/g after 24 h of drying at room temperature) were also determined by a modified end-point procedure (Mazzotta 2001). These almonds were treated in hot water as described above. Instead of plating, samples were enriched for *Salmonella* using a modification of the FDA-BAM method (Andrews & Hammack 2007). Almonds (40 g) and 360 mL of lactose broth were added to a sterile stainless steel blender jar (Waring Products, Torrington, CT, USA) and blended at low speed for 2 min. As described previously by Du et al. (2010), three 10-mL portions of the blender homogenate (each equivalent to 1 g of almonds) were placed into individual sterile test tubes (16 by 150 mm), incubated at 35 ± 2 °C for 24 ± 2 h, and 0.1 mL portions of this culture were placed into tubes containing 10 mL of Rappaport–Vassiliadis (RV) broth. 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, *Salmonella*-presumptive colonies on HE plates were picked and inoculated onto both TSI and LIA slants. The slants were incubated at 35 ± 2 °C for 24 ± 2 h. Cultures from slants showing a reaction typical of *Salmonella* were confirmed with a *Salmonella* latex test.

2.9. Evaluation of heat transfer to almond surface

To model microbial destruction on the surface of the almond, the temperature of the almond surface must be known. The model applied here assumes a constant surface temperature. To ensure this assumption was met, an aluminum almond was manufactured to simulate a Nonpareil-type almond, with dimensions of 2.5 × 1.4 × 0.6 cm. A type K thermocouple was embedded in the aluminum almond. The heat transfer coefficient from the hot water heating medium to the aluminum almond was determined by a lumped capacity analysis (Miller, Singh, & Farkas 1994). The same water bath used in the blanching experiments was used in this study. Time temperature profiles were recorded in triplicate at water temperatures of 60, 70, 80 and 88 °C. Data were acquired over 30 s at 1-s intervals (Daqpro 5100; Omega Engineering). Water temperature was recorded using a type K thermocouple. Water temperatures were consistently within 0.8 °C of the desired temperature.

The heat transfer coefficient was calculated from nonlinear regression of the time-temperature profile, using Matlab 7.10 Curve Fitting Toolbox (The MathWorks, Natick, MA, USA). The heat transfer coefficient, *h*, was determined from the equation

$$T = T_m - (T_o - T_m)\exp\left[-hAt \Big/ \left(\rho c_p V\right)\right]$$

where T is temperature in °C, T_m is the heating medium temperature (hot water) in °C, T_o is the initial thermocouple (e.g., aluminum almond) temperature in °C, t is time in seconds, ρ is the density of aluminum (2700 kg/m^3), c_p is the specific heat of aluminum (900 J/kg °C), A is the surface area of the almond, and V is the volume of the almond. The surface area was estimated as the surface area of an ellipsoid of the same dimensions. The volume was determined by the ratio of the mass of the aluminum almond (2.6 g) to the density.

2.10. Calculation of D and z values

Decimal reduction times (D values (min)) were determined as the negative inverse slope of the linear regression line for log survivors over the time heated in water, as determined by plating on TSA. The z value (C°) was calculated as the negative inverse slope of the linear regression line for the log D values over the range of heating temperatures tested.

2.11. Effect of variety on skin removal

A study was undertaken to determine how efficiently the skins could be removed from various almond varieties with a hot water treatment of 2 min. For blanching, 40-g samples of uninoculated almonds were placed in a wire mesh basket and heated for 2 min in deionized water (25 L) at 88 ± 0.2 °C, drained for 2 s, and immersed in 1 L of cold (4 °C) water. The loosened skins were removed by hand, and the kernels were sorted based on complete or partial removal of the skin. A defect rate (%) was calculated by counting the number of kernels with some adhering skin and dividing by the total number of kernels in the 40-g sample.

2.12. Statistical analysis

Data were analyzed using JMP (version 8.0) software (SAS Institute, Cary, NC, USA). Analysis of variance by the general linear model procedure and student's *t*-test were used to find the significant differences ($P<0.05$) among treatments.

