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Survival of foodborne pathogens on inshell walnuts ☆

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ABSTRACT

The survival of *Salmonella enterica* Enteritidis PT 30 or five-strain cocktails of *S. enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* was evaluated on inshell walnuts during storage. Inshell walnuts were separately inoculated with an aqueous preparation of the pathogens at levels of 10 to 4 log CFU/nut, dried for 24 h, and then stored at either 4 °C or ambient conditions (23–25 °C, 25–35% relative humidity) for 3 weeks to more than 1 year. During the initial 24-h drying period, bacterial levels declined by 0.7 to 2.4 log CFU/nut. After the inoculum dried, further declines of approximately 0.1 log CFU/nut per month of *Salmonella* Enteritidis PT 30 levels were observed on inshell walnuts stored at 4 °C; at ambient conditions the rates of decline ranged from 0.55 to 2.5 log CFU/nut per month. Rates of decline were generally greater during the first few weeks of storage, particularly at lower inoculum levels. The survival of the five-strain cocktails inoculated at very low levels (under 400 CFU/nut) was determined during storage at ambient conditions. The pathogens could be recovered by either enumeration or enrichment from most samples throughout the 3-month storage period; reductions in bacterial levels from the beginning to end of storage were 0.7, 0.2, and 2.3 log CFU/nut for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively. For 6% of all nut samples (14 of 234 samples), pathogens were isolated from the second but not first 24-h enrichment, suggesting that bacterial cells were viable but not easily culturable. *Salmonella*-inoculated walnuts were exposed for 2 min to water or a 3% solution of sodium hypochlorite (to mimic commercial brightening) either 24 h or 7 days after inoculation; treated nuts were dried for 24 h and held at ambient conditions. *Salmonella* levels were reduced by less than 0.5 log or 2.4 to 2.6 log CFU/nut on water– or chlorine– treated walnuts, respectively, regardless of postinoculation treatment time. Additional reductions of 2.6 and 2.1 log CFU/nut were observed for water- and chlorine-treated walnuts, respectively, after storage for 2 weeks at ambient conditions. Bacterial foodborne pathogens are capable of long-term survival on the surface of inshell walnuts even when initial levels are low.

1. Introduction

Tree nuts have been implicated in a number of foodborne outbreaks (Scott et al., 2009). Salmonellosis has been associated with consumption of nut kernels including almonds and pine nuts (CDC, 2004; Isaacs et al., 2005; Ledet Müller et al., 2007), and *Escherichia coli* O157:H7 gastroenteritis was epidemiologically linked to consumption of walnut kernels (CFIA, 2011a, 2011b). Although outbreaks with inshell nuts are less common, *E. coli* O157:H7 was isolated from inshell hazelnuts linked to a multi-state outbreak in the U.S. (CDC, 2011). Contaminants on the shell can presumably transfer to the kernel during cracking or result in cross contamination of hands or other foods.

Independent of reported illnesses, several Class I recalls initiated in the U.S. and Canada have resulted from isolation of *Salmonella* from nut kernels (hazelnuts, FDA, 2009c; macadamia, FDA, 2009a; pecans, Hitti, 2009; pine nuts, FDA, 2010a; and walnuts, FDA, 2010b) and inshell nuts (hazelnuts, CFIA, 2012a; pistachios, FDA, 2009b; walnuts, CFIA, 2012b). Walnut kernels also were recalled in 2009 after isolation of *Listeria monocytogenes* (Hughlett, 2009).

The microbiota of walnuts has not been well described in the literature. Limited surveys have isolated coliforms (Weinzirl, 1929) and *E. coli* (Entis et al., 1984; Kokal, 1965; Little et al., 2009, 2010; Meyer and Vaughn, 1969; Riyaz-Ul-Hassan et al., 2003) from walnut kernels. *Salmonella* was isolated from walnut kernels in India (10 g, $n = 50$) (Riyaz-Ul-Hassan et al., 2003) and from one pre-packed mixed nuts sample (25 g, $n = 329$) that also contained walnuts (Little et al., 2010), but was not detected in other surveys that included walnut kernels (25 g, $n = 74$ (Little et al., 2009); 25 g, $n = 441$ (Little et al., 2010); 25 g, $n = 80$ (NSW Food Authority, 2012)). In a 3-year survey

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of California inshell walnuts, *E. coli* O157:H7 was not detected; *Salmonella* was detected in none of the samples in 2010 (100 g, $n = 935$), in 0.2% of samples in 2011 (375 g, $n = 905$), and in 0.1% of samples in 2012 (375 g, $n = 999$) (Eidsath, 2012).

The United States is the leading exporter of the Persian or English walnut (*Juglans regia* L.); 99% of the U.S. production (470,000 metric tons projected in 2012) is grown in California (California Walnut Commission, 2012; USDA FAS, 2012; USDA NASS, 2012). Shortly after harvest, walnuts are hulled to remove the fleshy husk and then dried with forced air; dried inshell walnuts may be stored prior to packaging or shelling. In 2011, approximately 40% of edible California walnut kernels were sold in-the-shell (estimated average 44% kernel weight) and the majority of these inshell walnuts (94%) were exported (California Walnut Board, 2012); the remaining 60% were removed from storage as needed, then cracked and sold as kernels.

Traditionally, most of the inshell walnuts sold in North America undergo a shell-lightening (or "brightening") treatment by direct surface application of sodium hypochlorite at a concentration of 3 to 4% (30,000–40,000 μg/ml or ppm in solution). The solution is sprayed onto the nuts, which are then mechanically mixed for approximately 2 min in a barrel trommel, and dried with or without forced air (Lindsay, 2010). Although the purpose of this treatment is to lighten shells, sodium hypochlorite is also a common disinfectant; it is unknown to what extent brightening impacts the microbial load on walnut shells.

The routes of contamination of tree nuts have not been definitively determined, but there are a number of potential opportunities for introduction of foodborne pathogens to walnuts through direct contact with contaminated soil during harvest, during postharvest hulling and drying, during cracking and shelling, or during further processing (Blessington, 2011; Meyer and Vaughn, 1969; Weinzirl, 1929). Foodborne pathogens can survive for extended periods on walnut kernels (Blessington et al., 2012) and *Salmonella* has been shown to survive on the shells of pecans and hazelnuts (Beuchat and Heaton, 1975; Beuchat and Mann, 2010a, 2010b; Komitopoulou and Peñaloza, 2009). Survival of foodborne pathogens on inshell walnuts has not been documented. The objectives of this study were to evaluate the survival of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* during storage of inshell walnuts, and to determine the impact of a brightening treatment on reducing *Salmonella* levels on inoculated inshell walnuts.

2. Materials and methods

2.1. Walnut samples

Inshell walnuts, *J. regia* L. cv. Hartley and cv. Chandler, were obtained from a San Joaquin county processor in California. The walnuts had been hulled and dried (to <8% moisture) at a commercial huller-dehydrator and had been stored at the processor for 1 to 6 months after harvest. For the inoculation studies, the inshell walnuts were used within 1 month of receipt; for the brightening study, the walnuts were stored for up to 11 months at ambient conditions in the laboratory (23–25 °C, 25–35% relative humidity) in a closed container. Walnuts with missing shell or those with major visible cracks were discarded.

