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

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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.

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.

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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