3. Results and discussion

Salmonella Enteritidis PT 30 was chosen for the current study in order to make direct comparisons to inactivation in hot oil (Du et al. 2010), and because the Almond Board of California recommends the use of this specific strain of *Salmonella* for validation of almond thermal processes (ABC, 2007a). *Salmonella* Senftenberg 775W is considered unusually heat resistant (Ng, Bayne, & Garibaldi 1969), especially in aqueous solutions (Goepfert, Iskander, & Amundson 1970). On almonds, *Salmonella* Enteritidis PT 30 and *Salmonella*

Senftenberg 775W have the same heat resistance in hot oil (Du et al. 2010). The reported discrepancy in heat resistance of *Salmonella* Senftenberg 775W is the reason this strain was also included in the current study.

3.1. Almond surface temperature during exposure to hot water

Surface contamination of the almond kernel is considered most likely given the means of harvest and postharvest handling (Uesugi & Harris 2006). Thus, the surface temperature is considered more relevant to thermal processes than the internal product temperature (Du et al. 2010). In the present study the skins began to separate from the almond kernels shortly after immersion in the hot water; therefore, inserting the thermocouple into the skin was considered to be an unreliable method to monitor temperature. Readings from the thermocouples attached to the wire mesh basket used to submerge the almonds in the water reached the water temperature in 10 s (data not shown).

The average heat transfer coefficient, as determined with an aluminum almond, was 1288 W/m^2 °C. There was no significant difference ($P=0.74$) in heat transfer coefficient values due to heating medium temperature, based on a single factor ANOVA with three replicates. Based on this heat transfer coefficient and the thermal properties of almonds as calculated based on composition (Toledo 1991), the surface temperature of almonds was within 2 °C of the heating medium temperature within 30 s. Therefore, a come-up time of 30 s was used to model microbial destruction on the surface of the almonds. For all further experiments, water and almond surface temperatures were monitored by thermocouples attached to the basket containing the almonds.

3.2. Recovery methods

In preliminary experiments for both untreated and treated (70 °C, 4 min) samples, hand shaking and mechanical shaking methods resulted in significantly lower colony counts ($P<0.05$) compared with stomaching (Table 1). Sample sizes of 10 and 50 g were evaluated. For untreated samples, differences in counts between the two samples sizes were not significantly different in most cases ($P>0.05$) (Table 1). However, after treatment, *Salmonella* Enteritidis PT 30 counts were significantly higher ($P<0.05$) with the larger sample and the standard deviation among samples was often lower. For further experiments a larger sample size was used; 40 g was chosen over 50 g to provide greater room for the almonds in the basket used for the blanching studies. Stomaching of 40 g of almonds in 80 mL of cold diluent (Du et al. 2010) was used to recover *Salmonella* in subsequent experiments. An almond-to-diluent ratio less than the standard 1:9 was chosen to improve the limit of detection after heating. A 1:1 ratio was also assessed but this volume of liquid was insufficient for the almonds to break up adequately during stomaching (Du et al. 2010).

3.3. Microbial populations

Background counts for uninoculated almonds ranged from 2 to 3 log CFU/g on TSA (data not shown). After blanching, counts on TSA dropped to ≤2 log CFU/g at the end of the heating period for each temperature. Colonies appearing on TSA plates of uninoculated, untreated control samples varied in size, shape and color and sometimes included spreading colonies. Colonies appearing on TSA plates of inoculated samples were consistent in size, shape and appearance. With the exception of one or two individual plates, colonies were never detected on BSA in any of the trials for the uninoculated control samples. The colonies that were detected on BSA were atypical of *Salmonella* Enteritidis PT 30 on this medium. Selection of these isolates and streaking on TSI and LIA slants confirmed that they were not *Salmonella*. Colony counts for *Salmonella* Enteritidis PT 30 and *Salmonella* Senftenberg were 0 to 0.2 log CFU/mL higher on TSA than on BSA for the inoculum preparation, and higher by an average of 0.1 log CFU/g (maximum 0.4 log CFU/g) for inoculated almonds after drying for 24 h. Counts on TSA and BEA differed by 0.2 log CFU/mL (inoculum) or 0.2 log CFU/g (almonds); counts on BEA were sometimes higher.