2.2. Bacterial cultures

The pathogens used in this study were as follows: *S. enterica* Enteritidis PT 30 (ATCC BAA-1045), isolated from raw almonds associated with an outbreak (Isaacs et al., 2005); *S. enterica* Enteritidis PT 9c, a clinical isolate from an outbreak associated with raw almonds (CDC, 2004); *S. enterica* Anatum (CAHFS D0307231), isolated from an almond survey (Danyluk et al., 2007); *S. enterica* Oranienburg, isolated from pecans, (provided by Dr. Larry R. Beuchat, University of Georgia); *S. enterica* Tennessee (K4643), a clinical isolate from a peanut butter-associated outbreak (CDC, 2007); *E. coli* O157:H7 (H1730), a clinical isolate from a lettuce-associated outbreak; *E. coli* O157:H7 (CDC 658), a clinical isolate from a cantaloupe-associated outbreak; *E. coli* O157:H7 (F4546), a clinical isolate from an alfalfa sprout-associated outbreak; *E. coli* O157:H7 (Odwalla strain 223), isolated from an apple juice-associated outbreak; *E. coli* O157:H7 (EC4042), a clinical isolate from a spinach-associated outbreak (Kotewicz et al., 2008); *L. monocytogenes* (4b) (LJH552), isolated from tomatoes; *L. monocytogenes* (4b) (LCDC81-861), isolated from a raw cabbage–associated outbreak; *L. monocytogenes* (4b) (Scott A), a clinical isolate from a milk-associated outbreak; *L. monocytogenes* (1/2a) (V7), isolated from milk in a milk-associated outbreak; and *L. monocytogenes* (4b) (101 M), isolated from beef in a beef-associated outbreak. *E. coli* K12 was used as a pathogen substitute, for safety reasons and to mimic similar viscosity and chemical characteristics of inoculation liquid, in experiments in which the moisture content and water activity of the walnut shells and kernels were analyzed before, during, and after inoculation.

Many of the inshell walnuts used in this study had high initial populations of bacteria (>5 log CFU/nut) and yeasts and molds (>3 log CFU/nut), which necessitated the use of antibiotic-resistant strains. Mutants of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* able to grow in media supplemented with rifampicin (Rif) (Sigma-Aldrich, St. Louis, MO) at 50 μg/ml were isolated and used in experiments in which the inoculation level was near the indigenous microbiota level. Unless otherwise specified, culture media were obtained from BD (Franklin Lakes, NJ), and were supplemented with Rif. The isolates were stored at −80 °C in tryptic soy broth (TSB) supplemented with 15% glycerol (Fisher Scientific, Fair Lawn, NJ).

2.3. Inoculum preparation

The single-strain inocula were prepared as described by Uesugi et al. (2006). The frozen stock culture was streaked for isolation onto tryptic soy agar (TSA: tryptic soy broth plus 1.5% granulated agar) and incubated at 37 ± 2 °C for 24 ± 3 h. A 10-μl sterile loop of this culture was transferred into 10 ml of TSB and incubated at 37 ± 2 °C for 24 ± 3 h; this transfer procedure into TSB was repeated once. An aliquot (1 ml) of the second overnight culture was spread over large TSA plates (150 by 15 mm) and incubated at 37 ± 2 °C for 24 ± 3 h. The resulting bacterial lawn was collected by adding 9 ml of a 0.1% peptone to each plate and scraping the surface of the plate with a sterile spreader (Lazy-L Spreader, Andwin Scientific, Tryon, NC). The harvested cells (11 log CFU/ml) were diluted, as appropriate, with 0.1% peptone to inoculum levels ranging from 4 to 11 log CFU/ml. The five-strain mixtures of *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes* were prepared by growing each strain separately (under the conditions described above) and then combining equal volumes of each strain to produce the target inoculum. The populations in the individual and final mixed inocula were determined by serial dilution in Butterfield's phosphate buffer (BPB) and plating onto media as described below.

2.4. Inoculation procedures

Inshell walnuts were inoculated as described by Uesugi et al. (2006) for almond kernels. Inshell walnuts (400 g) were weighed into a sterile bag, inoculum (25 ml) was added, and the sealed bag was shaken and rubbed by hand for 2 min. Inoculated walnuts were spread onto four layers of filter paper (57 by 46 cm sheets; Qualitative P-5 Grade, Fisher Scientific) that was placed into a lidded plastic container (leaving a 3- to 5-cm gap to allow for air exchange). Walnuts were dried under ambient conditions for 24 ± 2 h. After drying, inshell walnuts were placed in sterile plastic bags and manually mixed by shaking for 2 min.

2.5. Storage conditions

To evaluate pathogen survival on inshell walnuts, inoculated and control nuts were stored in unsealed bags within closed plastic

containers held at refrigerator (4 °C) or ambient conditions for periods of 12 weeks to 3 years, depending upon the experiment. Condensate was not observed in the bags or on the walnuts during storage. Data loggers (TempTale 4, Sensitech Inc., Beverly, MA) were placed in each storage area to record temperature and relative humidity (RH).

2.6. Reduction of Salmonella on inshell walnuts washed in water or sodium hypochlorite

The reduction of *Salmonella* Entertidis PT 30 during ambient storage was determined after exposing the inoculated inshell walnuts to water (control) or sodium hypochlorite. The inshell walnuts were inoculated with *Salmonella* at 9 log CFU/nut (wet) and dried for 24 h at ambient conditions as described above. The inshell walnuts were treated either immediately after the 24-h inoculum-drying period or after 7 days of ambient storage. Groups of six nuts were placed into 500-ml lidded jars (Nalgene, Rochester, NY) with either 20 ml of sterile distilled water (pH: 6.3) or a 3% solution of sodium hypochlorite (30,000 μg/ml or ppm; pH: 9.6). This ratio of walnuts to liquid was sufficient to visibly coat the nuts without producing significant excess liquid. The jars were vigorously shaken in a 10-cm arc for 2 min to mimic agitation of the nuts under commercial conditions. Water-washed, sodium hypochlorite-treated, and non-treated nuts were spread onto four layers of filter paper and dried under ambient conditions for 24 ± 2 h. After drying, nuts were stored for up to 2 weeks at ambient conditions and analyzed as previously described.

2.7. Enumeration

For pathogen enumeration, an individual inshell walnut (approximately 12 g) was added to 10 ml of 0.1% peptone or, for those samples in the brightening study, D/E neutralizing broth (both without Rif) in a sterile 532-ml (18-oz) Whirl-Pak bag (Nasco, Modesto, CA). Each bag was rubbed by hand and periodically shaken in a 10-cm arc for 2 min.

The bacterial population density in the recovery liquid was determined by serial dilution in BPB and plated onto TSA for all inoculated organisms as well as on bismuth sulfite agar (BSA) for *Salmonella*, MacConkey sorbitol agar (SMAC; without Rif) for *E. coli* O157:H7, and Oxford medium base with modified Oxford antimicrobic supplement (MOX) for *L. monocytogenes*. TSA, SMAC, and MOX plates were incubated at 37 ± 2 °C for 24 ± 3 h; BSA plates were incubated at 37 ± 2 °C for 48 ± 3 h. Colonies were counted and bacterial populations were determined. The calculated CFU per millimeter of plated solution multiplied by 10 ml (the volume of diluent) was considered to be equivalent to the CFU recovered per nut.