Salmonella Enteritidis PT 30 levels determined for the undiluted inoculum preparation on TSA were 12 log CFU/mL. *Salmonella* Senftenberg was more sensitive to drying than was *Salmonella* Enteritidis. After drying of undiluted inoculum, *Salmonella* Enteritidis PT 30 and *Salmonella* Senftenberg levels were 8.7 ± 0.3 and 7.8 log CFU/g of almonds, respectively. For the modified endpoint procedure diluted inoculum concentrations of 7.7 and 8.7 log CFU/mL for *Salmonella* Enteritidis and *Salmonella* Senftenberg, respectively, yielded concentrations of 6.2 ± 0.1 and 7.4 ± 0.1 log CFU/g, respectively, on wet almonds and concentrations of 5.3 and 5.0 log CFU/g, respectively, on dried almonds.

Significant reductions of *Salmonella* Enteritidis PT 30 by 0.9, 1.1, 2.9 and 4.7 log CFU/g were observed in the first 30 s of heating at 60, 70, 80 and 88 °C, respectively (Table 2), which corresponded to the come-

Table 1
Recovery of *Salmonella* Enteritidis PT 30 and *E. faecalis* from inoculated Carmel variety almonds before and after exposure to hot water at 70 °C for 4 min comparing stomaching, mechanical shaking, and hand shaking methods.

Almonds[a]	Sample size (g)	Mean (±SD) plate counts (log CFU/g)[b,c]					
		Stomaching (100 mL BPB)		Mechanical shaking (50 mL BPB)		Hand shaking (50 mL BPB)	
		TSA	BSA/BEA[d]	TSA	BSA/BEA	TSA	BSA/BEA
Salmonella Enteritidis PT 30							
Untreated	10	A9.1 ± 0.2D	A9.0 ± 0.2 d	A8.7 ± 0.1E	A8.7 ± 0.1e	A8.8 ± 0.1E	A8.6 ± 0.1e
	50	B8.8 ± 0.1D	B8.8 ± 0.2 d	A8.6 ± 0.2E	B8.4 ± 0.1e	A8.7 ± 0.2DE	A8.7 ± 0.1 d
Treated	10	b5.0 ± 0.9D	b4.2 ± 0.9 d	b2.4 ± 1.7E	b0.9 ± 1.0e	b3.9 ± 1.0D	a3.0 ± 1.6 d
	50	a5.9 ± 0.3D	a5.1 ± 0.5 d	a5.0 ± 0.7E	a3.2 ± 1.3e	a4.7 ± 0.9E	b2.7 ± 1.7e
E. faecalis							
Untreated	10	A8.8 ± 0.6D	A8.8 ± 0.7 d	A7.8 ± 0.5E	A7.6 ± 0.4e	A8.2 ± 0.6DE	A8.0 ± 0.5e
	50	A8.7 ± 0.5D	A8.5 ± 0.6 d	A7.9 ± 0.6E	A7.6 ± 0.6e	A8.2 ± 0.6DE	A7.9 ± 0.5de
Treated	10	a4.9 ± 1.3D	a4.3 ± 1.6 d	b2.0 ± 0.6E	b0.4 ± 0.9e	a4.8 ± 0.4D	a4.3 ± 1.2 d
	50	a5.9 ± 0.4D	a5.7 ± 0.4 d	a3.8 ± 0.8E	a3.4 ± 0.9e	a4.0 ± 0.6E	a3.3 ± 1.3e

[a] Untreated (before blanching), $n=8$; treated (after blanching), $n=8$ (*Salmonella* Enteritidis PT 30) or $n=4$ (*E. faecalis*).
[b] For each organism, populations at both sample sizes were compared: for untreated samples, mean plate counts within columns for TSA or BSA preceded by different uppercase letters (A, B) are significantly different ($P<0.05$); for treated samples, mean plate counts within columns for TSA or BSA preceded by different lowercase letters (a, b) are significantly different ($P<0.05$).
[c] Within rows, mean plate counts for TSA followed by different uppercase letters (D, E) are significantly different ($P<0.05$) and mean plate counts for BSA followed by different lowercase letters (d, e) are significantly different ($P<0.05$).
[d] Enumeration on BSA for *Salmonella*; enumeration on BEA for *E. faecalis*.

Table 2
D values calculated from TSA data with initial data point at 30 s for *Salmonella* inoculated onto almonds and treated in hot water.

Strain	Water temp (°C)	Reduction during first 30 s (log CFU/g)	D value (min)	R^2 value
Salmonella Enteritidis PT 30	60	0.9	2.6	0.89
	70	1.1	1.2	0.83
	80	2.9	0.75	0.71
	88	4.7	0.39	0.81
Salmonella Senftenberg	88	3.4	0.37	0.81
E. faecalis	88	3.5	0.36	0.85

Colonies enumerated on TSA, *n* = 6 to 12.

up time. Thereafter, the reduction was linear but at a slower rate of decline. Counts on BSA were consistently, but not significantly, lower than counts on TSA at most time points (Fig. 1). Similar curves were observed for *Salmonella* Senftenberg and *E. faecalis* at 88 °C except that differences between TSA and BSA or BEA were greater (BSA and BEA lower; data not shown). Therefore, the TSA data were used for D-value calculations for all three organisms.

Possible explanations for the rapid initial reduction include wash-off of loosely attached bacteria as the almonds are placed in the water. However, the initial reduction increased with increasing temperature, which suggests the presence of a less protected outer layer of cells or a more sensitive subpopulation. Similar initial disproportionate reductions were noted on exposure of almonds to oil (Du et al. 2010). However, the survival curve associated with heating in oil remained curved and a Weibull model was considered most appropriate for analyzing those data. For the current study, the Weibull model did not accurately predict the observed survivor curve (data not shown).

The reduction of *Salmonella* Enteritidis PT 30 may be influenced by degree of attachment of the skin to the kernel. At 60, 70 and 80 °C, few skins had visibly separated from the kernels after 4 min of heating. At 88 °C, skin separation was clearly visible after 2 min of heating. The purpose of blanching is to remove the skin of the almond. Sufficient time/temperature treatment is needed for effective skin removal from the majority of almonds in a given process. Some differences in efficiency of skin removal were demonstrated among the seven different almond samples tested (data not shown). After 2 min of exposure to 88 °C water, the percentage of almonds still retaining skins ranged from 2.4 to 16.1% for the samples examined; all within a common industry standard of <20% skin retention during a blanching treatment (Gray 2005). In commercial blanching the almond kernels typically pass through a series of rubber rollers after heating to facilitate removal of the loosened skins. Free skins are rinsed away with a water spray and the wet almonds are dried with forced hot air.

To calculate D values a conservative approach was used that ignored reductions observed during the come-up time of 30 s. For *Salmonella* Enteritidis PT 30, D values of 2.6, 1.2, 0.75 and 0.39 min were determined for temperatures of 60, 70, 80 and 88 °C, respectively (Table 2). Log D values were plotted against temperature to determine a z value of 35 C° (R^2 = 0.99). The D values determined for *Salmonella* Senftenberg 775W and *E. faecalis* at 88 °C were 0.37 and 0.36 min, respectively (Table 2). Using the calculated D values at 88 °C, 4- and 5-log reductions of *Salmonella* Enteritidis PT 30, *Salmonella* Senftenberg 775W, and *E. faecalis* were predicted to occur in 1.6, 1.5, and 1.4 min, respectively, and 2.0, 1.9, and 1.8 min, respectively. The Almond Board of California currently recommends the use of a related strain, *Enterococcus faecium* (NRRL B-2354/ATCC 8459) for validation of dry and wet thermal processes (ABC, 2007d). Lee et al. (2006) exposed almonds inoculated with a three-strain cocktail of *Salmonella* to steam at 93 °C. D values of 0.2 to 0.3 min were observed, similar to an estimated D value of 0.3 min extrapolated from data in the current study.

Significantly higher temperatures are required to achieve comparable reductions under dry heat conditions. Other research in this laboratory (Du et al. 2010) demonstrated 4- to 5-log reductions in both *Salmonella* Enteritidis PT 30 and *Salmonella* Senftenberg on almonds exposed to hot oil at 127 °C for 0.74 to 1.3 min, respectively. For oil-roasted almonds, the Almond Board of California requires that processors demonstrate a minimum of 1.6 min at this temperature to meet a 4-log requirement, or 2.0 min at 127 °C (260 °F) for a 5-log reduction (ABC, 2007c).