For some studies, enrichment was conducted when sample results were expected to be below the limit of detection (LOD; 10 CFU/nut). For all pathogens, the sample remaining after plating (remainder of the 10-ml diluent and the inshell walnut) was added to 50 ml of TSB and incubated at 37 °C for 24 or 48 ± 3 h. Secondary enrichments for each pathogen (*Salmonella*: Rappaport-Vassiliadis R10 broth and tetrathionate broth, both without Rif; *E. coli* O157:H7: Brilliant Green Bile Lactose broth without Rif; *L. monocytogenes*: UVM Modified *Listeria* enrichment broth without Rif) and confirmations on differential/selective media (*Salmonella*: BSA, xylose lysine deoxycholate agar without Rif, and Hektoen enteric agar without Rif; *E. coli* O157:H7: BBL CHROMagar O157 (ChromO157; BD Diagnostic Systems); *L. monocytogenes*: MOX) were conducted exactly as described in detail previously (Blessington et al., 2012).

2.8. Moisture content and water activity of walnut shells and kernels

Moisture content and water activity were compared for shells and kernels obtained from uninoculated inshell walnuts and *E. coli* K12–inoculated inshell walnuts (10 log CFU/nut) immediately after inoculation or after drying on filter paper for 24 h under ambient conditions. Inshell walnuts were cracked with a culinary nut cracker, kernels and shells were separated, and pieces were reduced (to ~1 cm) with a mortar and pestle. Moisture content and water activity of the sieved samples was measured with a dual moisture content and water activity meter (AquaLab model 4TE DUO, Decagon Devices, Pullman, WA).

2.9. Experiment design and statistical analysis

Six replicates per experiment were used to enumerate the population density at each sampling time, and three replicates per experiment were used to estimate moisture and water activity of nut samples. When enumerated bacterial values obtained were below the LOD (10 CFU/nut) but positive through enrichment of the remaining sample, the bacterial concentration was analyzed with an assigned value of just below the LOD or 9 CFU/nut (0.9 log CFU/nut). When results were negative after enrichment, the bacterial concentration was analyzed with an assigned value of 0.1 CFU/nut (<1 CFU/nut) or −0.9 log CFU/nut. Population declines were normalized by the initial wet-nut level or dry-nut level. Analyses of variance and post-hoc Tukey's HSD multiple comparison tests were performed with the JMP 8 software package (SAS Institute, Cary, NC). Differences between the mean values were considered significant at $P < 0.05$. Baranyi, Gompertz, and linear regression models of microbial behavior were developed with the aid of DMFit (Baranyi and Roberts, 1994; Zwietering et al., 1991) and JMP 8. Rates of bacterial decline during storage were converted from log CFU per nut per day to log CFU per nut per month by multiplying by 30.4.

3. Results and discussion

3.1. Influence of inoculation on moisture content and water activity of walnut shells and kernels

Shell moisture content and water activity were affected by the aqueous inoculation procedure, initially increasing by more than 1% (from 3.9 to 5.1%) and 0.30 (from 0.28 to 0.60), respectively. After drying at ambient conditions for 24 h, inoculated shells differed from the uninoculated shells in moisture content and water activity by <0.05% (4.3%) and <0.01 (0.41 and 0.42), respectively. Kernel moisture and water activity for inoculated walnuts differed by <0.2% (from 3.9 to 4.1%) and <0.1 (from 0.28 to 0.34), respectively, from the uninoculated controls immediately after inoculation and no differences in moisture (4.3%) and water activity (0.42) were observed after drying.

3.2. Influence of temperature and inoculum level on survival of Salmonella Enteritidis PT 30 on inshell walnuts during storage

Inshell walnuts are typically stored in large silos or in warehouses in bins. Temperatures during storage are often at ambient during the cooler months after harvest; as ambient temperatures rise, walnuts may be transferred to cold storage (4 to 10 °C) to reduce the potential for development of rancidity. Inshell walnuts may also be stored, and are often distributed and retailed, at ambient temperature.

Inshell walnuts were inoculated with *Salmonella* at approximately 10 log CFU/nut and dried for 24 h at ambient conditions; after drying, population densities declined by 0.70 log CFU/nut to 9.5 log CFU/nut (Table 1). The inoculated dried walnuts were stored at 4 °C and ambient conditions. At 21 days of storage and all subsequent sampling times, *Salmonella* populations were significantly greater on walnuts stored at 4 °C (relative humidity ranged from 65 to 95%) than those stored under ambient conditions (Fig. 1A). After 20 weeks (139 days), populations on walnuts stored at 4 °C or ambient had declined by 0.5 or 2.7 log CFU/nut, respectively (Table 1, Fig. 1A). Although it is unusual for walnuts to be stored for more than 1 year, *Salmonella* levels were also determined on these nuts stored for 1.2 and 3.1 years (431 and 1143 days, respectively). After 1.2 and 3.1 years of storage, *Salmonella*

Table 1
Calculated rates of decline for inoculated pathogens on inshell walnuts after drying, during storage, and after treatment by shaking for 2 min in distilled water or 3% sodium hypochlorite.

Bacteria	Storage temp	Population level (log CFU/nut)[a]			Storage time (day)	ANOVA *P*-value	Model type[b]	Rate of change (log CFU/nut/month)[c]	R^2
		Wet nut (0 h)	Dry nut (24 h)	Nut at end of storage					
Salmonella Enteritidis PT 30	4 °C	10.2 ± 0.10	9.5 ± 0.12	9.0 ± 0.12	139	<0.001	Baranyi-linear	−0.11	0.40
				8.0 ± 0.29	431	<0.001	Baranyi-linear	−0.11	0.81
				6.1 ± 0.69	1143	<0.001	Baranyi-linear	−0.09	0.93
	Ambient	10.2 ± 0.10	9.5 ± 0.15	6.8 ± 0.27	139	<0.001	Baranyi-linear	−0.55	0.85
				5.9 ± 1.1	431	<0.001	Baranyi	−0.56	0.84
				3.9 ± 0.34	1143	<0.001	Gompertz	−0.59	0.85
	Ambient	10.0 ± 0.12	9.3 ± 0.04	7.0 ± 0.27	83	<0.001	Baranyi	−1.33	0.91
	Ambient	9.0 ± 0.10	7.6 ± 0.08	6.4 ± 0.46	14	<0.001	Linear	−2.3	0.48
	Ambient	7.5 ± 0.44	6.0 ± 0.37	2.2 ± 0.08	83	<0.001	Linear[d]	−1.24	0.60
	Ambient	5.7 ± 0.34	3.7 ± 0.76	1.3 ± 0.55	27[e]	<0.001	Linear[d]	−2.54	0.63
Salmonella cocktail	Ambient	3.9 ± 0.37	1.8 ± 0.33	1.1 ± 0.36	97	0.003	ND		
		3.9 ± 0.28	1.8 ± 0.38	1.1 ± 0.47		0.012	ND		
E. coli O157:H7 cocktail	Ambient	3.7 ± 0.57	1.5 ± 0.50	1.3 ± 0.84	97	0.049	ND		
L. monocytogenes cocktail	Ambient	4.4 ± 0.37	2.5 ± 0.77	0.0 ± 0.99	97	<0.001	ND		
		4.7 ± 0.33	2.3 ± 0.64	0.0 ± 0.99		<0.001	ND		

[a] *Salmonella* Enteritidis PT 30 values are based on data collected from BSA media; *Salmonella* cocktail values are based on data collected from TSA + Rif and BSA + Rif media, respectively; *E. coli* O157:H7 cocktail values are based on data collected from TSA + Rif media; *L. monocytogenes* cocktail values are based on data collected from TSA + Rif and MOX + Rif media, respectively.
[b] Baranyi (DMfit), Gompertz, and linear regression models were chosen based on R^2 value. Baranyi-linear: both models have equivalent R^2 and rates of change.
[c] Rate of change (log CFU/nut/month) was calculated from the slope of the regression model (log CFU/nut/day) multiplied by 30.4.
[d] Linear model was chosen over the Baranyi model, which had higher R^2 but predicted an unreasonable decline (6 to 7 log CFU/nut/month).
[e] Samples stored longer than 27 days had counts lower than the LOD (1 log CFU/nut) and were not enriched; population values beyond 27 days were not used in the regression analysis.

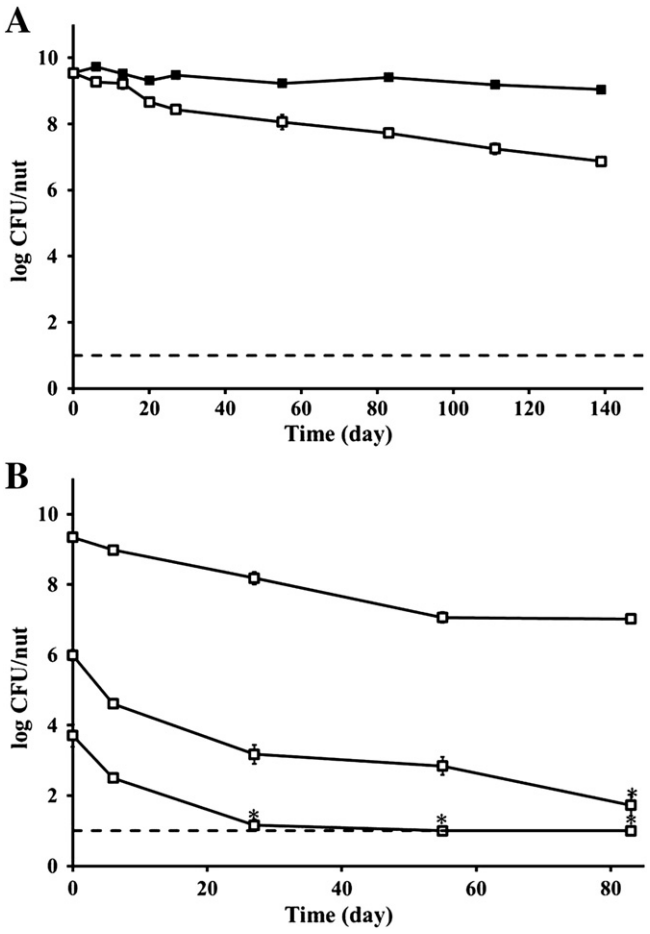

Fig. 1. Survival of *Salmonella* Enteritidis PT 30 on inshell walnuts during storage. Storage began on day 0 (24 h after inoculation). Results are mean plate counts (and standard error) on BSA + Rif: A) influence of storage temperature, 4 °C (closed square) and ambient conditions (open square); and B) influence of inoculum level (initial inoculum levels: 6, 8, and 10 log CFU/nut) on walnuts stored at ambient conditions. * indicates at least one replicate was below the LOD (1 log CFU/nut).

populations had declined by 1.5 and 3.4 log CFU/nut, respectively, at 4 °C and by 3.6 and 5.6 log CFU/nut, respectively, at ambient conditions (Table 1).

The long-term survival of *Salmonella* in tree nuts is well documented (Abd et al., 2012; Beuchat and Heaton, 1975; Beuchat and Mann, 2010a; Blessington et al., 2012; Kimber et al., 2012; Komitopoulou and Peñaloza, 2009; Uesugi et al., 2006). The survival of *Salmonella* at ambient conditions as observed in the current study for inshell walnuts was comparable to the survival in these previous studies. Survival of *Salmonella* in tree nuts is usually significantly better at colder temperatures; in some cases bacterial levels remain virtually unchanged for more than a year of storage at −20 or 4 °C (Beuchat and Mann, 2010a; Blessington et al., 2012; Kimber et al., 2012; Uesugi et al., 2006). Consistent with the current study, *Salmonella* levels have also been shown to slowly decline during low-temperature storage (−20 to 5 °C) on inoculated pecan kernels, inshell pecans, crushed hazelnut shells, and crushed cocoa shells (Beuchat and Heaton, 1975; Beuchat and Mann, 2010a; Komitopoulou and Peñaloza, 2009). The differences in low-temperature survival among different nuts may be linked to available nutrients and/or protectants on the surface of the inshell or kernel, the bacterial strain, the inoculation procedure, or other storage variables (e.g., humidity).

Natural levels of contamination of walnuts with *Salmonella* are not known but are likely to be very low (e.g., 1 MPN/100 g) based on levels measured in other tree nuts (Bansal et al., 2010; Danyluk et al., 2007; Lieberman and Harris, unpublished). One of the potential points of contamination of walnuts after harvest is when the outer hull is removed. After hulling, inshell walnuts pass through a "rock" or float tank that allows heavy materials like stones to separate from the product. Aerobic plate counts and coliform counts in the rock tank water can exceed 6 log CFU/ml (Blessington, 2011; Frelka and Harris, unpublished). Meyer and Vaughn (1969) reported hulling water with *E. coli* levels of 4.3 log CFU/ml at a black walnut (*Juglans nigra*) facility. Walnuts leaving the rock tank are often rinsed with potable water or sometimes with water containing an antimicrobial such as peroxiacetic acid. Even so, aerobic plate counts and coliform counts of more than 6 and 5 log CFU/nut, respectively, before dehydration are not uncommon (Blessington, 2011; Frelka and Harris, unpublished).

Inoculating product with pathogens at high levels allows for easier enumeration of microbial populations. This may be an appropriate

approach if the rates of decline of the pathogen are similar across a wide range of inoculum levels, however, the survival dynamics of various inoculation concentrations may be incongruent. Inshell walnuts were inoculated at 10, 8, and 6 log CFU/nut and stored for 90 days at ambient conditions. Inoculation level influenced the survival of *Salmonella* on inshell walnuts during both drying of the inoculum and subsequent storage. During the initial 24-h drying period, a greater reduction in *Salmonella* populations was observed for walnuts inoculated at 6 and 8 log CFU/nut (2.0- and 1.5-log CFU/nut reductions, respectively) than for walnuts inoculated at 10 log CFU/nut (0.7-log CFU/nut reduction) (Table 1). Inoculum level similarly impacted survival of *Salmonella* on walnut kernels (Blessington et al., 2012) and almond kernels and inshell pistachios (Kimber et al., 2012) during postinoculation drying but not during long-term storage of walnut and almond kernels (Blessington et al., 2012; Uesugi et al., 2006) or of inshell pecans (Beuchat and Mann, 2010a).

During the first 4 weeks of ambient storage after drying, bacterial populations declined more rapidly for inshell walnuts inoculated at 6 or 8 log CFU/nut than for walnuts inoculated at 10 log CFU/nut (Fig. 1B). Similarly for medium pecan pieces, the decline of *Salmonella* was greater within the first few weeks of storage when inoculated at moderate (5 log CFU/g) compared to high (7 log CFU/g) levels (Beuchat and Mann, 2010a). When walnuts were inoculated at 6 log CFU/nut, populations of *Salmonella* fell below the LOD (1 log CFU/nut) in three out of six samples after 4 weeks and in all six samples by 8 weeks of storage. At an initial inoculum of 8 log CFU/nut *Salmonella* levels were above the LOD through 8 weeks and fell below the LOD in two of six samples at 12 weeks of storage.