3.4. End-point determination

For calculating D values the 3- to 4-log reductions achieved during the come-up time were ignored, therefore, we elected to also use the end-point determination to evaluate reduction of *Salmonella* in hot water. Almonds were inoculated with *Salmonella* Enteritidis PT 30 and *Salmonella* Senftenberg 775W to an initial concentration of 5.3 and 5.0 log CFU/g after drying, respectively. Almonds (three 50-g samples) were heated in 88 °C water for 0.5 to 2.0 min and the equivalent of three 1-g quantities of treated product were enriched for *Salmonella*. Treatment at 88 °C for 0.5, 1.0, 1.5 and 2.0 min resulted in 9, 6, 3 and 0 of 9 tubes positive after enrichment, respectively, for *Salmonella* Enteritidis PT 30 and in 9, 9, 1 and 0 of 9 tubes positive after enrichment, respectively, for *Salmonella* Senftenberg 775W. Thus a 5- to 6-log reduction for both strains occurred between 1.5 and 2.0 min, which was shorter than the time predicted using only the D value

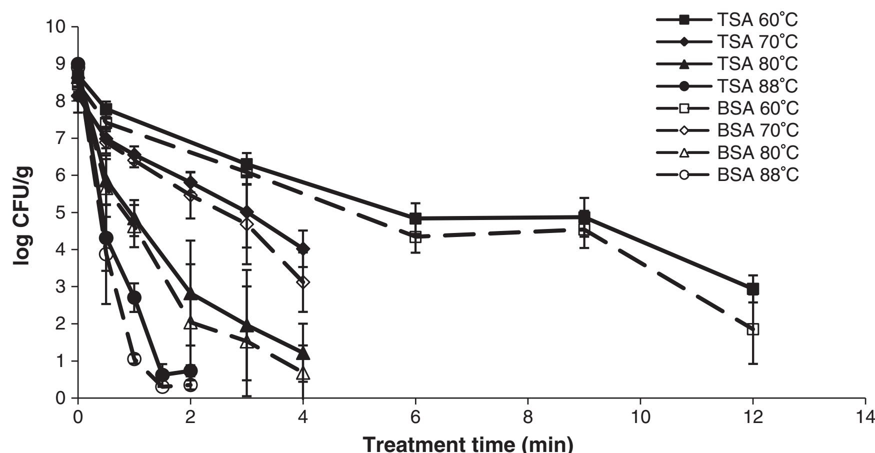

Fig. 1. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after exposure to hot water at 60, 70, 80, and 88 °C and enumerated on TSA and BSA (*n* = 6 to 12; limit of detection = 0.3 log CFU/g).

generated from plate count data. The significant 3.4- to 4.7-log reduction observed during the first 30 s of heating, and not counted in the D-value calculation, contributed to these results.

Beuchat and Heaton (1975) inoculated in-shell pecans with *Salmonella* Senftenberg 775W by dipping them in inoculum and holding overnight. A 3.5 log CFU/g reduction was observed in 2 min for shells and nutmeats cracked after heating in hot water at either 82 or 93 °C, which is less than what would be predicted for almonds heated to this temperature. However, movement during inoculation of the organism to the nutmeat through cracks in the shell and poor heat penetration through the shell were thought to significantly impact the efficacy of the heat process.

4. Conclusions

The time required to achieve 4- and 5-log reductions of *Salmonella* Enteritidis PT 30, *Salmonella* Senftenberg 775W and *E. faecalis* ATCC 29212 at 88 °C were similar and are consistent with the Almond Board of California recommendations for a blanching process of 1.6 to 2.0 min at a minimum temperature of 88 °C at the cold spot to ensure a 4- or 5-log reduction, respectively, of *Salmonella* Enteritidis PT 30. These times are conservative because (1) the times do not take into account the large reductions observed during the come-up time, (2) the skin was included in the microbiological analysis where it would be discarded in commercial practice, and (3) the impact of hot air drying used in commercial practice after skin removal was not assessed.

Acknowledgments

This research was supported by the Almond Board of California. We are grateful for the cooperation from members of the almond industry. We are thankful for the technical assistance of Wen-Xian Du, Chris Theofel and Frank Schlitt-Dittrich as well as the editorial assistance of Sylvia Yada.