3.3. Survival of low-level cocktails of Salmonella, E. coli O157:H7, and L. monocytogenes inoculated on inshell walnuts

Inshell walnuts were inoculated at 4 log CFU/nut with five-strain cocktails of *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes* (4 to 5 log CFU/ml); survival was evaluated over 14 weeks (97 days) of ambient storage (Table 2). During the 24-h drying period, *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* declined by 2.1, 2.2, and 1.9 log CFU/nut, respectively, as determined on TSA; declines among the genera were not significantly different. Thus at the beginning of ambient storage, bacterial populations were near the limit of detection by plating (10 CFU/nut) and average counts were estimated using a combination of plate counts and enrichment data. A range of bacterial levels was observed among samples within a single time point, sometimes resulting in a standard deviation that was larger than the average counts, in part, a product of the combination of enumerated and assigned values for samples (Table 2). This is an inherent limitation of microbiological data. Gathering statistically sound plate count data is only possible when using higher inoculum concentrations, but such treatments are less likely to mimic natural contamination scenarios. Lowering the limit of detection for enumeration by filtration or including MPN determinations would add time and cost to the analysis but should be considered for future studies. Differences among samples in nut shell topography and shell integrity (e.g., small cracks) may also have contributed to this variation by influencing our ability to remove inoculated organisms with our sampling procedure.

Bacterial decline at each sampling point during storage was calculated by subtracting the levels determined at the sampling point from the levels measured at the beginning of storage. The declines among the three genera were similar at all sampling points except for three; greater declines were observed in *L. monocytogenes* populations than in *Salmonella* and *E. coli* O157:H7 populations at 27, 83, and 97 days of storage. Over 97 days of ambient storage, declines of *Salmonella* and *E. coli* O157:H7 were estimated to be less than 1 log CFU/nut and the decline of *L. monocytogenes* was 2 log CFU/nut. These declines were less than the 2.8- to 3.8-log decline observed for *Salmonella* Enteritidis PT 30 on walnuts inoculated at 10 or 7.5 log CFU/nut, respectively, and stored for a similar length of time (83 to 139 days) (Table 1). These data are comparable to previous studies with other tree nuts; as populations decrease to near the standard LOD the rate of decline slows (Beuchat and Heaton, 1975; Beuchat and Mann, 2010a; Blessington et al., 2012; Kimber et al., 2012).

During the 97-day storage period, 78 inshell nuts were sampled per genera; of these, 73 *Salmonella*-, 66 *E. coli* O157:H7-, and 66 *L. monocytogenes*-inoculated nuts were positive by plate count or enrichment. Plate counts of at least 1 log CFU/nut were obtained for 49 *Salmonella*-, 23 *E. coli* O157:H7-, and 31 *L. monocytogenes*-inoculated nuts. At all time points during storage after the initial plating, all samples were subjected to a primary enrichment. Enriched broths were streaked

Table 2
Survival of five-strain cocktails of *Salmonella enterica*, *Escherichia coli* O157:H7, or *Listeria monocytogenes* on inshell walnuts stored at ambient conditions (23–25 °C, 25–35% RH).[a]

Storage day[b]	*Salmonella*						*E. coli* O157:H7					*L. monocytogenes*					
	TSA + Rif (log CFU/nut)	BSA + Rif (log CFU/nut)	E[c]	1°[d]	2°	3°	TSA + Rif[e] (log CFU/nut)	E	1°	2°	3°	TSA + Rif (log CFU/nut)	MOX + Rif[f] (log CFU/nut)	E	1°	2°	3°
−1	3.9 ± 0.37	3.9 ± 0.28	6	NA	NA	NA	3.7 ± 0.57	6	NA	NA	NA	4.4 ± 0.37	4.7 ± 0.33	6	NA	NA	NA
0	1.8 ± 0.33a	1.8 ± 0.38a	6	6	NA	NA	1.5 ± 0.52a	5	6	NA	NA	2.5 ± 0.77a	2.3 ± 0.64a	6	6	NA	NA
2	1.5 ± 0.48a	1.5 ± 0.45a	6	6	NA	NA	1.2 ± 0.34a	4	6	NA	NA	1.9 ± 0.39a	1.6 ± 0.39a	6	6	NA	NA
6	2.0 ± 1.1a	2.0 ± 1.2a	6	6	NA	NA	1.3 ± 0.64ab	3	5	6	6	1.1 ± 0.34b	0.92 ± 0.04b	4	6	NA	NA
13	1.7 ± 0.93a	1.4 ± 0.66a	5	6	NA	NA	1.0 ± 0.24a	1	6	NA	NA	1.8 ± 0.49a	1.4 ± 0.45a	6	6	NA	NA
20	1.8 ± 1.1a	1.7 ± 1.0ab	3	6	NA	NA	0.90 ± 0.00ab	0	6	NA	NA	1.4 ± 0.86ab	1.0 ± 0.24b	2	3	6	NA
27	1.7 ± 0.75a	1.5 ± 0.70a	5	6	NA	NA	0.93 ± 0.05a	2	6	NA	NA	1.0 ± 0.33b	0.90 ± 0.00b	1	4	6	NA
34	1.9 ± 1.2a	1.9 ± 1.1a	5	6	NA	NA	0.41 ± 1.6a	2	3	3	3	1.1 ± 0.47a	0.97 ± 0.16a	1	6	NA	NA
41	0.3 ± 0.9a	0.33 ± 0.96a	2	4	4	4	0.93 ± 0.05a	2	6	NA	NA	0.70 ± 0.82a	0.71 ± 0.82a	2	4	5	5
48	1.4 ± 0.99a	1.3 ± 0.94a	4	6	NA	NA	0.92 ± 1.6a	2	4	4	4	0.43 ± 1.1a	0.30 ± 0.93a	2	4	4	4
55	0.77 ± 0.91a	0.81 ± 0.96a	1	4	5	5	0.00 ± 0.99a	0	2	3	3	0.62 ± 0.74a	0.60 ± 0.74a	1	4	5	5
69	1.6 ± 1.2ab	1.7 ± 1.2a	2	6	NA	NA	0.00 ± 0.99ab	0	2	3	3	0.90 ± 0.00b	0.90 ± 0.00ab	0	4	6	6
83	0.38 ± 1.0a	0.50 ± 1.1a	2	4	4	4	0.60 ± 0.74a	0	4	4	5	−0.60 ± 0.74b	−0.60 ± 0.74b	0	1	1	1
97	1.1 ± 0.36a	1.1 ± 0.47a	2	6	NA	NA	1.3 ± 0.84a	2	6	NA	NA	0.00 ± 0.99b	0.00 ± 0.99b	0	3	3	3

[a] Values are means ± standard deviation. Within rows, means with different letters are significantly different on the basis of decline values calculated from the initiation of storage ($P < 0.05$).

[b] Days before (−1) or during (0 to 97) storage; walnuts were placed in storage 24 h after inoculation (time 0).

[c] E, number of replicates enumerated at or above the LOD (1 log CFU/nut).

[d] 1°, number of replicates that were positive after the primary enrichment; 2°, number of replicates that were positive after the secondary enrichment; 3°, number of replicates that were positive after the tertiary enrichment; NA, not applicable.

[e] Values obtained from SMAC cultures were disregarded because indigenous microbiota were not distinguishable from inoculated *E. coli* O157:H7. All replicate enrichments were streaked onto SMAC plates to confirm clear to white colonies typical of *E. coli* O157:H7 on this medium.

[f] Colonies isolated from TSA + Rif per replicate per sampling day were restreaked onto MOX + Rif to confirm values obtained from TSA + Rif. All restreaked colonies were black colonies typical of *L. monocytogenes* on this medium.

onto selective/differential media for confirmation if enumerated values were below the LOD or if the previous enrichment was negative. An additional number of walnuts were positive after secondary or tertiary enrichment (Table 2); 14 nut samples (6% of the 234 nut samples evaluated) required additional enrichment beyond the initial 24 h for positive isolation.

Recovery of pathogens from dry foods presents a challenge as the cells may be severely injured. It is possible that standard and, especially, more rapid enrichment techniques may deliver false negative results (D'Lima and Suslow, 2009). As rapid methodologies are adapted to dry products, they should be validated using samples inoculated at low levels and held under dry conditions that may promote populations of difficult-to-culture cells that reflect naturally-contaminated samples. The influence of desiccation stress and injury on bacterial cell virulence is unknown, thus at this time the assumption is that the health risk from injured cells is similar to that from healthy cells (Lesne et al., 2000).

3.4. Rates of decline for inoculated pathogens on inshell walnuts during storage

As noted above, the decline of inoculated bacteria approaches a nonlinear pattern at lower inoculum levels and the most significant reductions occur within the first month of storage. Similar survivor curves have been observed for *Salmonella* inoculated on walnut kernels (Blessington et al., 2012), on inshell pecans (Beuchat and Heaton, 1975), and on inshell pistachios (Kimber et al., 2012). Nonetheless, rates of decline were calculated to allow for more direct comparison among a range of experiments. In each single-strain inoculation study, an analysis of variance was conducted and time was analyzed as a factor in determining bacterial populations during storage. In each study, the variance between time points exceeded that within time points, allowing for further analysis to assess trends to predict bacterial levels. The data for *Salmonella* Enteritidis PT 30 were fit to linear, Baranyi, and Gompertz regression models. Best-fit models were selected based on their respective R^2 values. For comparison purposes the rates of decline for the non-linear curves of these models (DMFit and Gompertz) were developed from a potential maximum rate of the model rather than an average (Baranyi and Roberts, 1994), which most closely represented die-off during initial storage. In two cases (*Salmonella* Enteritidis PT 30 inoculation levels of 7.5 and 5.7 log CFU/nut), the DMFit model resulted in the greatest R^2 value; however, the shapes of these models were unreasonable due to a greatly exaggerated predicted rate of decline (6 to 7 log CFU/nut/month). Thus, the linear model was chosen for these two data sets (Table 1).

Rates of decline for *Salmonella* Enteritidis PT 30 (inoculated at log 10 CFU/nut) from 139 to over 3 years of storage at 4 °C and ambient conditions were approximately 0.1 and 0.6 log CFU/nut per month, respectively (Table 1, Fig. 1A). In a separate 83-day ambient storage study the calculated rates of decline for inoculation levels of 10, 8, and 6 log CFU/nut were 1.3, 1.2, and 2.5 log CFU/nut per month, respectively (Table 1, Fig. 1B). When inoculated at 6 log CFU/nut, *Salmonella* levels on some of the samples fell to or below the LOD upon storage for 27 days. At 8 and 12 weeks all six samples initially inoculated at 6 log CFU/nut had *Salmonella* levels that were below the LOD. Enrichment procedures were not performed on these samples and data beyond 27 days were not included in the model determinations. The calculated decline rate for this inoculation level was 2.5 log CFU/nut per month. A similar rate of decline (2.3 log CFU/nut per month) was calculated for the 14-day storage of untreated inoculated inshell walnuts within the water washing/brightening study even though the inoculum level in that experiment was 9 log CFU/nut. In general, shorter storage times and lower inoculum levels resulted in greater calculated rates of decline.

The survival of *Salmonella* on inshell nuts has been described in a limited number of nut crops including pecans (Beuchat and Heaton, 1975; Beuchat and Mann, 2010a, 2010b), hazelnuts (Komitopoulou and Peñaloza, 2009), and pistachios (Kimber et al., 2012); the survival of *E. coli* O157:H7 and *L. monocytogenes* on nuts (inshell or shelled) has only recently been reported in almond and walnut kernels, and for inshell pistachios (Blessington et al., 2012; Kimber et al., 2012). The association between low-moisture foods and *Salmonella* contamination has been well described (Scott et al., 2009). Due to the number of outbreaks and recalls resulting from *Salmonella* contamination, it has been assumed that this bacterium has a greater ability to survive in dry environments. However, recent low-moisture food or ingredient outbreaks associated with pathogenic *E. coli* (CDC, 2011; CFIA, 2011a, 2011b; Neil et al., 2012) and the long-term viability of this pathogen on the surface of inshell walnuts and walnut kernels suggest that this organism should be considered in hazard assessments for the production and processing of walnuts and other tree nuts. *L. monocytogenes* populations declined more rapidly than either *Salmonella* or *E. coli* O157:H7 on both inshell walnuts and walnut kernels. *L. monocytogenes* would be of concern in products that support the growth of this pathogen and that use raw nuts as an ingredient.

Data generated from cocktail inoculations were not modeled because they were a combination of enumerated and assigned values based on positive and negative enrichments. The LOD was reached at 0, 6, and 13 days of storage for *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*, respectively and one or more samples were negative upon enrichment by day 34, 41, and 41, respectively. Given the 1 to 2 log CFU/month reductions calculated for low-level inoculum and short storage time samples (Table 1), the detection of *Salmonella* by plate count was not expected and the results further suggest rates of decline at these lower levels are not congruent with those observed at higher inoculation levels. It is not known whether low levels of indigenous bacterial contaminants would survive in a manner similar to this low-level inoculation, but normal commercial storage should not be assumed to significantly reduce bacterial contaminants on inshell walnuts. In addition, caution should be taken when calculated decline values from a limited number of studies at high inoculation levels are used in the development of risk assessments.

3.5. Impact of shell washing or brightening on Salmonella Enteritidis PT 30 survival

High concentrations of sodium hypochlorite (3%) are currently used to cosmetically lighten a small proportion of inshell walnuts (primarily markets in U.S. and Canada) to meet appearance standards. Alternative brightening methods such as 5% sodium hydroxide under alkaline conditions (pH 8–9) have also been explored (Fuller and Stafford, 1992, 1993) but were not evaluated in this study. Inshell walnuts were inoculated with *Salmonella* and exposed to water or sodium hypochlorite at 1 or 8 days after inoculation. In both cases, when compared to the corresponding untreated samples, *Salmonella* levels declined by 0.3 to 0.4 log CFU/nut after 2 min of exposure to water and by 2.4 to 2.6 log CFU/nut after 2 min of exposure to sodium hypochlorite (Fig. 2A). Additional population declines of approximately 1 log CFU/nut were observed after the treated nuts were dried at ambient conditions for 24 h. *Salmonella* levels continued to decline by a further 1.2, 2.7, and 2.1 log CFU/nut during 2 weeks of storage at ambient conditions on the untreated, water-washed, and hypochlorite-treated samples; total reductions were 1.2, 3.1, and 4.7 log CFU/nut, respectively.