References

[ABC] Almond Board of California. (2007a). *Considerations for proprietary processes for almond pasteurization and treatment.* v1.0, April 13, 2007. Available from: http://www.almondboard.com/Handlers/FoodQualitySafety/Pasteurization/PasteurizationProgram/ValidationGuidelines/. Accessed Nov 1, 2010.

[ABC] Almond Board of California. (2007b). *Guidelines for validation of blanching processes.* v1.0, April 13, 2007. Available from: http://www.almondboard.com/Handlers/FoodQualitySafety/Pasteurization/PasteurizationProgram/ValidationGuidelines/. Accessed Nov 1, 2010.

[ABC] Almond Board of California. (2007c). *Guidelines for validation of oil roasting processes.* v1.0, April 13, 2007. Available from: http://www.almondboard.com/Handlers/FoodQualitySafety/Pasteurization/PasteurizationProgram/ValidationGuidelines/. Accessed Nov 1, 2010.

[ABC] Almond Board of California. (2007d). *Guidelines for process validation using Enterococcus faecium NRRL B-2354.* v1.2, October 24, 2007. Available from: http://www.almondboard.com/Handlers/FoodQualitySafety/Pasteurization/PasteurizationProgram/ValidationGuidelines/. Accessed Nov 1, 2010.

[ABC] Almond Board of California. (2010a). *California almonds — Forms.* Available from: http://www.almondboard.com/Handlers/Documents/Technical%20%20Information%20Kit.pdf. Accessed Sept 1, 2010.

[ABC] Almond Board of California. (2010b). *The 2010 almond almanac.* Available from: http://www.almondboard.com. Accessed Jan 12, 2011.

Andrews, W. H., & Hammack, T. S. (2003). Food sampling and preparation of sample homogenate, chap. 1. *Food and Drug Administration bacteriological analytical manual* [online]. Available from: http://vm.cfsan.fda.gov/~ebam/bam-toc.html. Accessed Sept 1, 2010.

Andrews, W. H., & Hammack, T. S. (2007). *Salmonella*, chap. 5. *Food and Drug Administration bacteriological analytical manual* [online]. Available from: http://vm.cfsan.fda.gov/~ebam/bam-toc.html. Accessed Sept 1, 2010.

Bari, M. L., Nei, D., Sotome, I., Nishina, I. Y., Hayakawa, F., Isobe, S., & Kawamoto, S. (2010). Effectiveness of superheated steam and gas catalytic infrared heat treatments to inactivate *Salmonella* on raw almonds. *Foodborne Pathogens and Disease*, *7*, 845–850.

Bari, M. L., Nei, D., Sotome, I., Nishina, I., Isobe, S., & Kawamoto, S. (2009). Effectiveness of sanitizers, dry heat, hot water, and gas catalytic infrared heat treatments to inactivate *Salmonella* on almonds. *Foodborne Pathogens and Disease*, *6*, 953–958.

Beuchat, L. R., & Heaton, E. K. (1975). *Salmonella* survival on pecans as influenced by processing and storage conditions. *Applied Microbiology*, *29*, 795–801.

Brandl, M., Pan, Z., Huynh, S., Zhu, Y., & McHugh, T. H. (2008). Reduction of *Salmonella* Enteritidis population sizes on almond kernels with infrared heat. *Journal of Food Protection*, *71*, 897–902.

[CDC] Centers for Disease Control, Prevention. (2004). Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds—United States and Canada, 2003–2004. *MMWR Weekly*, *53*(22), 484–487.

Chang, S. -S., Han, A. R., Reyes-De-Corcuera, J. I., Powers, J. R., & Kang, D. -H. (2010). Evaluation of steam pasteurization in controlling *Salmonella* serotype Enteritidis on raw almond surfaces. *Letters in Applied Microbiology*, *50*, 393–398.

Danyluk, M. D., Uesugi, A. R., & Harris, L. J. (2005). Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. *Journal of Food Protection*, *68*, 1613–1622.

Du, W. -X., Abd, S. J., McCarthy, K. L., & Harris, L. J. (2010). Reduction of *Salmonella* on inoculated almonds exposed to hot oil. *Journal of Food Protection*, *73*, 1238–1246.

Federal Register (2007). Almonds grown in California; outgoing quality control requirements. *Federal Register*, *72*, 15021–15036 (7 C.F.R. part 981). Available from: http://www.gpoaccess.gov/fr/retrieve.html. Accessed July 1, 2009.

Goepfert, J. M., Iskander, I. K., & Amundson, C. H. (1970). Relation of the heat resistance of *Salmonellae* to the water activity of the environment. *Applied Microbiology*, *19*, 429–433.

Gray, G. (personal communication, 2005). Blanching standards in industry [E-mail to LJ Harris, 2005 Feb 16]. Available from: the author at ljharris@ucdavis.edu.

Gray, G. (personal communication, 2010). Industry practices for drying blanched almonds [E-mail to LJ Harris, 2010 Sept 26]. Available from: the author at ljharris@ucdavis.edu.

Isaacs, S., Aramini, J., Ciebin, B., Farrar, J., Ahmed, R., Middleton, D., Chandran, A. U., Harris, L. J., Howes, M., Chan, E., Pichette, A. S., Campbell, K., Gupta, A., Lior, L. Y., Pearce, M., Clark, C., Rodgers, F., Jamieson, F., Brophy, I., & Ellis, A. (2005). An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. *Journal of Food Protection*, *68*, 191–198.

Jeong, S., Marks, B. P., & Orta-Ramirez, A. (2009). Thermal inactivation kinetics for *Salmonella* Enteriditis PT 30 on almonds subjected to moist-air convection heating. *Journal of Food Protection*, *72*, 1602–1609.

King, A. D., Jr., & Jones, T. (2001). Nut meats. In F. P. Downes, & K. Ito (Eds.), *Compendium of methods for the microbiological examination of foods* (pp. 561–563). (4th ed.). Washington, DC: American Public Health Association.

Ledet Müller, L., Hjertqvist, M., Payne, L., Pettersson, H., Olsson, A., Plym Forshell, L., & Andersson, Y. (2007). Cluster of *Salmonella* Enteritidis in Sweden 2005–2006 — Suspected source: Almonds. *Eurosurveillance*, *12*(6), 153–155.

Lee, S. -Y., Oh, S. -W., Chung, H. -J., Reyes-De-Corcuera, J. I., Powers, J. R., & Kang, D. H. (2006). Reduction of *Salmonella enterica* serovar Enteritidis on the surface of raw shelled almonds by exposure to steam. *Journal of Food Protection*, *69*, 591–595.

Mazzotta, A. S. (2001). Thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. *Journal of Food Protection*, *64*, 315–320.

Miller, K. S., Singh, R. P., & Farkas, B. E. (1994). Viscosity and heat transfer coefficients for canola, corn, palm, and soybean oil. *Journal of Food Processing and Preservation*, *18*, 461–472.

Ng, H., Bayne, H. G., & Garibaldi, J. A. (1969). Heat resistance of *Salmonella*: the uniqueness of *Salmonella senftenberg* 775W. *Applied Microbiology*, *17*, 78–82.

Toledo, R. T. (1991). *Fundamentals of food process engineering* (2nd ed.). New York: Van Nostrand Reinhold.

Uesugi, A. R., Danyluk, M. D., & Harris, L. J. (2006). Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at −20, 4, 23, and 35 °C. *Journal of Food Protection*, *69*, 1851–1857.

Uesugi, A. R., & Harris, L. J. (2006). Growth of *Salmonella* Enteritidis phage type 30 in almond hull and shell slurries and survival in drying almond hulls. *Journal of Food Protection*, *69*, 712–718.

Willford, J., Mendonca, A., & Goodridge, L. D. (2008). Water pressure effectively reduces *Salmonella enterica* serovar Enteritidis on the surface of raw almonds. *Journal of Food Protection*, *71*, 825–829.

Yang, J., Bingol, G., Pan, Z., Brandl, M. T., McHugh, T. H., & Wang, H. (2010). Infrared heating for dry-roasting and pasteurization of almonds. *Journal of Food Engineering*, *101*, 273–280.