Both the water and sodium hypochlorite treatments reduced the levels of inoculated *Salmonella* on the surface of inshell walnuts, especially after drying and storage. Water washing of dry inshell walnuts is not a current commercial practice. Introduction of water into a dry food facility without adequate controls to prevent both the cross contamination within the facility and the establishment of harborage sites for *Salmonella* would be problematic (Scott et al., 2009). Although adding appropriate levels of a suitable antimicrobial to maintain water quality might overcome some of these issues, an aqueous pre-shelling treatment for kernel production would face additional challenges. Walnuts are sorted into inshell and shelling streams prior to brightening to

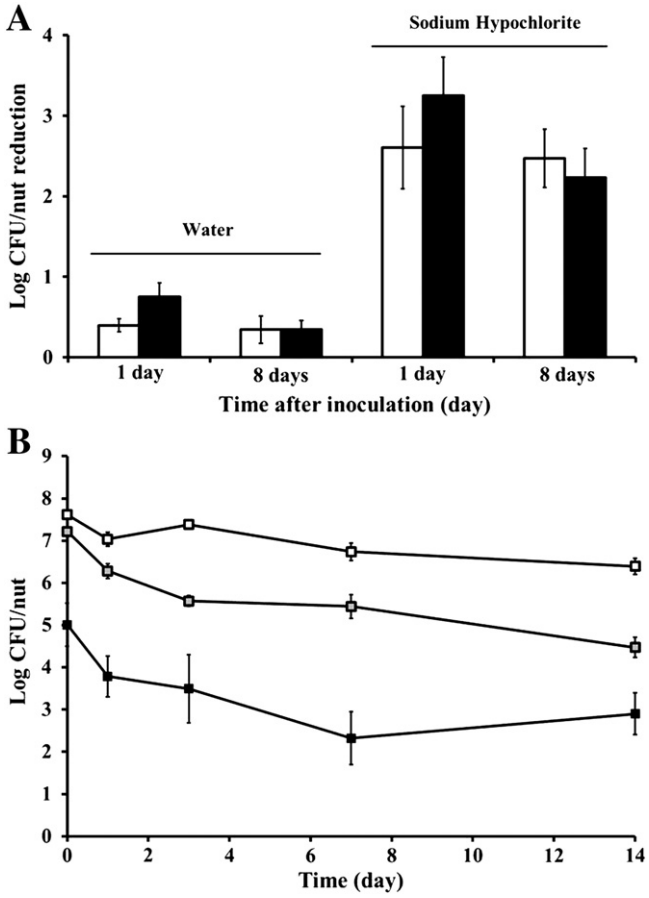

Fig. 2. Reductions of *Salmonella* Enteritidis PT 30 on inshell walnuts during water and brightening treatments and subsequent storage. Results are mean plate counts (and standard error) on BSA + Rif: A) reductions compared to corresponding untreated samples on inshell walnuts 1 or 7 days after inoculation after shaking for 2 min in water or 3% sodium hypochlorite immediately after treatment (open bars) and 24 h after treatment (black bars); and B) bacterial reductions on untreated (open squares), water treated (gray squares), and 3% sodium hypochlorite treated (black squares) inshell walnuts (1 day after inoculation) during storage at ambient conditions.

remove those with significantly cracked or broken shells from the inshell stream. This sorting leaves a significant portion of exposed nutmeats in the shelling stream and contact of the kernel with an antimicrobial might negatively impact kernel flavor.

Salmonella, *E. coli* O157:H7, and *L. monocytogenes* are capable of long-term survival on the surface of inshell walnuts even when initial levels are low. Walnut producers, processors, and those using walnuts as ingredients should consider these organisms when developing food safety plans and strive to minimize the opportunities for contamination and cross-contamination. Brightening treatments with sodium hypochlorite can reduce *Salmonella* levels and may, in some cases, be an appropriate preventative control measure.

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References

Abd, S.J., McCarthy, K.L., Harris, L.J., 2012. Impact of storage time and temperature on thermal inactivation of *Salmonella* Enteritidis PT 30 on oil-roasted almonds. Journal of Food Science 77, M42–M47.

Bansal, A., Jones, T.M., Abd, S.J., Danyluk, M.D., Harris, L.J., 2010. Most-probable-number determination of *Salmonella* levels in naturally contaminated raw almonds using two sample preparation methods. Journal of Food Protection 73, 1986–1992.

Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology 23, 277–294.

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

Beuchat, L.R., Mann, D.A., 2010a. Factors affecting infiltration and survival of *Salmonella* on in-shell pecans and pecan nutmeats. Journal of Food Protection 73, 1257–1268.

Beuchat, L.R., Mann, D.A., 2010b. Survival and growth of *Salmonella* in high-moisture pecan nutmeats, inshell pecans, inedible nut components, and orchard soil. Journal of Food Protection 73, 1975–1985.

Blessington, T., 2011. A Microbiological Hazard Analysis of California Walnut Production and Handling. (PhD dissertation) University of California, Davis.

Blessington, T., Mitcham, E.J., Harris, L.J., 2012. Survival of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on inoculated walnut kernels during storage. Journal of Food Protection 75, 245–254.

California Walnut Board, 2012. California Walnut Board monthly management report. Available at: http://www.walnuts.org/tasks/sites/walnuts/assets/File/MMR_Dec11.pdf (Accessed 13 November 2012).

California Walnut Commission, 2012. Walnut history and cultivation. Available at: walnuts.org/about-walnuts/walnut-history/ (Accessed 13 November 2012).

[CDC] Centers for Disease Control and Prevention, 2007. Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter—United States, 2006–2007. MMWR Weekly 56, 521–524.

[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, 484–487 (Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5322a8.htm. Accessed 4 May 2013).

[CDC] Centers for Disease Control, Prevention, 2011. Investigation update: multistate outbreak of *E. coli* O157:H7 infections associated with in-shell hazelnuts. Available at: www.cdc.gov/ecoli/2011/hazelnuts0157/ (Accessed 17 February 2012).

[CFIA] Canadian Food Inspection Agency, 2011a. Raw shelled walnuts sold from certain retail stores in London, Ontario and Calgary, Alberta may contain *E. coli* O157:H7 bacteria. Available at: www.inspection.gc.ca/english/corpaffr/recarapp/2011/20110411be.shtml (Accessed 17 February 2012).

[CFIA] Canadian Food Inspection Agency, 2011b. Certain walnuts and snack products may contain *E. coli* O157:H7 bacteria. Available at: www.inspection.gc.ca/english/corpaffr/recarapp/2011/20110909e.shtml (Accessed 17 February 2012).

[CFIA] Canadian Food Inspection Agency, 2012a. Certain pre-packaged and bulk in-shell filberts (hazelnuts) may contain *Salmonella* bacteria. Available at: www.inspection.gc.ca/english/corpaffr/recarapp/2012/20121202e.shtml (Accessed 20 December 2012).

[CFIA] Canadian Food Inspection Agency, 2012b. Certain compass brand in-shell California walnuts may contain *Salmonella* bacteria. Available at: www.inspection.gc.ca/english/corpaffr/recarapp/2012/20121130be.shtml (Accessed 20 December 2012).

D'Lima, C.B., Suslow, T.V., 2009. Comparative evaluation of practical functionality of rapid test format kits for detection of *Escherichia coli* O157:H7 on lettuce and leafy greens. Journal of Food Protection 72, 2461–2470.

Danyluk, M.D., Jones, T.M., Abd, S.J., Schlitt-Dittrich, F., Jacobs, M., Harris, L.J., 2007. Prevalence and amounts of *Salmonella* found on raw California almonds. Journal of Food Protection 70, 820–827.

Eidsath, C., California Walnut Board, 2012. Walnut survey results. Available from: the author at ljharris@ucdavis.edu (personal communication).

Entis, P., Bennett, B., Brodsky, M.H., Burgener, D.M., Carlson, V.L., Carson, M., Catherwood, K., Ciebin, B.S., Cox, N.A., Dahiya, R.S., Daley, H., Devenish, J., Erickson, J., Fettig, P., Foster, R., Gorski, J., Hall, P.A., Hunt, S.D., Jagow, J., Jenkins, P., Jordan, P.R., Krieger, F., Kwan, P., Lanier, J.M., Lillard, H.S., Martin, A., Milling, M.E., Nakanis, H., Nath, E., Oggle, J., Okrend, A., Pongchairerks, V., Posey, D., Rodriguez, J., Rose, B.E., Sayfie, J., Taylor, C., Teramoto, T., Vandellen, E., 1984. Enumeration of total coliforms, fecal coliforms, and *Escherichia coli* in foods by hydrophobic grid membrane filter: collaborative study. Journal of the Association of Official Analytical Chemists 67, 812–823.

[FDA] U.S. Food and Drug Administration, 2009a. Enforcement report for November 11, 2009. Available at: www.fda.gov/Safety/Recalls/EnforcementReports/ucm190285.htm (Accessed 17 February 2012).

[FDA] U.S. Food and Drug Administration, 2009b. Update on pistachio product recall. Available at: www.fda.gov/Safety/Recalls/MajorProductRecalls/Pistachio/Update/default.htm (Accessed 17 February 2012).

[FDA] U.S. Food and Drug Administration, 2009c. Willamette Shelling recalls shelled hazelnuts because of possible health risk. Available at: www.fda.gov/Safety/Recalls/ucm194806.htm (Accessed 17 February 2012).

[FDA] U.S. Food and Drug Administration, 2010a. Hines Nut Company announces recall of pine nuts because of possible health risk. Available at: www.fda.gov/Safety/Recalls/ucm197825.htm (Accessed 17 February 2012).

[FDA] U.S. Food and Drug Administration, 2010b. Tropical Nut & Fruit of Charlotte, NC announces a nationwide voluntary recall of walnut products because of possible health risk. Available at: www.fda.gov/Safety/Recalls/ucm236656.htm (Accessed 17 February 2012).

Fuller, G., Stafford, A.E., 1992. Quality and safety of unshelled walnuts. Walnut Research Reports 1992, pp. 288–292 (Available at: http://walnutresearch.ucdavis.edu/Topic_results.asp?txtSecNo=69. Accessed 17 February 2012).

Fuller, G., Stafford, A.E., 1993. Quality and safety of unshelled walnuts. Walnut Research Reports 1993, pp. 225–236 (Available at: http://walnutresearch.ucdavis.edu/Topic_results.asp?txtSecNo=69. Accessed 17 February 2012).

Hitti, M., 2009. *Salmonella* risk spurs Nature Valley recall — General Mills recalls certain 'Nut Lovers' flavor of Nature Valley Granola Nut Clusters. Available at: http://www.webmd.com/food-recipes/food-poisoning/news/20090710/salmonella-risk-spurs-nature-valley-recall (Accessed 17 February 2012).

Hughlett, M., 2009. Elgin nut company Sanfilippo issues recall. Available at: articles.chicagotribune.com/2009-10-15/news/0910140663_1_john-b-sanfilippo-nut-listeria (15 October, Accessed 17 February 2012).

Isaacs, S., Aramini, J., Ciebin, B., Farrar, J.A., 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.

Kimber, M.A., Kaur, H., Wang, L., Danyluk, M.D., Harris, L.J., 2012. Survival of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on inoculated almonds and pistachios stored at −19, 4, and 24 °C. Journal of Food Protection 75, 1394–1403.

Kokal, D., 1965. Viability of *Escherichia coli* on English walnut meats (*Juglans regia*). Journal of Food Science 30, 325–332.

Komitopoulou, E., Peñaloza, W., 2009. Fate of *Salmonella* in dry confectionery raw materials. Journal of Applied Microbiology 106, 1892–1900.

Kotewicz, M.L., Mammel, M.K., LeClerc, J.E., Cebula, T.A., 2008. Optical mapping and 454 sequencing of *Escherichia coli* O157:H7 isolates linked to the US 2006 spinach-associated outbreak. Microbiology 154, 3518–3528.

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), 718 (1 June).

Lesne, J., Berthet, S., Binard, S., Rouxel, A., Humbert, F., 2000. Changes in culturability and virulence of *Salmonella typhimurium* during long-term starvation under desiccating conditions. International Journal of Food Microbiology 60, 195–203.

Lindsay, S., 2010. Walnut brightening. Available from: the author at ljharris@ucdavis.edu (personal communication).

Little, C.L., Jemmott, W., Surman-Lee, S., Hucklesby, L., de Pinna, E., 2009. Assessment of the microbiological safety of edible roasted nut kernels on retail sale in England, with a focus on *Salmonella*. Journal of Food Protection 72, 853–855.

Little, C.L., Rawal, N., de Pinna, E., McLauchlin, J., 2010. Survey of *Salmonella* contamination of edible nut kernels on retail sale in the UK. Food Microbiology 27, 171–174.

Meyer, L.T., Vaughn, R.H., 1969. Incidence of *Escherichia coli* in black walnut meats. Applied Microbiology 18, 925–931.

Neil, K.P., Biggerstaff, G., MacDonald, J.K., Trees, E., Medus, C., Musser, K.A., Stroika, S.G., Zink, D., Sotir, M.J., 2012. Novel vehicle for transmission of *Escherichia coli* O157:H7 to humans: multistate outbreak of *E. coli* O157:H7 infections associated with consumption of ready-to-bake commercial prepackaged cookie dough—United States, 2009. Clinical Infectious Diseases 54, 511–518.

NSW Food Authority, 2012. Report on the prevalence of *Salmonella* and *E. coli* in ready to eat nuts and nut products sold in Australia, July 2012. Available at: www.foodauthority.nsw.gov.au/science/market-analysis/ready-to-eat-nuts/#.UNIEf6WE4dI (Accessed 9 December 2012).

Riyaz-Ul-Hassan, S., Verma, V., Malik, A., Qazi, G.N., 2003. Microbiological quality of walnut kernels and apple juice concentrate. World Journal of Microbiology and Biotechnology 19, 845–850.

Scott, V.N., Chen, Y.U.H., Freier, T.A., Kuehm, J., Moorman, M., Meyer, J., Morille-Hinds, T., Post, L., Smoot, L., Hood, S., Shebuski, J., Banks, J., 2009. Control of *Salmonella* in low-moisture foods I: minimizing entry of *Salmonella* into a processing facility. Food Protection Trends 29, 342–353.

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.

[USDA FAS] U.S. Department of Agriculture, Foreign Agricultural Service, 2012. Tree nuts: world markets and trade. Available at: usda01.library.cornell.edu/usda/fas/treenutwm//2010s/2012/treenutwm-10-26-2012.pdf (Accessed 13 November 2012).

[USDA NASS] U.S. Department of Agriculture, National Agricultural Statistics Service, 2012. 2012 California walnut objective measurement report. Available at: www.nass.usda.gov/Statistics_by_State/California/Publications/Fruits_and_Nuts/201209walom.pdf (Accessed 13 November 2012).

Weinzirl, J., 1929. Nuts as a possible source of *Escherichia coli* found in candy. American Journal of Hygiene 10, 265–268.

Zwietering, M.H., de Koos, J.T., Hasenack, B.E., de Witt, J.C., van't Riet, K., 1991. Modeling of bacterial growth as a function of temperature. Applied and Environmental Microbiology 57, 1094–1101.